THE OCCURRENCE AND TREATMENT OF POIKILOCYTOSIS IN DAIRY CATTLE

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by

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INTRODUCTION

Poikilocytosis, the term used to designate the pathological conditions in which deformed red corpuscles appear in the blood, is related to the physical phenomenon of crenation only insofar as the shape of erythrocytes is somewhat similarly distorted.

No single disease of the blood is characterized by poikilocytosis although it is generally noticeable in grave forms of primary anemia. Many workers describe infinitely variable shapes and sizes of poikilocytes and the degree of deformity, as marked or slight, depending upon the nature and severity of the disease with which it is associated.

The greater majority of these reports, however, concern human pathology. In addition to these, other investigations have suggested a possible relationship of poikilocytosis in animals to nutrition. This investigation was undertaken to study the occurrence, symptoms, possible causes and treatment of poikilocytosis in dairy cattle.

I. THE OCCURRENCE OF POIKILOCYTOSIS IN DAIRY CATTLE

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THE OCCURRENCE OF POIKILOCYTOSIS IN DAIRY CATTLE

The term "poikilocytosis" was first used by Quinke (45) in 1880 to designate a pathologic condition of the blood in which variously shaped deformed erythrocytes called poikilocytes appeared. The poikilocytic condition is characterized by various bizarre-shaped erythrocytes which vary greatly in size and shape. The degree of distortion may be extensive or slight, depending on the nature and the severity of the blood disease. Many shapes of poikilocytes have been observed which do not fall into any named class characterized by their particular form. However, various forms and shapes have been described: tailed (27, 31, 40), filamented (13, 41, 46, 50), club-shaped (27), dumbbell (10), sickle (8, 9, 13, 21, 22, 31, 48), spherical (18, 44), anvil (10), tennis racquet (10, 11, 40), stellate (54), tomahawk or hatchet (10, 11), gourd (11), teardrop (40), kidney (10, 11), cigar (10), sausage shape (40), horseshoe (11), cat shape (40), dagger (11), pencil (10), target (6, 12), dimpled (18), and boot shaped (40).

There are no reports in the literature which indicate that poikilocytosis is a primary disease in itself, and no uniform explanation accounts for the formation or the presence of the poikilocytes in the blood. A number of theories have been proposed, however: (a) Arneth (cited by Kanellis (26) and Haden (17)) expressed the belief that poikilocytes are produced in the bone marrow; (b) Isaacs (25), Cunningham (10), Dameshed (12) and Sydenstricker (49) were in agreement on the hypothesis that the cells are "congenitally deformed"; (c) according to Osler (41), they are produced by an altered condition of the serum, whereas (d) Penati (42) and Emmel (13) favored the belief

that erythrocytes are transformed to poikilocytes in the circulating blood.

Bohrod (7) indicated, however, that there is an accentuation of the eccentricity of the elliptic cell after its removal from the body which explains the formation of this type of malshapeness after exposure to the atmosphere. Whether or not this theory explains the production of poikilocytes, it necessitates caution in handling the blood specimen. Other investigators (10, 11, 40, 55) have also suggested the necessity of careful technic in the manipulation of the blood sample in order to avoid confusing the poikilocytes with artefacts. Da Costa (11) stated that poikilocytosis is akin to crenation so far as the cells in both conditions may be similarly distorted and misshapen. It is unlike crenation, however, for the reason that poikilocytosis is a pathologic condition, demonstrable the moment the blood is withdrawn from the body, whereas crenation is a physical phenomenon dependent on external influences for its production. Crenation never occurs until the blood has remained exposed to the air for some time. Osgood and Ashworth (40) regarded crenation as an artefact produced by faulty preparation of the blood specimen and stated that the crenated forms may be differentiated from the poikilocytes by the regularly notched outline and nodular surface which are characteristic of all of the cells in a particular area. Kanellis (26), Cunningham (10), and Wintrobe (55) called attention to the possible confusion of poikilocytes with crenated erythrocytes produced artificially by trauma during the preparation of the blood film. Other physical influences which distort the shape of the blood cell are: (a) diluting the blood sample in saline solution (3, 16), (b) drying blood films slowly (40), (c) heat (19, 26, 30, 34, 43, 50), (d) hemorrhage (50) and (e) irradiation with roentgen rays (26). Different chemical substances have been shown to produce changes in normal erythrocytes resulting in shapes which resemble poikilocytes. So-

dium chloride (3, 46), alkali dissolved from glass (53), mercuric chloride (46), lecithin and cholesterol (26), and alanine (38) are some of the substances which will alter the shape of the red cells.

Research on poikilocytes occurring alone in animals is quite limited and its occurrence in connection with human diseases has been studied in a secondary manner. Although little is known concerning its cause and mechanism, the appearance of poikilocytes has been observed and described in different species of animals and in human beings not only in disease but also in health. Many workers (10, 11, 25, 26, 28, 45, 50) observed poikilocytes with many bizarre shapes in blood from patients with pernicious anemia. Takeuchi (50) found filamented red blood cells and detached filaments in 69 per cent of observed cases of tuberculosis, carcinoma, intestinal disorder and fever, in addition to those seen in cases of pernicious anemia. Kilduffe (28) pointed out that abnormally shaped erythrocytes may appear in any case of severe or long-standing anemia. Elliptic red corpuscles, according to Florman and Wintrobe (14), are noted in most cases of anemia, and sometimes more than 25 per cent of the cells are affected. Da Costa (11) reported the general occurrence of small, slightly deformed poikilocytes in milder types of anemia and larger, extremely distorted poikilocytes in the more severe types. Penati (42) associated elliptically deformed erythrocytes with pernicious anemia, secondary anemia and hemolytic icterus, but he found no relationship between the intensity of cell deformation and the severity of the anemia, although the deformed cells became less noticeable with amelioration of the anemia.

Sickle cell anemia (first named by Mason (35) in 1922 but first described by Herrick (21)) is a condition in which a specific and constant type of poikilocytosis is associated with anemia. Huck (22), Sydenstricker (49)

and Alden (1) observed the sickle cells in the blood of male and female patients characterized by anemia and a tendency to have ulcers on the legs. Sydenstricker (49) reported sickle cell anemia occurring with abdominal pains and pathologic changes of the spleen. Cells of peculiar sickle shapes were noted in tissue sections and blood films by Landon and Lyman (32) in 1929. According to Emmel (13), the sickle cell trait appears to be hereditary and occurs most commonly in the negro race although the reports of Hunter and Adams (23), Lawrence (33) and Cooley and Lee (9) showed the occurrence of this condition in other races. Van den Bergh (51) and Huck (22) demonstrated that this condition may be inherited by either sex as a dominant trait.

Dameshek (12) reported a type of anemia characterized by target cells, which constituted as much as 32 per cent of the erythrocytes, and resembling somewhat Cooley's sickle cell anemia. He designated this condition "target-cell anemia" although this type of cell was found to be associated with other types of anemia. Bohrod (6) stated that the target cells appear for a short time in acute types of anemia but disappear as the erythrocyte count rises. In chronic types of anemia the target cells exist for a long period in quantities as great as 10 per cent of the erythrocytes. Haden and Evans (18) observed target cells in several different types of anemia but found them in great numbers in sickle cell anemia. Wintrobe (55) associated various undifferentiated poikilocytes and target cells with Mediterranean anemia. Isaacs (25) in studies of blood films of patients having miliary lesions of the bone marrow observed abnormal shapes in some of the red blood cells. The presence of poikilocytes in myelogenous leukemia and chlorosis was indicated by Emmel (13).

Mrowka (36) reported the presence of red corpuscles of spindle or elliptic shape in the blood of horses suffering from infectious anemia. Poisonous substances elaborated by many helminths were regarded by Hutyra and Marek (24) as a cause of poikilocytosis and anemia. Bernhard (4) reported what he termed "oval poikilocytosis", which was accompanied by anemia and splenic tumor. Apinis (2) observed filamented red blood cells in the blood of horses, cattle, sheep, dogs, cats, rabbits, pigs and chickens. The filaments varied for different animals in a distinctive manner. Thin filaments were noted on the erythrocytes of hogs, very long filaments on those of chickens and button-like structures on those of sheep and cats, and granulation was noticeable in the red blood cells of the horse. He gave no description of the erythrocytes observed in the blood of cattle. O'Roke (39) and Whitlock (54) observed sickle cells, stellate cells, rod-shaped cells and other poikilocytes in the blood of deer. Hanke and Koessler (20) reported poikilocytes in the blood of guinea pigs showing symptoms of scurvy. The observation of spindle-shaped poikilocytes in fowl's blood was made by Gordon (15).

Vawter (52) made microscopic examinations of the blood of dairy cattle and found poikilocytosis associated with anaplasmosis. He concluded that this is one of several clinical findings which distinguish anaplasmosis from bacillary hemoglobinuria (or red water disease) in dairy cattle. Blount (5) noted a few sickle cells in the blood of a day old calf which he thought were artefacts. He observed no poikilocytes in the normal adult bovine. Neal and Ahmann (37) reported that anisocytosis and poikilocytosis were observed in their studies of anemia in dairy calves of Florida. Knoop and others (29) showed a photomicrograph of a blood smear from a milk-fed calf with severe anemia which demonstrated extensive poikilocytosis.

From a review of the literature it is obvious that only limited studies have been made of poikilocytosis in bovine animals. The object of this study is to report the occurrence and the symptoms of poikilocytosis and the association of the condition with health and disease of dairy animals receiving various farm rations.

EXPERIMENTAL PROCEDURE

Calves of different ages representing Holstein, Guernsey, Jersey and Ayrshire breeds, cows representing Holstein, Guernsey and Jersey breeds, 5 cows with fistula of the rumen in the herd of the Michigan State College and dairy animals in herds on farms of Michigan were used in this investigation. The major portion of the data, however, was derived from calves in the experimental herd of the Michigan State College.

The animals in most cases were bled weekly and often several times during the week. Jugular blood was used for microscopic examination, hemoglobin determinations and other tests employed in this investigation. All blood samples except those used for microscopic examination were collected in test tubes containing lithium citrate as anticoagulant. Since several authors have indicated that contamination with certain reagents and faulty manipulation in preparing a specimen for examination may result in artefacts which resemble the poikilocyte, all known precautions were employed to avoid these errors. Only freshly shed blood was used since the common anticoagulants are salts capable of increasing the osmotic pressure sufficiently to effect possible alterations of shape. Immediately after the blood was drawn, a small drop was placed on a clean slide, spread over its surface with a clean coverslip and dried as rapidly as possible.

Some hanging drop preparations were made also as a check on the blood smears, with an isotonic solution of sodium chloride or blood plasma being used as the suspension medium. A few drops of blood were drawn through a paraffin-lined rubber tube attached to a paraffin-lined bleeding needle which was submerged below the surface of the suspension medium contained in a receptacle. This apparatus prevents direct contact of the blood with the atmosphere. The blood was mixed in the medium and the hanging drop was prepared and sealed in a depression slide for microscopic examination.

Weekly hemoglobin determinations were made by the method of Sanford, Sheard and Osterberg (47). Standard procedures were employed for the blood cell count and the determination of cell volume.

RESULTS

Incidence of Poikilocytosis

During the course of this investigation the blood from 423 animals was studied, 190 of which showed no evidence of poikilocytosis. The number and the percentage of animals afflicted with poikilocytes and the percentage of poikilocytes in the blood are shown in table 1. In 78 animals the poikilocytes constituted more than 51 per cent of the total red blood cells; in 53 animals, more than 61 per cent; in 44, more than 71 per cent; in 32, more than 81 per cent, and in 14, more than 91 per cent.

Table 1. Distribution of Given Degrees of Poikilocytosis in Affected Animals

Percentage of Poikilocytes Range	Affected Animals	
	Number	Percentage
1 - 10	81	34.7
11 - 20	31	13.2
21 - 30	21	9.0
31 - 40	13	5.5
41 - 50	19	8.1
51 - 60	15	6.8
61 - 70	9	3.8
71 - 80	12	5.1
81 - 90	18	7.8
91 - 100	14	6.0
Total	233	100.0

It was observed that one or more herds in a community would tend to show a high incidence of poikilocytosis while the neighboring herd or herds would be entirely free from this condition. Table 2 shows data on two herds with poikilocytosis and an unaffected herd in the same community.

Table 2. Incidence of Poikilocytosis in Three Different Herds in One Community

Animal no.	Poikilocytes %	Hb. gm. %	R. B. C. C. mill./mm.^{3}
	Unaffected Herd		
R1	0	13.7	.....
R2	0	12.5	.....
R3	0	11.2	7,840,000
R4	0	12.5	.....
R5	0	10.1	.....
R6	0	11.7	.....
R7	0	12.8	6,940,000
R8	0	11.3	.....
R9	0	10.9	.....
R10	0	10.5	.....
	Affected Herds		
N1	78	10.5	8,380,000
N2	95	11.4	8,100,000
N3	95	11.6	.....
N4	50	11.6	.....
T1	50	14.3	7,480,000
T2	65	13.3	.....
T3	95	10.5	10,100,000

Biconcave Disk-Shaped Erythrocytes

The microscopic observations of the blood smears from the 423 animals reveal that 190 had normal biconcave disk-shaped erythrocytes. Symmetric regularly outlined round corpuscles with transparent-appearing centers suggesting biconcavity were noted. By the use of the hanging drop preparation, in which the erythrocytes could be observed from many different angles, it was found that the predominant shape of the erythrocytes in normal, healthy dairy cattle is the biconcave disk. Figure 1 shows a photomicrograph of a blood smear of a representative animal showing the normally shaped red corpuscles.

Poikilocytes

Almost all of the shapes of the poikilocytic cell described in the literature were observed in this study. In addition, many other bizarre forms were seen to which no descriptive name has been applied. The most prevalent poikilocyte encountered consists of a "body" of variable shape and size from which tail-like structures protrude. The tail-like protrusions varied from 2 to 7 microns in length. The number of tails per cell commonly varied from one to five and were arranged in a haphazard fashion around the body of the cell. Frequently, cells possessing a round "body" were observed to have one or two of the protruding structures. The tail-like structures were a definite part of the cell and were stained homochromically with the rest of the cell. The severity of the poikilocytic condition seemed to be associated with the length of the tail-like protrusions. Figure 2 is a photomicrograph of a blood smear showing many shapes of poikilocytes in the blood of a representative animal.

Many other shapes suggestive of the following objects were seen: urn,

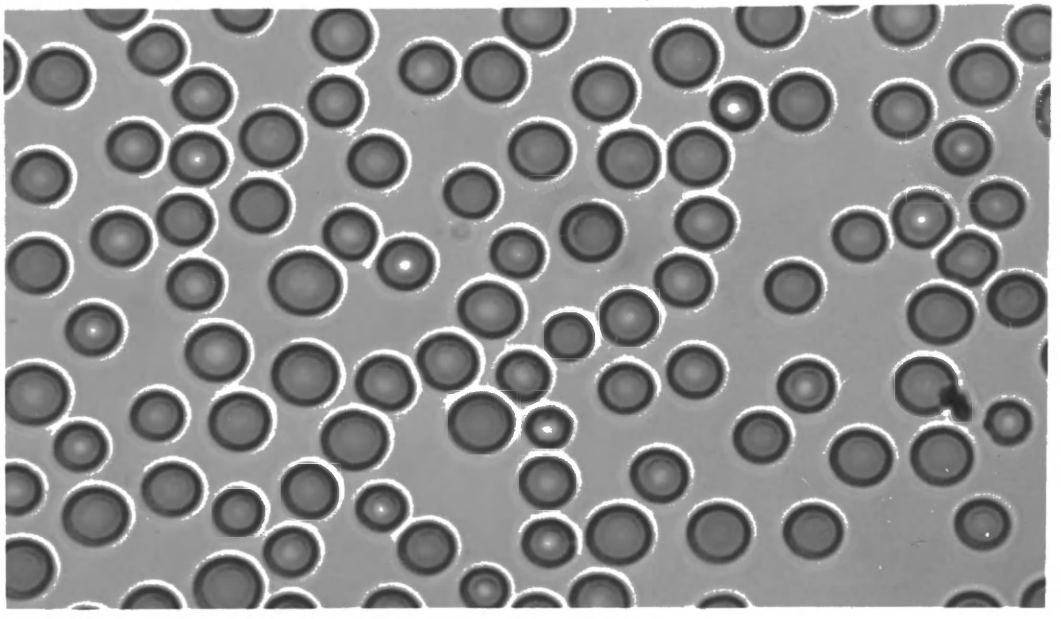

Figure 1. Photomicrograph of a blood smear showing normal bovine biconcave disk-shaped erythrocytes; x 1,270.

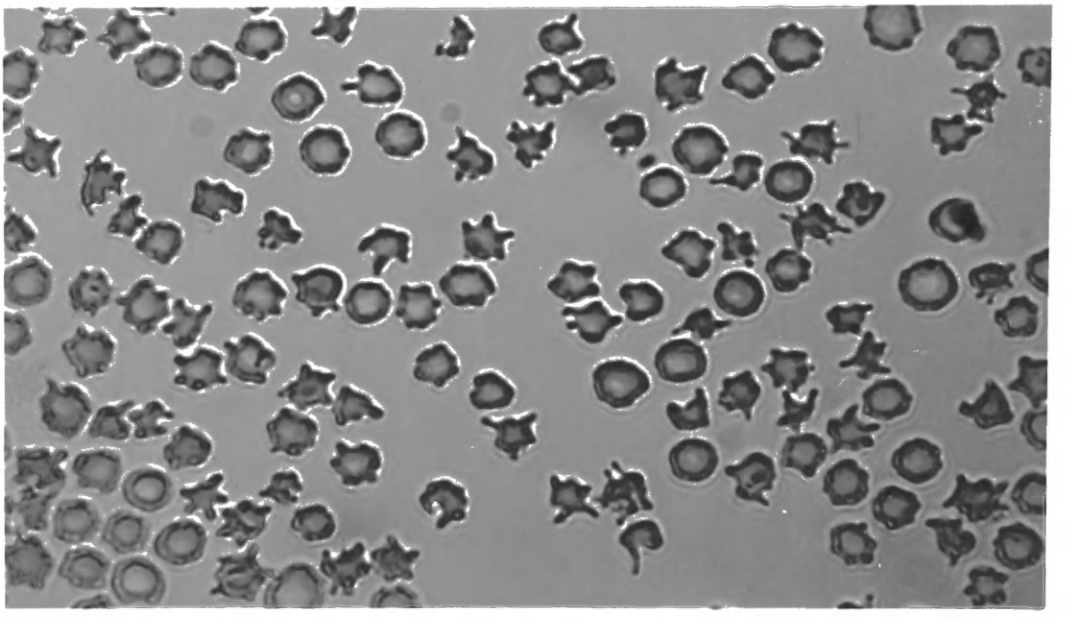

Figure 2. Photomicrograph of a blood smear showing poikilocytic cells; x 1,270.

half moon, star, oval cell, sickle, tear drop, pipe, horseshoe, kidney, tennis racquet, Christmas tree, gondola, tomahawk, dagger, oat, sausage and gourd. All of them often appeared with tail-like or filamented structures identical with those already described. The degree of distortion of cell and irregularity of shape was more accentuated in the bloods that contained the larger numbers of poikilocytes. The extreme contrasting appearance of normal erythrocytes and severely poikilocytic cells is best illustrated by an examination of figures 1 and 2.

Cells with Crenation Induced by Hypertonic Saline Solution

It was observed early in this investigation that the possibility of confusing poikilocytes with crenated corpuscles was slight. Crenation induced in normally shaped corpuscles with 2 per cent saline solution is manifested by a somewhat symmetric and regular arrangement of nodular or short spinelike structures around the periphery of the corpuscle when viewed in a blood smear. In hanging drop preparations this phenomenon was seen on the surface of the corpuscle as well as at the periphery. A comparison of the poikilocytes in figure 2 with the crenated forms in figure 3 evidences this observation. The crenated shapes in Figure 3 are those induced in the normal corpuscles shown in Figure 1 with 2 per cent saline solution. The regular arrangement of nodules and other features of the crenated forms offer a sharp contrast to the different bizarre forms exhibited by the poikilocytes in Figure 2.

Several stages of crenation were observed which were apparently due to the severity of the process. In the early stage the corpuscle displays a slight wrinkling and bulging of the surface and the periphery. Later in the process the wrinkled and bulged foci of the cell become more intensified in

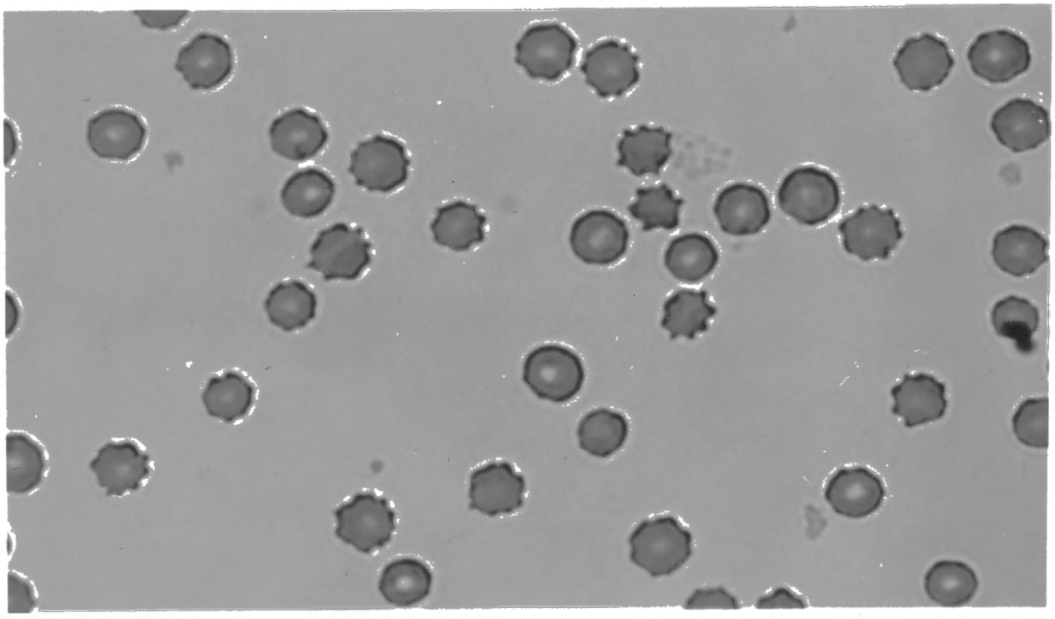

Figure 3. Photomicrograph of a blood smear showing crenated cells produced from the same normal corpuscles shown in figure 1; x 1,270.

the form of knobs or spinelike nodules. These structures appear to develop into longer, protruding coarse filaments, which are arranged in a regular fashion around the cell, and later finer, fuzzy, hairlike processes develop in place of the coarser protrusions.

Health of Animals Affected with Poikilocytosis

The health of the animals whose blood showed 50 per cent or more of the total red corpuscles to be poikilocytes was usually subnormal. Anorexia was the outstanding symptom that accompanied high incidence of poikilocytosis. In some instances, however, depraved appetite was manifested by the animal's chewing of wood. Other symptoms shown by affected animals were general thinness and roughness. The hair was long and shaggy and had a hard, dry appearance. Noticeable features shown by the young animals were a marked retardation of growth and a general unthrifty condition.

The poikilocytic status of the affected animals was not influenced by the plasma concentration of calcium, inorganic phosphorus or magnesium or by the hemoglobin content or the number of or the volume of red cells, since the values obtained did not reveal any constant feature that could be associated with the degree of severity of the poikilocytosis.

DISCUSSION OF RESULTS

In order to make a study of poikilocytosis in dairy cattle it was necessary to establish a standard for the normal shape of the erythrocyte, especially since the reports in the literature are controversial and quite limited with regard to bovine animals. The red blood corpuscles of animals which were apparently normal in all respects showed a circular biconcave disklike shape when observed in hanging drop or blood smear preparations. The hanging drop preparation in which the corpuscles were suspended in

either isotonic solution of sodium chloride or blood plasma was particularly adapted to the study of corpuscular shape "in vitro" since it permitted the observation of the corpuscles from many different viewpoints. The stained red cells in blood smears showed a round, regular outline and a heavily stained rim at the periphery of the cell with a light or nonstaining inner portion, suggesting a biconcave disk shape as the normal erythrocyte shape. No investigation has definitely established the normal in vivo shape of the bovine erythrocyte, and the studies of the normal shape in vitro are also recorded in a somewhat contradictory manner. The form observed in this study, however, is in close agreement with the bovine corpuscular shape described by Blount (5).

Many investigators have shown that normally shaped corpuscles manifest alterations in conformation as a result of having been contaminated by different physical and chemical agents during the preparation of the blood specimen. Since the occurrence of such artefacts would result in confusion in the study of cell shape, every precaution was taken to avoid those agents which are capable of effecting changes in the shape of the cells. Crenated corpuscles were reported (11, 40) to be the artefacts most commonly encountered and those most nearly resembling the poikilocytes. It was found during this investigation that the chances of confusing the crenated forms with the poikilocytes were slight, especially since only those cells in the latter stages of crenation bear any similarity to the poikilocytes. The outstanding distinguishing characteristic observed was the regular arrangement of the protrusions from the crenated cell as compared with the haphazard arrangement of those of the poikilocyte.

Most of the studies of poikilocytosis reported in the literature concerned its occurrence in man, and included the condition mainly as a secon-

dary interest in connection with other diseases. Although the deformed red cells have been observed in the blood of several of the lower animals, the reports of the occurrence of these cells in the bovine type of animal are limited to their association with anemia in calves and with anaplasmosis in dairy cattle.

Assuming the normal "in vitro" erythrocyte of dairy cattle to be a circular biconcave disk-shaped cell, many of the bizarre shapes of poikilocytes described in the literature were observed in the blood of 233 of the 423 animals studied during this investigation. Many of the deformed corpuscles were so irregular in shape that no particular name could describe them. The majority of the poikilocytes observed in the blood of the animals severely affected with the disease were generally classifiable in the latter nondescript group of poikilocytes. Deformed cells classes in this category consisted of a body portion from which pseudopodium-like processes protruded which gave the poikilocyte a striking configuration. The degree of cell distortion seemed to be correlated directly with the severity of the condition, the erythrocytes of more severely affected animals manifesting the greater deformation. This observation is in agreement with Da Costa's (11) finding that the blood of human beings shows poikilocytosis.

In a given vicinity it was found that one entire herd would manifest the condition while a neighboring herd would be completely unaffected. The condition appeared rather widespread, and the incidence of affected animals was high--55.1 per cent of the total number of animals studied.

Although an occasional mature animal manifested poikilocytosis, the young animals and the calves were more frequently affected. Usually health was subnormal in animals whose blood showed 50 per cent or more of the total corpuscles as poikilocytes, and at least one animal showed unthriftiness when only 35 per cent of the cells were poikilocytes.

The blood pictures of the affected animals failed to disclose any item that could be associated constantly with poikilocytosis. Anisocytosis appeared frequently in the blood of animals with normal erythrocytes as well as in the blood of those showing poikilocytes and was, therefore, not considered of any consequence. Although the photomicrographs published by Knoop and co-workers (29) indicated the occurrence of poikilocytosis in association with severe anemia in calves, the present investigation revealed that the hemoglobin content, the red blood cell count, the red blood cell volume and the average corpuscular hemoglobin values were independent not only of the incidence of poikilocytosis but also of the degree and severity of cell distortion. The hemoglobin content of the blood of the affected animals ranged from 4.4 to 15.9 gm. per hundred cubic centimeters of blood. The blood of the animal which had the lowest hemoglobin value showed only 25 per cent as many poikilocytes as the animal whose blood had the highest hemoglobin value. It was observed, however, that many of the animals whose blood had a low hemoglobin concentration showed the presence of poikilocytes but that not all of the animals affected with poikilocytosis had a low concentration of hemoglobin in their blood.

In this study observation was made of some dairy herds manifesting poikilocytosis while neighboring herds were unaffected. Since the rations fed to these animals varied greatly in kind and quality, it is assumed that the presence of poikilocytes in the blood was to a large extent attributable to a nutritional deficiency.

SUMMARY AND CONCLUSIONS

Dairy cattle ranging from young calves to mature cows were used in a study of the occurrence and the symptoms of poikilocytosis.

The normal erythrocytes of dairy cattle "in vitro" have a circular biconcave disk shape when observed from a surface view and have the appearance of a dumbbell when observed from an edge view.

The possibility of confusing poikilocytes with the artefacts produced in crenation is negligible.

The distribution of poikilocytes among the 233 affected animals varied up to more than 91 per cent of the red corpuscles (see table 1).

The symptoms manifested by the animals with severe poikilocytosis were: anorexia, thinness, unthriftiness, a dry and harsh condition of the hair coat and, in young animals, a retarded rate of growth. Depraved appetite was frequently observed.

The occurrence of poikilocytosis in dairy cattle is independent of the hemoglobin content, the number and the volume of red blood cells and the calcium, inorganic phosphorus and magnesium contents of the blood.

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II. THE TREATMENT OF POIKILOCYTOSIS IN DAIRY CATTLE

II.

THE TREATMENT OF POIKILOCYTOSIS IN DAIRY CATTLE

The work of several investigators has suggested a relationship of nutrition to the occurrence of poikilocytosis in animals. The study reported in this paper was initiated for the purpose of investigating the relationship of anti-anemia mineral mixtures, yeast, pyridoxine, riboflavin and nicotinic acid to the alleviation of a poikilocytic condition found to be prevalent in some farm herds and produced experimentally in young calves by feeding semi-restricted rations. The results are of additional interest because of the absence in the literature of specific data on the production of poikilocytosis in dairy animals.

Hanke and Koessler (7) produced scurvy symptoms associated with a marked poikilocytosis by feeding guinea pigs a diet consisting exclusively of autoclaved soybeans and minerals. The abnormal erythrocytic condition was frequently associated with anemia. Further study offered no evidence that a diet deficient in vitamin A is conducive to the production of abnormal red blood cells in the guinea pig. In a study of the anemia of suckling pigs, Kernkamp (9) found a marked poikilocytosis associated with oligochromemia and oligocythemia. Hogan and associates (8) developed a poikilocytic condition and anemia in pigeons by feeding a diet deficient in all of the members of the vitamin B-complex. The abnormally shaped cells resembled sickle cells and frequently constituted 50 per cent of the total red blood cells. Knoop and others (10) published a photomicrograph of a blood smear from an anemic milk-fed calf which showed extensive poikilocytosis. Although these investigators offered no explanation or description of the condition, they also pub-

lished a photomicrograph of a blood smear prepared from a calf fed milk, iron and copper in which the erythrocytes were normal in shape. Poikilocytosis and anisocytosis were occasionally observed in the blood of cobalt deficient calves (Neal and Ahmann (13)).

In a recent study Reid, Huffman and Duncan (14, 15) found a high incidence of poikilocytosis in Michigan dairy cattle accompanied by anorexia, retarded growth and general unthriftiness. Some herds manifested poikilocytosis while neighboring herds were completely unaffected. Many of the affected animals were anemic while others were normal in this respect. Since the kind and quality of rations fed to these animals varied greatly, it was believed that the condition was associated with a nutritional deficiency.

EXPERIMENTAL PROCEDURE

Most of the data reported in this paper were derived from animals receiving a whole milk and cereal breakfast food* ration although many other calves received the individual farm ration. Some calves were treated with only one supplement, others were treated with several supplements simultaneously, while others received several supplements for varying periods but only one supplement at a time.

Two mineral mixtures, No. 1 and No. 2, were used. The No. 1 mixture supplemented the ration of 7 affected calves with 250 mg. of iron, 250 mg. of manganese and 25 mg. of copper daily, while the No. 2 mixture furnished 5 mg. of cobalt daily to 20 affected calves in addition to the minerals supplied in the No. 1 mixture.

Two calves received 200 gm. of dry brewers' yeast per day while a third calf received 200 gm. and later 450 gm. of the same yeast per day. Two calves

*Rice Krispies

received 100 gm. of live yeast daily in addition to the No. 2 mineral mixture. Several of the B-complex vitamins were administered to ascertain the anti-poikilocytic factor(s) provided by yeast. One calf received 50 mg. of nicotinic acid per day and another calf received 25 mg. of riboflavin per day. These vitamins were administered for a period of 70 days after which time they were replaced with 40 mg. of pyridoxine daily. Pyridoxine was fed to eight other calves in amounts ranging from 5 to 40 mg. per day. Some of the calves also received the No. 1 or No. 2 mineral supplement at the same time.

The microscopic examination of the red blood cells of four rumen fistula cows, all having open or unplugged fistulas, revealed the presence of from 15 to 99 per cent poikilocytes. The condition of the most severely affected rumen fistula cow was corrected on two occasions by feeding brewers' yeast. The poikilocytic condition reoccurred, however, sometime after the yeast treatment was terminated.

The progress of recovery of the affected animals was followed by the microscopic examination of blood smears and occasionally by hanging drop preparations. Weight gains were used also as a criterion of the animal's general status of health. The hemoglobin, red blood cell count, red cell volume, and the plasma calcium, inorganic phosphorus and magnesium concentrations were determined by methods previously reported (Reid, et. al.(15)).

Forty-two calves and four rumen fistula cows were employed in this study.

RESULTS

The results obtained from feeding the various supplementary factors in addition to the regular ration of the calves are shown in tables 1-6. Many animals were encountered during the course of this investigation that showed a typical anemia blood picture uncomplicated with poikilocytosis.

The data obtained from calf A in table 1 illustrate a case of this type. Although the blood of most of the poikilocytosis affected animals revealed low hemoglobin concentrations, some of the animals were normal in this respect. The data for B in table 1 show a severely affected animal whose hemoglobin level is nearly normal. The data also show the loss in weight accompanying the course of a typical case of poikilocytosis when no therapeutic agent was administered.

Table 1

Blood picture of a calf (A) showing oligochromemia but unaffected by poikilocytosis and a calf (B) affected with poikilocytosis but not showing oligochromemia

Calf	Age	Body Wt.	Hb	R.B.C.V.	R.B.C.C.	Poikilocytes	Plasma		
							Ca	Inorg. P	Mg
no.	days	kg.	gm.%	%	mill./mn.^{3}	%	mg. per 100 ml.		
A	240	103.2	6.7	18.0	5.40	0	9.9	7.02	1.24
	250	102.6	6.5	18.0	5.07	0	10.2	5.34	1.30
	260	112.5	8.0	23.0	3.97	0	11.6	7.86	1.98
	270	115.7	8.0	23.0	5.55	0	11.0	6.51	1.72
	280	116.4	9.5	24.7	6.93	0	10.9	5.70	1.57
B	80	36.7	10.9	-	-	90	11.1	6.32	1.87
	90	33.0	10.9	-	-	90	8.8	6.32	0.99
	100	33.4	10.9	-	-	95	10.2	5.34	1.03
	110	30.2	11.3	-	-	90	11.0	5.90	1.03
	120	30.0	10.3	-	-	94-97	11.1	4.96	1.01

The values included in several tables for plasma calcium, inorganic phosphorus and magnesium indicate no relationship of these constituents to the poikilocytosis anomaly.

Effect of mineral supplementation on poikilocytosis

Table 4 shows the effect of mineral supplementation of the ration of a representative animal. These data show a gradual increase in the hemoglobin concentration without any change in the severity of poikilocytosis.

Table 2

Effect of adding brewers' yeast to the ration

Calf no.	Date	Age days	Body Wt. kg.	Poikilocytes %	Hb gm.%
C-3	9/24	348	-	75	9.8
	10/15	369	142.2	80	9.1
	11/13*	397	146.3	75-80	9.1
	11/28	412	142.2	50-75	8.8
	12/18	432	165.3	5	9.6
C-4	9/24	237	-	25	11.6
	10/15	258	175.3	20	11.7
	11/13*	286	179.8	23-25	11.9
	11/28	301	197.5	10-15	11.2
	12/18	321	202.1	0	11.7

*Calf received 200 gm. of brewers' yeast daily after this date.

Treatment with dry brewers' yeast

Table 2 shows the effectiveness of brewers' yeast in the treatment of poikilocytosis in two calves. Since these calves exhibited symptoms similar to those observed in cobalt deficiency, this element was administered for seven weeks without response prior to the feeding of yeast. (The symptoms of cobalt deficiency disease are always alleviated within a week after the initial treatment.) The cobalt supplement was then discontinued and 200 gm. of brewers'

yeast was fed daily. The distortion of the red blood cells of the calves was markedly ameliorated although no change in the level of hemoglobin was observed. From the percentage of poikilocytes recorded in the table it appears that the distorted red cells can be restored to normally shaped corpuscles by feeding dry brewers' yeast for three to four weeks. The data indicate also the stunted condition of the calves prior to the feeding of yeast. An increased appetite and an impetus in weight gained was observed as poikilocytosis subsided. Three calves received the yeast supplement and all responded in the same manner. These findings are similar to those reported by Hogan et al. (8) in that poikilocytosis in pigeons was corrected after five weeks of yeast supplementation.

Table 3

Data obtained from a representative poikilocytosis affected animal treated with live yeast

	Body	Daily intake per kg. body wt.					Poikilo-	Plasma		
Age	Wt.	Fe	Cu	Mn	Co	Hb	cytes	Ca	Inorg. P	Mg
days	kg.	mg.	ug.	mg.	ug.	gm.%	%	mg. per 100 ml.		
10	36.7	0.25	43.7	13.2	3.8	6.3	75	11.5	6.19	1.84
15	Added 100 gm. live yeast and No. 2 mineral mixture daily									
15	38.9	8.86	711.6	16.2	137.7	5.7	70-75	11.2	7.10	1.69
20	41.1	8.39	673.8	18.0	147.6	7.3	20-25	11.0	7.02	2.13
30	46.2	7.47	601.0	16.6	123.0	8.2	4	11.7	4.19	1.93
40	50.5	6.86	554.5	13.7	113.0	9.8	3	11.3	9.06	1.88
50	53.6	7.79	539.7	15.6	111.6	10.7	1	11.2	7.96	1.99
60	61.4	6.91	473.6	15.5	98.1	12.6	0	11.1	8.12	1.72

Treatment with live yeast

The effect of live yeast in correcting the deformed red cell condition in calves is shown in table 3. These data show the correction of the poikilocytic condition to nearly normal-shaped erythrocytes after a 4-week period during which time the calf consumed 100 gm. of live yeast daily. A remarkable decrease in the number of poikilocytes was noted after the first and second week of yeast feeding as well as a striking transformation in the shape of the poikilocytes--the degree of distortion became less extreme. An increased

Table 4

Data obtained from a representative poikilocytosis affected animal treated with pyridoxine

Age*	Body Wt.	Daily intake per kg. body wt.				Hb	Poikilocytes	Plasma		
		Fe	Cu	Mn	Co			Ca	Inorg.P	Mg
days	kg.	mg.	ug.	mg.	ug.	gm.%	%	mg. per 100 ml.		
50	67.3	2.52	60.4	12.0	11.9	9.0	85	11.8	6.44	2.40
70	69.8	1.29	42.6	9.9	6.9	7.0	85-90	11.9	8.56	1.18
72	Added No. 1 mineral mixture									
83	75.9	5.97	392.6	9.3	12.5	9.3	90	11.2	6.69	0.99
83	Discontinued No. 1 mineral mixture and added No. 2 mineral mixture									
100	80.1	6.26	380.6	8.9	63.9	9.5	90	11.7	5.76	1.10
120	86.4	5.09	346.3	9.3	68.5	8.9	90	11.7	6.01	1.01
137	99.1	5.73	313.7	8.1	50.9	11.6	90	11.9	7.35	2.09
137	Added 40 mg. pyridoxine daily									
140	101.6	5.73	313.7	8.1	50.9	11.6	85	11.9	7.35	2.09
150	118.9	4.21	308.6	8.0	50.0	13.3	40	11.8	6.95	1.28
160	126.8	4.03	307.3	8.5	49.8	11.3	1-5	12.2	8.56	1.14
168	134.4	3.85	300.1	7.9	47.1	10.7	0	12.2	5.74	1.03

*Data obtained at 7 day intervals and on days when supplements were changed.

growth rate accompanied the disappearance of the poikilocytes in the blood. Only two calves were treated with live yeast but the results were uniformly the same. Since both of the calves had received the No. 2 mineral mixture simultaneously with the live yeast, it was necessary to eliminate one or the other of the supplements in order to derive the correction factor. The correction of the poikilocytic condition is attributed entirely to the ingested yeast since the mineral supplements failed to alter the extreme poikilocytosis of animals for which the data in table 4 are representative.

Treatment with pyridoxine

Eight animals varying in the severity of poikilocytosis were fed different amounts of pyridoxine. Although the daily ingestion of 5, 10, 15 or 20 mg. of pyridoxine was effective in ameliorating the poikilocytic condition, the recovery was slow. Forty milligrams of pyridoxine daily, however, resulted in the complete absence of poikilocytes within three or four weeks after treatment was begun. Tables 4-6 show the rapidity with which the poikilocytes disappeared from the blood. The disappearance of the poikilocytes was accompanied by an improvement in the general health of the animals and was also reflected by an accelerated growth rate.

Table 5 shows the ineffectiveness of feeding 25 mg. of riboflavin per day on the poikilocytosis of a representative animal. After a 70-day period, riboflavin feeding was discontinued, and 40 mg. of pyridoxine was added to the animal's ration. Although riboflavin treatment did not affect the number of poikilocytes, the pyridoxine treatment corrected the condition to normal.

Table 6 shows that 50 mg. of nicotinic acid daily for 70 days failed to correct poikilocytosis in a representative animal, whereas the transformation to normal was attained after treatment with 40 mg. of pyridoxine daily. It should be emphasized that the decreased rate of growth and the poikilocytic

Table 5

Effects of riboflavin and pyridoxine on poikilocytosis

Date*	Body Wt.	Hb	R.B.C.C.	R.B.C.V.	Poikilocytes	Plasma Ca	Plasma Inorg. P	Plasma Mg
	kg.	gm.%	mill./m.^{3}	%	%	mg. per 100 ml.		
4/11	48.2	8.7	9.70	25.0	5	11.3	6.87	1.65
5/9	58.2	8.7	10.72	28.0	30	12.1	8.39	1.64
5/15	Added 100 mg. Fe daily							
6/6	75.0	12.5	13.96	44.0	40	9.8	9.06	2.20
6/14	Discontinued Fe							
6/14	75.0	11.9	12.15	32.0	40	11.7	9.47	2.11
7/11	84.5	10.1	10.27	27.0	45-50	11.4	6.79	2.08
8/8	96.8	8.7	8.21	25.5	55	12.5	7.44	2.06
8/29	105.5	9.1	7.27	28.0	50	12.3	8.56	2.06
9/5	Added 25 mg. of riboflavin daily							
9/5	108.2	7.3	7.05	21.0	40	11.1	5.63	1.63
10/6	138.1	9.1	11.60	26.5	40	10.5	9.06	1.54
10/24	143.1	9.5	-	30.0	45	10.9	8.12	1.18
11/7	152.7	9.5	9.55	27.0	45	11.5	6.72	1.16
11/13	168.6	Discontinued riboflavin and added 40 mg. pyridoxine daily						
11/15	170.5	9.2	13.04	26.5	40	11.3	7.67	1.73
11/22	176.8	8.3	9.65	25.0	15	11.5	9.33	2.08
11/27	181.8	8.6	6.70	23.5	5-7	10.8	7.91	1.68
12/5	186.8	8.8	7.65	25.0	1	10.8	7.44	1.96
12/11	189.6	9.4	8.31	26.0	0	11.5	6.76	1.87

*Data obtained at 7 day intervals and on days when supplements were changed. status of the animals remained unchanged during the mineral supplement, riboflavin and nicotinic acid feeding periods, but rapid response was obtained when pyridoxine was added to the ration.

Table 6

Effects of nicotinic acid and pyridoxine on poikilocytosis

Date*	Body Wt.	Hb	R.B.C.C.	R.B.C.V.	Poikilocytes	Plasma Ca	Plasma Inorg. P	Plasma Mg
	kg.	gm.%	mill./mm.^{3}	%	%	mg. per 100 ml.		
8/8	153.9	9.0	9.06	26.0	35	12.5	6.44	2.33
8/29	164.5	10.4	8.38	31.0	35	12.1	9.19	1.76
9/5	Added 50 mg. nicotinic acid daily							
9/5	-	9.8	-	26.5	30	11.3	7.86	2.10
10/6	187.5	9.8	7.24	29.5	30-35	11.1	7.67	1.73
10/24	195.0	9.0	-	28.0	35	10.9	7.14	2.11
11/7	205.1	9.4	6.95	26.0	45	11.2	6.57	1.37
11/13	213.7	Discontinued nicotinic acid and added 40 mg. pyridoxine daily						
11/15	218.5	9.0	6.50	26.0	40	11.4	6.79	1.35
11/22	221.6	8.7	8.05	24.0	15	11.2	6.87	2.05
11/27	226.6	8.7	5.81	24.5	7	11.2	5.19	2.15
12/5	231.48	9.1	7.86	25.5	1	9.9	4.77	2.02
12/11	241.36	9.1	7.15	25.0	0	10.9	6.51	2.19

*Data obtained at 7 day intervals and on days when supplements were changed.

DISCUSSION OF RESULTS

Different agents known to stimulate hemapoiesis were fed in an effort to correct the poikilocytic status of calves and rumen fistula cows. The mineral supplements had no effect on poikilocytosis although a noticeable elevation in the hemoglobin level resulted from their supplementation. The feeding of either brewers' yeast or live yeast was effective in ameliorating the poikilocytic condition within three to four weeks.

Several of the B-complex vitamins contained in yeast were used to treat the poikilocytic animals in an attempt to derive the curative factor(s) for

this anomaly. The use of riboflavin and nicotinic acid did not alter the shape of the poikilocytes but pyridoxine administration resulted in a specific response. In cases where severe poikilocytosis and retarded growth were found, the ingestion of pyridoxine resulted in an increase in appetite and an accelerated growth rate coincident with the subsidence of the poikilocytes. In the less severe cases the increase in the rate of growth after the administration of pyridoxine was not appreciable. Although the daily ingestion of 5 to 20 mg. of pyridoxine brought about slow recovery, the ingestion of 40 mg. accelerated the complete restoration of the poikilocytes to normal erythrocytes. Recovery was effected within three to four weeks. The prognosis of the animals treated with 40 mg. of pyridoxine was excellent and the cure was permanent.

In most of the cases observed, young calves were the animals most commonly affected with poikilocytosis whereas the incidence in mature animals was quite limited. Observation was made also of the apparently spontaneous recovery of previously affected calves. It seems logical to suppose that recovery occurred concomitant with the development of the rumen flora and fauna.

Since the blood of four rumen fistula cows revealed the presence of poikilocytes, the existence of the open rumen fistulas offered the possibility that a derangement in ruminal function may have been responsible for the occurrence of the poikilocytes. Since the young calves receiving a restricted ration of whole milk and a cereal breakfast food generally manifested poikilocytosis and the older animals did not, it appeared that a deficiency of some factor(s) necessary for the maintenance of corpuscular integrity may be synthesized in the normal mature rumen. The early studies of Theiler et al.(17) and Bechdel et al. (2,3) demonstrated that cattle were healthy and normal on vitamin B-complex deficient rations, while rumen synthesis of the complex was

reported by Scheunert and Schieblich (16), Bechdel et al. (4) and Wegner and coworkers (19, 20). The latter group of investigators believed that the increase in B-vitamins in the rumen ingesta, as compared to the ration fed, was due to synthesis and not to a concentration effect. Arnold and Elvehjem (1) stated that ruminant animals are able to satisfy their requirements for at least some members of the B-complex by virtue of the fermentation which occurs in the digestive tract. The synthesis of pyridoxine by the bacteria in the rumen has been pointed out by several workers, McElroy and Goss (11). Wegner and coworkers (19, 20) showed that the pyridoxine content of the rumen ingesta is higher than that of the ration fed and concluded that the increase was due to bacterial synthesis. These findings support the earlier work of McElroy and Goss (11) in that the vitamin B_6 potency of the rumen contents is 6 to 8 times as great as that found in the ration. These investigators favored a true synthesis of the vitamin related to growth of the rumen flora rather than a concentration caused by differential rates of passage of different nutrients from the rumen. No mention was made as to whether the rumen fistulas were open or closed. They showed that a normal amount of vitamin B_6 is found in the milk of a cow on a vitamin B_6 deficient ration which seems to indicate that the cow was able to synthesize enough of this vitamin to satisfy her maintenance needs and in addition transfer a normal amount into the milk.

No reports concerning the rumen synthesis of pyridoxine have indicated that the rumen of young calves has flora developed sufficiently to synthesize this vitamin in quantity large enough to satisfy the animal's maintenance requirements or that the vitamin is synthesized at all. It seems logical, therefore, to suppose that the ration fed, the age of the calf, and the condition of the rumen of both rumen fistula cows and normal calves are factors concerned in the synthesis of pyridoxine.

Although no known case of vitamin B_6 deficiency in cattle has been reported, a deficiency of this vitamin has been reported in other animals. Fouts and associates (5) successfully treated a severe microcytic hypochromic anemia in dogs with vitamin B_6 after iron therapy had failed to relieve the anemia. Later Fouts et al. (6) showed that adult dogs as well as puppies developed vitamin B_6 deficiency anemia which did not respond to iron and copper therapy. McKibbin and coworkers (12) reported that the ingestion of a low level of vitamin B_6 is necessary for growth but the ingestion of a higher level is necessary to protect pyridoxine deficient dogs against hypochromic microcytic anemia. They suggested 60 micrograms of vitamin B_6 as an anemia-curative dose for dogs.

The ability of pyridoxine to stimulate the hemapoietic system of deficient patients has been extensively demonstrated by increased blood regeneration and the restoration of the red corpuscles to normal size. The vitamin was used by Vilter and associates (18) to treat three pellagrins with macrocytic anemia and two patients with pernicious anemia. A subjective relief and a slight reticulocytosis occurred. Although these investigators stated that vitamin B_6 definitely affects the hemapoietic system of humans who have the macrocytic anemia of pellagra or pernicious anemia, they did not imply that it is an antipernicious anemia factor. It should be emphasized, however, that pernicious anemia is one of the diseases constantly associated with poikilocytosis. Wintrobe et al. (21) produced a severe anemia in pigs by feeding a pyridoxine deficient diet. When pyridoxine was administered, a rapid regeneration of blood accompanied the restoration of normal corpuscular size. The reports in the literature indicate that simple stomach animals and humans may suffer from a lack of vitamin B_6 whereas a deficiency of this vitamin in the normal ruminant is unlikely to occur by virtue of bacterial synthesis.

Poikilocytosis, however, has not been associated with pyridoxine deficiency in simple stomach animals.

This investigation has shown that when poikilocytes do occur in the blood of calves they can be replaced with normal erythrocytes following the administration of brewers' yeast, live yeast or vitamin B_6. The results of this study have indicated also the possibility of a lack of or a derangement of the normal rumen activity necessary for the synthesis or utilization of pyridoxine either for the direct maintenance of corpuscular integrity or for the factor(s) responsible for this function.

SUMMARY AND CONCLUSIONS

Young calves on semi-restricted rations and cows with large open fistulas of the rumen were found to be the dairy animals most commonly affected with poikilocytosis. Dry brewers' yeast, live yeast, or pyridoxine were effective therapeutic agents for the treatment of this condition, whereas the ingestion of nicotinic acid, riboflavin or a mineral mixture containing iron, copper, cobalt and manganese did not elicit any curative effects on the disease.

The occurrence and the degree of severity of poikilocytosis were independent of the hemoglobin content, red blood cell count, red blood cell volume and the plasma concentration of calcium, inorganic phosphorus and magnesium.

As a result of this investigation the hypothesis is offered that the primary defect responsible for the occurrence of poikilocytosis in dairy animals is due to a lack of or an interference with normal ruminal activity.

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III. THE EFFECT OF POIKILOCYTOSIS ON THE OXYGEN AND CARBON DIOXIDE CONTENT AND OXYGEN CARRYING CAPACITY OF THE BLOOD

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THE EFFECT OF POIKILOCYTOSIS ON THE OXYGEN AND CARBON DIOXIDE CONTENT AND OXYGEN CARRYING CAPACITY OF THE BLOOD

Many investigators (2,5,6,16,17,23,24,25,33,34,35,39,40) have demonstrated that the transport of oxygen to the tissues from the lungs and the removal of carbon dioxide from the tissues and its transport to the lungs is the outstanding function of blood. Meyer (22) stated that the ability of blood to "absorb and take up oxygen depends upon its hemoglobin content". Although Harrop's study (14) of the oxygen content of arterial blood in anemia showed that normal oxygen consumption was maintained, he found low values for the oxygen content of venous blood. The investigations of Henderson (16), Haggard and Henderson (13), Smith et al. (32) and Van Slyke (35) indicate that hemoglobin plays as important a role in the transport of carbon dioxide as it does for oxygen. Van Slyke and Neill (36) have outlined an indirect method for deriving the hemoglobin concentration by measuring the oxygen carrying capacity of blood.

Barcroft (3) pointed out that a solution of hemoglobin in the concentration in which it occurs in the blood possesses certain physical properties which would make its occurrence in circulating blood harmful to the organism. Nature has precluded these disadvantages by enclosing the hemoglobin in corpuscles with a diameter commensurate with that of the capillary bore.

Although several different forms have been described for the normally shaped red corpuscle, the most commonly accepted shape in mammals is the biconcave disk (7,8,10,11,19,20,21,29). Two investigations (4,28) have also shown this to be the predominant shape of red blood cell in healthy cattle.

Several attempts have been made to offer an explanation for the red blood corpuscle having the biconcave disk form. Emmel (8) believed that the relatively thin, flattened disks tended to give oxidation efficiency. Although Howell (18) believed that biconcavity offers an advantage from the standpoint of absorptive surface, Hartridge (15) was the first to definitely associate the respiratory physiology of the red blood corpuscle with its shape. He concluded that the biconcave disk form is the shape that functions most efficiently in allowing all the cell contents to be saturated with oxygen in the same interval of time. In a sphere shaped corpuscle, diffusing gases entering the periphery at different points would reach the center at the same time; likewise, an infinitely thin disk cell would allow the gases to reach different parts of the cell at the same time. Hartridge (15) pointed out that these two shapes of cells possess proportions necessary for uniform diffusion, but that they lack the proper surface-volume ratio for efficient respiration. He believed that the biconcave disk shape is a compromise between the two extremes and offers optimum respiratory efficiency. These conclusions were substantiated later by Haden and Evans (12), Ponder (27) and Wintrobe (41). The observation by Erlich and Lazarus (9) of malshaped corpuscles appearing in the blood of individuals going to high altitudes is of especial interest. Whether or not the appearance of poikilocytes in the blood under these conditions is a compensatory response of the hemapoeitic system to respiratory demands is not known.

It was the object of this investigation to determine whether or not poikilocytosis affects the oxygen and carbon dioxide content and the oxygen carrying capacity of the blood of dairy cattle.

EXPERIMENTAL PROCEDURE

The venous blood of normal and poikilocytosis affected animals was collected under neutral paraffin oil in tubes containing potassium oxalate and analyzed to determine the oxygen and carbon dioxide content and the oxygen carrying capacity according to the method outlined by Van Slyke and Neill (36). A manometric Van Slyke gas analysis apparatus was employed for the gas measurements.

The principles involved in the method are: (a) The hemolysis of the blood corpuscles by saponin allows the lactic acid to free carbon dioxide from the bicarbonate salts and potassium ferricyanide to expel the oxygen contained in oxyhemoglobin with the formation of methemoglobin. (b) The absorption of carbon dioxide is accomplished by sodium hydroxide while the oxygen is absorbed by sodium hyposulfite. (c) In each case pressure readings (representative of the total gas expelled and the amount of each gas absorbed) are obtained at a constant volume. (d) The quantities of oxygen and carbon dioxide are calculated from the standard tables suggested by Peters and Van Slyke (26).

Samples of blood analyzed for oxygen carrying capacity were equilibrated with air for three minutes previous to the analysis.

RESULTS

Oxygen Content

Table 1 shows data for the content of oxygen in the blood of 6 normal and 8 poikilocytosis affected animals. These figures show that a greater quantity of oxygen is contained per gm. of hemoglobin, per ml. of red blood cells and per million of red blood cells in normal animals than is contained

Table 1. The Oxygen Content of the Blood of Normal and Poikilocytosis Affected Animals

Animal No.	Oxygen Content vol. %	Oxygen per gm. Hb* vol. %	Oxygen per ml. R.B.C. vol. %	Oxygen per mill. R.B.C. vol.% x 10^{-5}	Poikilocytes %
13	10.64	.958	.333	1.186	0
9	8.30	.659	.252	1.263	0
12	6.85	.642	.249	1.065	0
7	5.38	.596	.224	0.884	0
10	8.34	.565	.214	.843	0
3	8.12	.615	.203	.829	0
5	5.26	.628	.219	.867	5-8
11	4.51	---	---	.832	10
14	5.59	.532	.200	---	10
2	4.98	.520	.199	.771	10-15
4	4.14	.425	.159	.682	30-35
6	2.03	.317	.123	.472	40-50
1	4.56	.558	.194	.867	85
8	1.19	.203	.066	.373	90-95

*Determined by method of Sanford, Sheard and Osterberg (31).

in the same quantities of hemoglobin and red blood cells from poikilocytosis affected animals. Although the cause for the failure of these factors to correlate in every case with the percentage of the cells consisting of poikilocytes is not known, it seems possible that the degree of cell distortion may be concerned in this problem.

Oxygen Carrying Capacity

An examination of table 2 shows that bloods containing a large percentage of poikilocytes have a greater oxygen carrying capacity than the blood of animals which are normal with respect to poikilocytosis. Although the differences are marked for the quantity of oxygen carried on a per gm. of hemoglobin and million of red blood cell basis, the differences per ml. of red blood cells are not appreciable.

Table 2. The Oxygen Carrying Capacity of the Blood of Normal and Poikilocytosis Affected Animals

Animal No.	Oxygen Capacity vol. %	Oxygen per gm. Hb* vol. %	Oxygen per ml. R.B.C. vol. %	Oxygen per mill. R.B.C. vol. % x 10^{-5}	Poikilocytes %
3	17.55	1.33	.439	1.791	0
10	19.67	1.34	.504	1.989	0
7	12.46	1.37	.519	2.046	0
9	17.01	1.35	.515	2.589	0
5	11.92	1.42	.497	2.069	5-8
2	11.56	1.44	.502	1.944	10-15
4	13.76	1.41	.529	2.267	30-35
6	9.51	1.49	.576	2.216	40-50
1	11.42	1.40	.486	2.171	85
2	8.72	1.49	.484	2.734	90-95

*Determined by method of Sanford, Sheard and Osterberg (31).

Oxygen Uptake upon Equillibration with Air

Table 3 shows the content of oxygen, oxygen carrying capacity (i.e. the total amount of oxygen contained after saturation), the increase in oxygen content upon saturation and the percentage increase in oxygen volume

upon saturation of the blood of normal and poikilocytosis affected animals. These figures indicate that the blood of animals affected with poikilocytosis is capable of taking up more oxygen than is that of normal animals.

Table 3. The Amount of Oxygen Taken up by the Blood of Normal and Poikilocytosis Affected Animal during Equillibration with Air

Animal No.	Oxygen Content vol. %	Oxygen Capacity vol. %	Oxygen Increase upon Saturation vol. %	Oxygen Increase upon Saturation %	Poikilocytes %
9	8.30	17.01	8.71	104.94	0
3	8.12	17.55	9.43	116.13	0
7	5.38	12.46	7.08	131.60	0
10	8.34	19.67	11.33	135.85	0
5	5.26	11.92	6.66	126.62	5-8
2	4.98	11.56	6.58	132.13	10-15
4	4.14	13.76	9.62	232.37	30-35
6	2.03	9.51	7.48	368.47	40-50
1	4.56	11.42	6.86	150.44	85
8	1.19	8.72	7.53	632.77	90-95

Ratio of Carbon Dioxide to Oxygen

Table 4 shows that in most of the cases the ratio of carbon dioxide to oxygen contained in the blood of animals affected with poikilocytosis is wider than that for normal animals. Poikilocytosis does not seem to affect the carbon dioxide content of the blood in a regular manner.

Table 4. The Ratio of the Contents of Carbox Dioxide to Oxygen in the Blood of Normal and Poikilocytosis Affected Animals

Animal No.	Blood Content		Ratio	Poikilocytes
	Carbon Dioxide vol. %	Oxygen vol. %	CO_2 / O_2	%
13	43.96	10.64	4.13	0
9	41.98	8.30	5.02	0
10	42.64	8.34	5.11	0
3	45.13	8.12	5.56	0
12	46.37	6.85	6.77	0
7	50.75	5.38	9.43	0
5	40.48	5.26	7.70	5-8
14	51.46	5.59	9.21	10
11	53.25	4.51	11.81	10
2	51.39	4.98	10.32	10-15
4	47.47	4.14	11.47	30-35
6	36.77	2.03	18.11	40-50
16	48.41	3.60	13.45	45
1	49.88	4.56	10.94	85
15	56.58	3.56	15.89	85
8	42.18	1.19	35.45	90-95

DISCUSSION

The results of this study indicate that the venous blood of dairy cattle affected with poikilocytosis contains less oxygen than the blood of normal animals. However, the data for the oxygen carrying capacity expressed on a per gm. of hemoglobin or on a per million of red blood corpuscles basis show that bloods containing the larger numbers of poikilocytes have a greater capacity for taking up oxygen than do the bloods of animals not affected with the disease. Generally speaking, the respiratory function of erythrocytes is carried on by their solid constituents. Recent studies have shown that the surface of the red corpuscle may participate in this function. The occurrence of protein, lecithin and cholesterol in the cell envelope has been demonstrated (19,41) and Alexander (1) has pointed out the ability of these substances to increase surface activity. Warburg (38) further showed that on shaking cystine and tyrosine with erythrocytes, carbon dioxide is formed among other products, which proves that a partial oxidation has occurred. Warburg (38) was successful in demonstrating that this phenomenon is a surface reaction. Since the poikilocyte possesses a larger surface area relative to volume than the normal erythrocyte, it is believed that this relationship increases the adsorptive capacity of the blood of poikilocytosis affected animals. The greater adsorption by the blood of animals so affected would explain the greater uptake of oxygen per gm. of hemoglobin and per million of red blood cells in these bloods than in those of normal animals. Although this study does not attempt a demonstration of the poikilocyte's capacity for giving up oxygen (a study of both venous and arterial blood as well as permeability appears to be an imperative approach to this angle of the problem), its results are nevertheless of scientific interest.

Although a definite relationship was observed between the number of poikilocytes and the oxygen content and carrying capacity, the failure of the oxygen values to correlate with the percentage of poikilocytes in a few cases was attributed to the differences in the degree of cell distortion. It is known that a greater degree of distortion and a greater number of processes on the poikilocyte will increase the surface area and therefore the adsorption capacity.

There is no known significance for the wide carbon dioxide-oxygen ratio found in the blood of animals affected with poikilocytosis; however, this relationship seemed to be a function of the oxygen content, for the content of carbon dioxide showed no relationship to the number of poikilocytes in the blood.

SUMMARY AND CONCLUSIONS

The results of this study have shown that the blood of animals affected with poikilocytosis contains less oxygen than the blood of normal animals. However, the blood of animals so affected manifested a greater capacity for taking up oxygen than the blood of normal animals. It is believed that the greater amount of oxygen taken up during equillibration with air is due to adsorption by virtue of the greater surface area of poikilocytes as compared to normally shaped erythrocytes.

A wider ratio of the content of oxygen to carbon dioxide was found in the blood of poikilocytosis affected animals than in that of animals not affected by the disease.

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