Glycogen in Leukocytes from Bovine Blood and Milk

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ABSTRACT

Glycogen content was determined quantitatively by the Anthrone reagent method in leukocytes obtained from blood and milk of five cows. Distribution of glycogen in leukocytes was studied by microscopic examination of slides stained by Periodic acid-Schiff (PAS) reaction. Blood glucose concentrations were investigated in these animals by standard procedures. In two of five cows both blood glucose levels and blood leukocyte glycogen levels on the same day were determined for six consecutive days. One hundred and two blood leukocyte samples from five cows had a mean glycogen content of 1.32 ± 0.04 (S.E.) mg/10^9 WBC, and 6.11 ± 0.17 (S.E.) mg/10^9 PMNs. Leukocyte preparations from 80 samples of milk comprising 97 to 98% PMNs, contained 3.81 ± 0.18 (S.E.) mg glycogen/10^9 milk leukocytes. In PAS preparations of blood and milk leukocytes glycogen was found almost exclusively in PMNs. Glycogen granules, present frequently in PMNs and occasionally in monocytes and large lymphocytes from blood, were not observed in those from milk. The glycogen level in milk leukocytes was significantly lower ($P = <0.01$) than that of the blood PMNs in every cow, and the overall mean difference between levels for milk leukocytes and blood PMNs was highly significant ($P = <0.001$). Mean blood glucose concentration in the five cows was 44.46 ± 0.66 (S.E.) mg%. There was no significant relationship between blood glucose and blood leukocyte glycogen levels in the five corresponding cows; nor between blood glucose and blood PMN glycogen levels on the same day in either of two cows investigated. Leukocyte preparations from milk samples obtained on the second day following intramammary infusion of endotoxin consistently contained markedly less glycogen than the leukocyte preparations from first day post-infusion samples. These tended to level off and became intermediate between first and second day levels. It is postulated that the poor phagocytic competence of leukocytes from bovine mammary glands compared to their counterparts in blood observed by various workers may be due partially to low energy reserves in these cells.

RÉSUMÉ

Les auteurs ont effectué la détermination quantitative du glycogène des leucocytes du sang et du lait de cinq vaches, en se servant de l'épreuve d'Anthrone. Ils recherchèrent la localisation du glycogène dans les leucocytes, par l'examen microscopique de préparations teintes à l'acide périodique de Schiff (PAS). Ils déterminèrent la concentration sanguine du glucose chez ces bêtes, en utilisant des méthodes conventionnelles. Pendant six jours consécutifs, ils déterminèrent concurremment, chez deux des cinq vaches, la teneur du sang en glucose et celle de ses leucocytes en glycogène. Cent deux échantillons de leucocytes sanguins, prélevés chez les cinq vaches, révélèrent une teneur moyenne en glycogène de 1.32 ± 0.04 (S.E.) mg/10^9 globules blancs, et de 6.11 ± 0.17 (S.E.) mg/10^9 neutrophiles. Des préparations leucocytaires de 80 échantillons de lait contenant de 97 à 98% de neutrophiles recélaient 3.81 ± 0.18 (S.E.) mg de glycogène/10^9 leucocytes. Les préparations au PAS de leucocytes du sang et du lait révélèrent la présence de glycogène presqu'uniquement dans les neutrophiles. Les granules de glycogène observés souvent dans les neutrophiles et rarement dans les mononucléaires et les grands lymphocytes du sang ne se retrouvaient pas dans ceux du lait. La concentration de glycogène s'avéra significativement plus basse ($P<0.01$) dans les leucocytes du lait que dans les neutrophiles du sang, chez toutes les vaches; la différence moyenne entre la teneur en glycogène des leucocytes du lait

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et celle des neutrophiles du sang se révéla très appréciable ($P<0.001$). La teneur moyenne du sang des cinq vaches en glucose s'établissait à 44.46 ± 0.66 (S.E) mg%. Il n'existait pas de relation appréciable entre la teneur du sang en glucose et celle de ses leucocytes en glycogène, chez les cinq vaches considérées individuellement, pas plus qu'entre celle du glycose sanguin et du glycogène des neutrophiles du sang, le même jour, chez l'une ou l'autre des deux vaches éprouvées en ce sens. Les préparations leucocytaires d'échantillons de lait, deux jours après l'infusion intramammaire d'endotoxine, contenaient régulièrement beaucoup moins de glycogène que celles des échantillons prélevés le lendemain de l'infusion. La quantité de glycogène tendait cependant à se stabiliser et à devenir intermédiaire entre celle du premier et du deuxième jour. On considère que la piètre activité phagocytaire des leucocytes de la glande mammaire bovine, comparée à celle des leucocytes du sang et telle qu'observée par plusieurs chercheurs, serait partiellement due à leurs faibles réserves énergétiques.

INTRODUCTION

The presence of leukocytes in milk from normal and diseased bovine mammary glands is well known. The roles of these, particularly the polymorphonuclear leukocytes or neutrophils (PMNs) which predominate in milk from mastitic mammary glands, has not been examined in detail until recently. Pre-existing leukocytosis protected 75% of mammary quarters against intramammary exposure to 10^3 C.F.U. of *Staphylococcus aureus* (3). Several other workers (18, 20) have produced evidence of the protective value of preexisting and experimentally induced leukocytosis against natural and induced baterial infections of the bovine mammary gland. It has, however, been pre-supposed in most or all of these cases that the PMNs appearing in the bovine mammary gland in health or in disease are capable of performing normal physiological functions similar to those of blood leukocytes. Significant difference, however, has been observed in the phagocytic competence of PMNs from blood and milk of cows by some workers (12, 13, 15, 33). Fewer efficient phagocytes were found among milk leukocytes than among those from blood of the same animal.

There seems to be general agreement that phagocytosing PMNs depend heavily on glycogen reserves for energy requirements, particularly in a glucose-poor medium such as the inflammatory site (11, 25). Phagocytosis by PMNs is an activated physiological process requiring the utilization of large quantities of energy (27). PMNs found in the peripheral blood are provided with adequate quantities of reserve energy in the form of intracellular glycogen to meet these requirements (30). Addition of either serum or glucose to the suspending medium improves significantly the phagocytic competence of milk leukocytes *in vitro* (16). This observation suggests that in the mammary gland adequate energy sources may not be available to these cells to carry out their functional activities to their full potential. Since leukocytes in milk do not appear to possess the ability to utilize lactose, the only carbohydrate available in significant amounts in milk, they must depend on their intracellular glycogen reserves for their energy requirements. Most studies on the glycogen content in leukocytes have been done on human blood leukocytes of normal and leukemic subjects (21, 22, 23, 26, 30, 31). Published reports on the quantitative glycogen content of leukocytes of domestic animals, particularly the dairy cow, have not yet appeared. It was therefore considered worthwhile to investigate qualitative and quantitative distribution of glycogen in leukocytes from blood and milk of dairy cows. In addition, the relationship of blood glucose levels to leukocyte glycogen levels in the blood of these animals has been investigated.

MATERIALS AND METHODS

Experimental Animals

Five purebred Holstein cows from the O.V.C. Mastitis Research Herd were selected for the present investigations, because leukocytes from their blood could be isolated rapidly by a Fibrinogen Sedimentation Method. They were kept under standard feeding and management conditions, and all remained clinically normal during the experimental period. The five animals, however, were in different stages of reproductive and lactation cycles.

Blood Leukocyte Preparations

Blood samples were routinely collected in 'vacutainer' tubes[1] containing 20 mg of sodium salt of EDTA with matching sterile, disposable needles, from jugular or tail veins of each cow. All subsequent procedures were carried out in sterile, siliconized glassware.

Leukocytes were isolated from bovine blood by a modification of a Fibrinogen Sedimentation Method described for human blood (24). Three point three ml of a 6% solution of Bovine Fibrinogen Fraction — I[2] containing approximately 60% protein and 40% citrate was mixed with 1.7 ml of blood, and the mixture was allowed to settle in a conical centrifuge tube. After complete sedimentation of the red cells, which took approximately three hr, the leukocyte-rich fibrinogen supernatant was removed with a pipette and centrifuged at 1000xg for 15 min. The leukocytes were washed twice and resuspended in an appropriate volume of 0.85% saline to contain 10-20x10^6 leukocytes/ml. These leukocyte preparations were used for glycogen determinations and contained less than 0.5% of the total red cells of the blood. The percentages of PMNs were determined in smears made from blood and leukocyte preparations stained with Leishman's stain.

Collection of Leukocytes from Milk

Leukocytes were obtained in large numbers in milk from the mammary glands by infusing into the right hind quarter of each of the five cows 50 ml of Sterile 0.85% NaCl containing 25 μg of Bacto Lipopolysaccharide-B from *E. coli* 0111:B4[3], using a sterile 35″ long 'simplex' intramammary infusion tube with plastic cannula[4], following the technique described for ewes (14). For four consecutive weeks each cow was infused on Monday soon after the afternoon milking, and the milk samples were collected daily for the next four days. Milk samples were routinely screened through cheese cloth to eliminate clots, and centrifuged at 100xg for 15 min in 40 ml siliconized glass tubes. The sedimented white cells were washed twice and finally resuspended in sufficient 0.85% saline to contain 10-20x10^6 leukocytes/ml. This cell suspension constituted the milk leukocyte preparation for further studies. Smears were prepared from leukocyte preparations and stained with Leishman's stain and the percent PMNs determined.

Glycogen Determination

Glycogen content of the leukocyte preparations from bovine blood and mammary glands were determined employing the Anthrone reagent method (21) with a minor modification consisting of the use of KOH pellets in the place of 60% KOH. Since only PMNs contained a significant amount of glycogen, the assumption was made that all glycogen found in the leukocyte preparations originated exclusively in the PMNs, and hence the glycogen content was expressed as mg/10^9 blood PMNs, in each case based on the differential counts of the leukocyte preparations. Glycogen content in leukocytes from milk was expressed as mg/10^9 milk leukocytes because 97 to 98% of these cells were PMNs in preliminary differential counts.

PAS Reaction in Leukocytes

Distribution of glycogen in different leukocyte cell types from bovine blood and mammary glands was studied by the Periodic acid-Schiff (PAS) reaction method described for smears prestained with Romanowsky staining techniques (7). Blood smears and smears made from leukocyte preparations from blood and milk prestained with Leishman's stain were restained for PAS reaction. Control smears were treated with fresh undiluted human saliva for 30 min at 37°C preceding staining for PAS reaction.

Blood Glucose Determination

Glucose levels in deproteinized blood samples were determined in the five cows employing a standard method (17). In order to study the effect of daily blood glucose concentration on the blood leukocyte glycogen level on the same day in the same cow, both were determined on two of the five cows for six consecutive days.

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[1]Becton, Dickinson & Company Canada Ltd., Mississauga, Ontario.

[2]Sigma Chemical Company, St. Louis, Mo.

[3]Difco Laboratories, Detroit, Mich.

[4]Peder Pederson, Gueph, Ontario.

Statistical Analysis

Statistical analysis was made on the data wherever necessary, employing Student's t-test.

RESULTS

Blood Leukocyte Glycogen Levels

The mean percent PMNs in the blood samples and leukocyte preparations from blood were 29.20 ± 7.32 (S.D.) and 22.75 ± 4.54 (S.D.) respectively.

In a total of 102 determinations on the leukocyte preparations from blood, mean glycogen levels of 1.32 ± 0.04 (S.E.) mg and 6.11 ± 0.17 (S.E.) mg10^9 WBC and 10^9 PMNs respectively were obtained. The mean glycogen values for individual cows are presented in Table I.

Milk Leukocyte Glycogen Levels

Differential counts on smears from milk leukocyte preparations revealed that 97 to 98% of the leukocytes were morphologically similar to blood PMNs. Very few mononuclear cells seen in leukocyte preparations resembled the monocytes of bovine blood. For this reason, the glycogen values in leukocyte preparations were expressed in mg/10^9 milk leukocytes, and for all practical purposes constituted glycogen/10^9 PMNs from bovine milk.

From a total of 80 determinations, a mean glycogen level of 3.81 ± 0.18 (S.E.) mg/10^9 milk leukocytes was obtained. Individual determinations in leukocytes of the five cows ranged from 1.10 to 9.5 mg/10^9 milk leukocytes. Mean glycogen levels for individual cows are shown in Table II.

The mean glycogen level in milk leukocytes of each cow was compared with that of the blood PMNs (Fig. 1). The difference between the overall milk leukocyte glycogen level (3.81 mg/10^9 cells) and that for the blood PMNs (6.11 mg/10^9 cells) was highly significant ($P = <0.001$).

Variations were observed in the glycogen content of leukocytes from milk samples obtained on different days following intramammary infusion. These changes were explored in detail in experiments carried out on three of five cows under study. These investigations revealed that there was a precipitous drop in the glycogen content of leukocytes obtained on day 2 post-infusion, compared to that of day 1 samples in all three cows. Levels on third and fourth days tended to reach values intermediate between those found on the first and second days following intramammary infusion (Fig. 2).

TABLE I. Mean Glycogen Values in the Blood Leukocytes of Five Cows

Cow #	No of Tests	Glycogen — mg/10^9 WBC		Glycogen — mg/10^9 PMN	
		Mean ± S.D.	Range	Mean ± S.D.	Range
3505	13	1.64 ± 0.29	1.31 – 2.20	5.64 ± 0.98	4.50 – 7.59
3565	22	1.19 ± 0.20	0.89 – 1.58	4.87 ± 0.85	3.64 – 6.24
3574	19	1.35 ± 0.36	0.76 – 1.20	6.92 ± 1.84	3.88 – 9.84
3892	25	1.59 ± 0.40	1.07 – 2.32	7.57 ± 1.92	5.11 – 11.03
4131	23	0.93 ± 0.10	0.73 – 1.13	5.29 ± 0.56	4.51 – 6.45

TABLE II. Glycogen Content in Leukocytes from Milk of Five Cows

	Cow 3505	Cow 3565	Cow 3574	Cow 3892	Cow 4131
Mean Glycogen — mg/10^9 cells	3.35[a]	3.95[a]	3.33[a]	4.96[a]	3.45[a]
S.D.	1.41	1.37	1.28	2.02	1.14
Range	1.10 – 6.50	1.80 – 6.90	1.70 – 6.20	2.50 – 9.50	1.60 – 5.90

[a]Mean value for 16 tests in duplicate

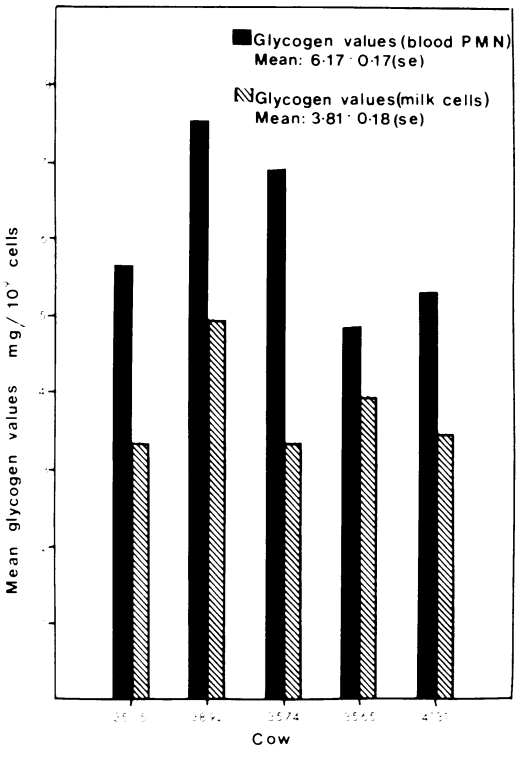

Fig. 1. Comparison of the mean glycogen values of bovine blood PMNs with values of milk leukocytes in five cows.

Distribution of Glycogen in Bovine Leukocytes

Smears from blood and leukocyte preparations stained for PAS reaction revealed that the PAS positive material was found almost exclusively in PMNs. Control smears treated with human saliva failed to reveal PAS positive material in any of the cell types, indicating that the PAS positive material observed in PMNs was glycogen. Glycogen was distributed uniformly throughout the cytoplasm of the PMNs both in blood and leukocyte preparation smears. In addition, PMNs in smears from blood frequently contained glycogen granules in varying numbers and dimensions. These were seen both along the periphery of the PMNs and scattered throughout the cytoplasm. Variations in the intensity of staining could be observed in individual cells both in different smears and in different fields of a single smear. PAS stained smears from sedimented preparations differed somewhat from those of the blood in that a slightly higher number of PMNs in the former revealed weak staining properties, and the glycogen granules were not observed in them.

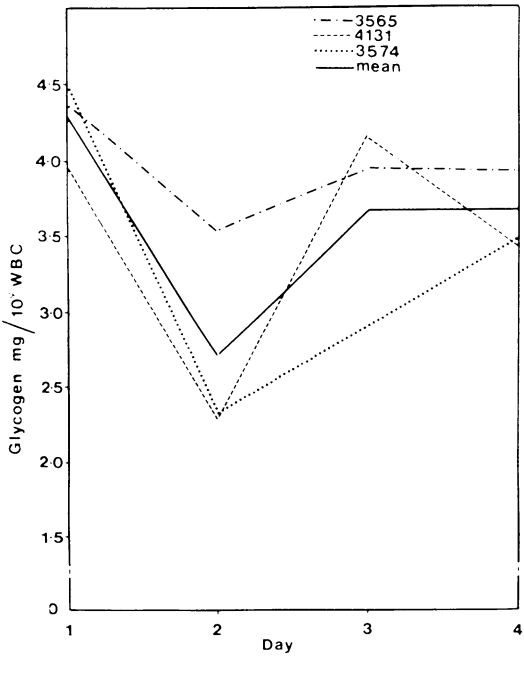

Fig. 2. Distribution of glycogen in leukocytes from milk obtained from first through fourth day following intramammary infusion of E. coli endotoxin into three cows.

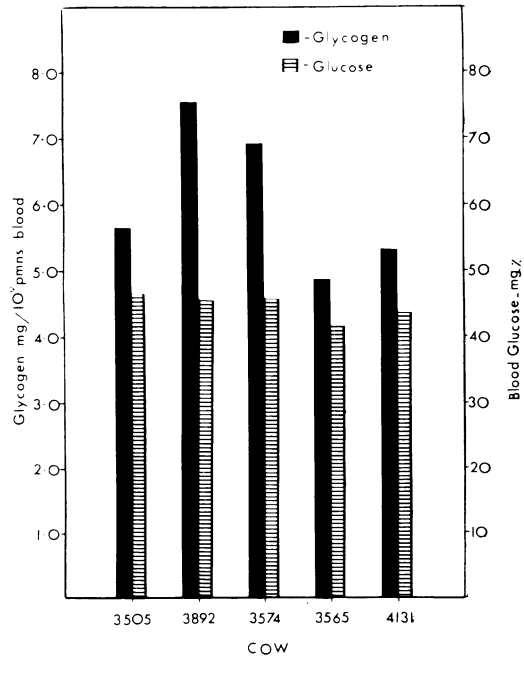

Fig. 3. Relationship of the blood PMN glycogen values to the blood glucose value in five cows.

TABLE III. Blood Glucose Levels in Five Cows

Cow #	Blood Glucose — mg% ± S.D.
3505	46.40 ± 5.60[a]
3565	41.36 ± 6.01[a]
3574	45.50 ± 3.48[a]
3892	45.40 ± 3.55[a]
4131	43.76 ± 4.90[a]

[a]Mean of 12 duplicate tests

Monocytes, large and small lymphocytes, and basophils were generally devoid of any demonstrable glycogen. However, a very few monocytes and large lymphocytes occasionally revealed glycogen granules in their cytoplasm. Eosinophils were somewhat difficult to evaluate for their PAS staining properties as the specific eosinophilic granules made differentiation impossible. The intergranular space in the cytoplasm revealed a weak positive reaction in some smears, while the control preparations revealed none. Erythrocytes did not reveal the presence of glycogen in any of the smears examined, and a few thrombocytes seen in clumps in leukocyte preparations generally revealed a weak positive reaction.

PAS reaction of the milk leukocyte preparations was similar to those of the blood and was, in general, more uniform throughout the cytoplasm of the PMNs from milk than those from blood. Glycogen granules were notably absent in milk leukocytes.

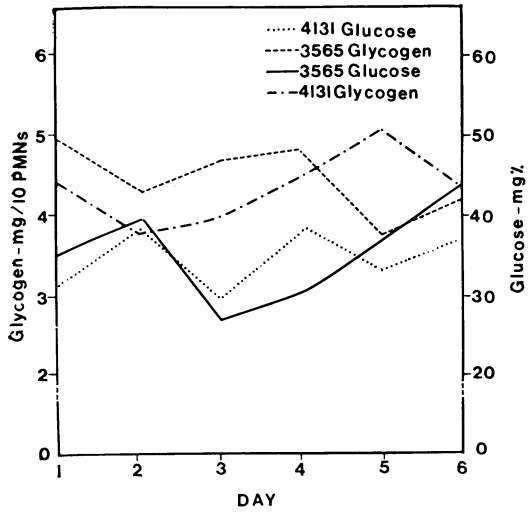

Fig. 4. Relationship of the daily blood PMN glycogen values to the daily blood glucose levels in two cows.

Blood Glucose Levels

In a total of 60 tests on the blood samples of five cows, a mean blood glucose concentration of 44.46 ± 0.66 (S.E.) mg/100ml was obtained. Mean values for individual cows are presented in Table III.

The mean blood glucose levels in the individual cows were compared with their respective blood PMN glycogen values in Fig. 3. The PMN glycogen value in blood apparently bore no relationship to the blood glucose concentration in any of the five cows studied. In tests carried out on two of five cows for six consecutive days, the glycogen level in the blood PMNs on any one day remained independent of the blood glucose level on the same day in the same cow (Fig. 4). The daily blood glucose levels varied considerably while the blood PMN glycogen levels in any single cow remained relatively constant.

DISCUSSION

As no published report is available on the blood leukocyte glycogen contents in animals other than guinea pig, results obtained in the present studies could only be compared with those of human blood leukocytes. Glycogen values of 2.54 mg and 4.23 mg/10^9 human leukocytes and granulocytes respectively were reported by an earlier worker (31). More recent workers, however, have reported values of 7.36, 7.30 and 7.51 mg/10^9 PMNs (21, 23, 26) using the Anthrone reagent method for glycogen determinations. In the present studies the 1.32 mg/10^9 bovine WBC obtained is approximately 50% of that reported for human leukocytes (31). Presumably this low level is due to lower percentage of PMNs (approximately 50% of those in human blood) normally found in bovine blood. The mean glycogen content of 6.11 mg/10^9 PMNs from cows is slightly higher than reported earlier in human PMNs (31) and is lower than the accepted values for human PMNs reported more recently (21, 23, 26). The delay of approximately three hr involved in the isolation of leukocytes from bovine blood by the fibrogen sedimentation method may

have resulted in considerable depletion of glycogen as these cells were suspended in a relatively glucose-poor medium during the separation process. This view gains support from the observations that glycogen losses amounting to 30% to 38% in a two hr period occurred in human PMNs when incubated in a medium devoid of glucose (21, 23). On the other hand, the possibility exists that the glycogen content in bovine PMNs may be inherently lower than that of human counterparts and that the delay in leukocyte separation may not have been responsible. The observation in human PMNs that they do not depend on their glycogen reserves for their normal glucose metabolism and that their glycogen stores are unaffected by fasting (27) would tend to support the latter hypothesis. Guinea pig exudate leukocytes containing a preponderence of PMNs were reported to contain 14 to 20 mg of glycogen/10^9 cells (1). This value is considerably higher than those reported for human PMNs by different workers and the value observed in bovine PMNs in the present investigations.

PAS positive material completely digestible by human salivary amylase has been considered to be a reasonably definite identification of glycogen in the leukocytes by most workers (6, 7, 8, 29). The present experimental findings of glycogen being present almost exclusively in PMNs of bovine leukocytes is in complete conformity with the published reports in animals (9) and human leukocytes (7, 29, 32). These observations give a reasonable justification for expressing glycogen values in mg/10^9 PMNs in the present studies. The observations of the presence of glycogen granules in bovine PMNs and occasionally in monocytes and large lymphocytes are in good agreement with those in animals (9) and humans (34), as is the presence of moderate amounts of glycogen in platelets from bovine blood observed in the present studies (7, 9).

Direct comparison of glycogen content in bovine milk leukocytes could not be made with those of other workers due to lack of published reports. An indirect comparison can, however, be made with the results of Cecil, Bitman and Wood (4) who reported a value of 125 μg of glycogen/ml of mastitic milk containing, on the average, 25 x 10^6 leukocytes. Seventy percent of their Anthrone positive material was shown to be glucose, and lactose did not react with Anthrone reagent. From these observations it can be estimated that the leukocytes from their mastitic milk contained on the average 3.50 mg of glycogen/10^9 leukocytes, which is in close agreement with the mean glycogen value of 3.81 mg/10^9 milk leukocytes obtained in the present studies. Even though RNA and milk proteins such as lactalbumin, β-lactoglobulin and casein have been reported to react with Anthrone reagent (4), these substances are not likely to be present in significant amounts on the surface of leukocytes used in the present studies as these were repeatedly washed in normal saline. There was a great variation among day to day milk leukocyte glycogen values as seen by a large standard deviation in each cow. Great difference in the glycogen values in leukocytes from first and second day milk samples may have contributed substantially to this.

The overall mean milk leukocyte glycogen level amounted to about 62% of that in blood PMNs. This suggests that the blood PMNs had lost approximately 38% of their initial glycogen content by the time these cells were isolated from the mammary gland. There was a minimum of ten hr interval between endotoxin infusion and collection of milk samples in the present studies. If the initial leukocyte response to endotoxin can be assumed to be at approximately three hr post-infusion (19, 20) then some leukocytes had been inhabiting the mammary gland for a minimum period of seven hr. Thus loss of glycogen from leukocytes in the bovine mammary gland is comparatively less than those reported for human PMNs (21, 23). This may be due to one or both of the following reasons: i) the leukocytes may be slowing down their metabolic processes in unfavourable environments thus curtailing the expenditure of their reserve glycogen (21); or (ii) some glucose may be escaping with blood serum into the mammary gland as a result of increased permeability, thus exerting a sparing effect on the intracellular glycogen reserves in the milk leukocytes.

The reasons for the variations in the glycogen levels in leukocyte preparations obtained from the first through fourth day following intramammary infusion of endotoxin (Fig. 2) are not clear from the present studies. One plausible explanation may be that leukocytes freshly infiltrating the mammary gland contain large quantities of glycogen, almost equalling those of blood PMNs, and that the rate of leuko-

cyte migration may be slowing down after the first milking as a result of elimination of endotoxin through milk and, to a lesser extent, by absorption into the blood. Thus the leukocytes appearing in the second day's milk sample may still be those that entered the mammary gland in initial response to endotoxin and hence had been inhabiting the udder for longer than 24 hr. As has been reported from studies involving experimental *Aerobacter aerogenes* infection of the udder (10), fresh leukocytes may emigrate into the mammary gland following the second day's milking, probably as a secondary response, and that these relatively fresh cells with average glycogen reserves may constitute the majority of leukocyte population in third and fourth day milk samples.

The mean blood glucose levels in five cows investigated are well within the range suggested for normal cows (5). No report could be found in the literature on the exact relationship between blood glucose concentration and leukocyte glycogen level in humans or animals. High blood glucose levels are known to result in moderate increases in muscle and liver glycogen in humans (2). It is not known whether a similar increase occurs in leukocyte glycogen. The leukocyte glycogen levels remain relatively unaffected by high blood sugar levels in poorly controlled diabetic subjects (27) and under experimental conditions where leukocytes were incubated in media with glucose concentrations significantly above those seen in blood (21). Information is likewise not available on the effect of hypoglycemia on leukocyte glycogen levels in man and animals. In view of the foregoing, it may not be unreasonable to presume that while leukocyte glycogen in itself is synthesized from blood glucose, extreme variations in blood glucose levels do not result in corresponding changes in normal blood leukocyte glycogen levels; an assumption which would tend to support the lack of relationship between the two observed in present investigations on cattle.

And finally, the results from the present studies revealed highly significant differences between mean blood PMN glycogen content and that of milk leukocytes. This observed difference in the energy resources of the two cell populations may, in part, account for some of the difference in phagocytic ability of these two groups of cells reported in literature.

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REFERENCES

1. **BAZIN, S. and C. AVICE.** Le métabolisme glycogenique des polynucléaeres au cours de la phagocytose **in vitro.** C.r. Séanc. Soc. Biol., Paris **14:** 1025-1027. 1953.
2. **BEST, C. H. and N. B. TAYLOR.** The Physiological Basis of Medical Practice: Eighth edition. Baltimore: Williams and Wilkinson Co. 1966.
3. **BLOBEL, H. and Y. KATSUBE.** Effect of experimentally induced leukocytosis in bovine mammary glands upon infection with **Staph. aureus, Strep. agalactiae** and **Aerobact. aerogenes.** Am. J. vet. Res. **25:** 1085-1089. 1964.
4. **CECIL, H. C., J. BITMAN and J. R. WOOD.** Changes in milk glycogen during mastitis. J. Dairy Sci. **48:** 1607-1611. 1965.
5. **COLES, E. H.** Veterinary Clinical Pathology: Philadelphia and London: W.B. Saunders Co. 1967.
6. **GIBBS, R. P. and R. H. STOWELL.** Glycogen in human blood cells. Blood **4:** 569-579. 1049.
7. **HAYHOE, F. G., D. QUAGLINO and R. J. FLEMANS.** Consecutive use of Romanowsky and Periodic acid-Schiff techniques in the study of blood and bone marrow cells. Br. J. Haemat. **6:** 23-25. 1960.
8. **HOTCHKISS, R. D.** A microchemical reaction resulting in staining of polysaccharide structures in fixed tissue preparations. Archs Biochem. Biophys. **16:** 131-141. 1948.
9. **JAIN, N. C.** Glycogen content of leukocytes of some animal species. Acta haemat. **4:** 249-253. 1959.
10. **JAIN, N. C., J. LASMANIS and O. W. SCHALM.** Influence of egg albumin-induced leukopenia on experimental **Aerobact. aerogenes** mastitis and on natural infection of mammary gland with Coagulase-negative Staphylococci in a cow. Am. J. vet. Res. **28:** 1243-1250. 1967.
11. **KARNOVSKY, M. L.** Metabolic basis of phagocytic activity. Physiol. Rev. **42:** 143-168. 1962.
12. **KENT, G. M.** Some aspects of the phagocytic competence of metabolism of leukocytes from cow's milk. Thesis. Univ. of Guelph. 1968.
13. **KENT, G. M. and F. H. S. NEWBOULD.** Phagocytosis and related phenomena in polymorphonuclear leukocytes from cow's milk. Can. J. comp. Med. **33:** 214-219. 1969.
14. **McDOWELL, G. H., C. S. LEE and A. K. LASCELLES.** Collection of polymorphs and macrophages from the involuted mammary gland of the ewe. Res.. vet. Sci. **10:** 13-17. 1969.
15. **NEWBOULD, F. H. S.** Some aspects of the source of bovine milk leukocytes and strain of Staphylococcus on their interaction **in vitro.** Can. J. comp. Med. **31:** 303-308. 1967.
16. **NEWBOULD, F. H. S.** Enhancement of phagocytosis in bovine leukocytes **in vitro.** Can. J comp. Med. **34:** 261-264. 1970.
17. Procedure for blood, plasma or serum with deproteinization. Sigma Tech. Bull. **510:** 4-8. St. Louis. Missouri: Sigma Chemical Co. 1967.
18. **REITER, B. and J. D. ORAM.** Bacterial inhibitors in milk and other biological fluids. Nature, Lond. **216:** 328-330. 1967.
19. **SCHALM, O. W., J. LASMANIS and N. C. JAIN.** Effects of humoral and cellular phases of acute inflammation in the bovine mammary gland on **Aerobact. aerogenes** introduced **en masse** and on leukocytes existing in milk. Am. J. vet. Res. **28:** 1251-1256. 1967.
20. **SCHALM, O. W. and J. LASMANIS.** The leukocytes: Origin and function in mastitis. J. Am. vet. med. Ass. **153:** 1688-1694. 1968.
21. **SCOTT, R. B.** Glycogen in human peripheral blood leukocytes. I. Characteristics of the synthesis and turnover of glycogen **in vitro.** J. clin. Invest. **47:** 344-352. 1968.
22. **SCOTT, R. B.** The role of glycogen in blood cells. New Engl. J. Med. **278:** 1436-1439. 1968.

23. **SEITZ, I. F.** Biochemistry of normal and leukemic leukocytes, thrombocytes and bone marrow cells. Advances in Cancer Research. 9: 303-410. New York and London: Academic Press. 1965.
24. **SKOOG, W. A. and W. S. BECK.** Isolation of leukocytes from whole blood. Blood 11: 436-454. 1956.
25. **STAHELIN, H., E. SUTER and M. L. KARNOVSKY.** Studies on the interaction between phagocytes and tubercle bacilli. I. Observations on the metabolism of guinea pig leukocytes and the influence of phagocytosis. J. exp. Med. 104: 121-136. 1956.
26. **VALENTINE, W. N., W. S. BECK, J. H. FOLETTE, H. MILLS and J. S. LAWRENCE.** Biochemical studies in chronic myeloid leukemia, polycythemia vera and other idiopathic myeloproliferative disorders. Blood 7: 959-977. 1952.
27. **VALENTINE, W. N., J. H. FOLETTE and J. S. LAWRENCE.** The glycogen content of human leukocytes in health and in various disease states. J. clin. Invest. 32: 251-257. 1953.
28. **VERCAUTEREN, R. E.** On the intracellular distribution of glycogen and phosphatases in leukocyte homogenates. Enzymologia 29: 45-52. 1965.
29. **WACHSTEIN, M.** The distribution of histochemically demonstrable glycogen in human blood and bone marrow cells. Blood 4: 54-59. 1949.
30. **WAGNER, R.** The estimation of glycogen in whole blood and white blood cells. Archs Biochem. Biophys. 11: 249-258. 1946.
31. **WAGNER, R.** Studies on the physiology of white blood cells: The glycogen content of leukocytes in leukemia and polycythemia. Blood 2: 235-243. 1947.
32. **WISLOCKI, G. B., H. BUNTING and E. W. DEMPSEY.** Further studies on chemical cytology of megakaryocytes and other cells of hemopoitic tissues. Anat. Rec. 98: 527-537. 1947.
33. **WISNIOWSKI, J., K. ROMANIUKOWA and H. GRAJEWSKI.** Phagocytic phenomena in the mammary glands of cows. I: The opsonizing factor and phagocytic activity of leukocytes in milk and blood. Bull. vet. Inst. Pulawy (Poland) 140-144. 1965.
34. **WULF, H. R.** Histochemical studies of leukocytes from inflammatory exudate: Glycogen and phosphorylase, Acta haemat. 28: 86-94. 1962.