Concentration of Sugars, Ketone Bodies and some Organic Acids in Udder Lymph and Plasma of Cattle

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Heyndrickx & Peeters (1958*a, b*) and Heyndrickx (1959) have described the electrolyte, enzyme, lipid and protein composition of the udder lymph and blood plasma in cattle. Because of the importance of the udder lymph in the synthesis of milk, the present paper extends their findings to include the sugars, ketone bodies and some organic acids.

MATERIALS AND METHODS

Biological material. East Flemish and Friesian cows in good lactation and with normally healthy udders were used. A blood sample was taken from the udder veins and, after slaughter of the animal, the udder lymph was sampled as previously described (Heyndrickx & Peeters, 1958*a*). Blood and lymph were heparinized and then centrifuged. The interval elapsing between taking the blood and lymph samples was about 15 min.

Analytical methods

Sugars. These were estimated by circular and descending chromatography with the solvent mixtures butanol-acetic acid-water (4:1:5, by vol.) and phenol (Partridge, 1948). The samples were first deproteinized and desalted as described by Odin & Werner (1949) and Wallenfels, Bernt & Limberg (1953). Glucose and lactose were determined on the developed chromatograms with *o*-aminodiphenyl (Piper & Bernardin, 1958). Fructose was identified by comparison with a control chromatogram and determined after extraction by the method of Honer & Tuckey (1953). Since the content of fructose is very low in lymph and plasma a sensitive method is essential.

Ketone bodies. Acetone plus acetoacetic acid was determined with 2,4-dinitrophenylhydrazine according to Mayes & Robson (1957). Acetoacetic acid was determined by the method of Walker (1954) and the free acetone was calculated by difference.

Organic acids. Pyruvic acid and α -oxoglutaric acid were estimated by chromatography of the 2,4-dinitrophenylhydrazones (Cavallini & Mondovi, 1957); the solvent systems phenol (Partridge, 1948) and 0.1N-glycine buffer, pH 8.4 (Virtanen, Miettinen & Kunttu, 1953), were used. Citric acid and lactic acid were determined by the method of Natelson, Pincus & Lugovoy (1948) and Barker & Summerson (1941) respectively. The results obtained were in agreement with the semi-quantitative paper-chromatographic methods of Nordmann, Marty, Tholozan & Nordmann (1957) and Osteux & Laturaze (1956).

Proteins. Protein content was calculated from the refractive index (Heyndrickx, 1959).

The results have been evaluated statistically according to Snedecor (1950). To calculate the significance of the difference between the means for each constituent in lymph and plasma, each of the plasma samples was paired with a corresponding sample of the lymph taken from the same cow, and the paired *t* test was used.

RESULTS

Table 1 shows the mean values and standard deviations for 11 samples of udder lymph and of the corresponding plasma from the udder vein; the degree of significance of the difference between the means of each constituent is also mentioned.

Sugars. The mean concentration of glucose in lymph was significantly lower than that in plasma. The figures for plasma confirm the results of Barros (1942) and Bach & Hibbit (1959). The content of fructose was lower in lymph than in plasma, but lactose is present in higher concentration in lymph.

Ketone bodies. The acetone concentration is higher in lymph than in plasma, although the concentration of acetoacetic acid is practically the same in the two fluids.

Organic acids. Pyruvic acid occurs in lower concentration in lymph than in plasma, whereas the reverse is true for α -oxoglutaric acid, citric acid and lactic acid. The concentrations in the plasma of the second and third acids are in agreement with the values reported by Blosser, Niedermeier & Smith (1951), Van den Berg (1946) and Powell & Shaw (1942).

Proteins. In agreement with our previous work, the protein content was much lower in lymph than in plasma.

DISCUSSION

There is no literature on the composition of udder lymph, but Yoffey & Courtice (1956) write that the readily diffusible non-protein constituents of the plasma are present in the lymph from other sources in concentrations which are approximately the same as in the plasma. Our results, however, show that in udder lymph the concentrations of most of the constituents differ significantly from those in plasma.

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Table 1. *Mean composition of 11 samples of udder lymph and plasma of the udder veins*

Constituent	Mean concn. (mg./100 ml.) $\pm$ s.d.		<i>P</i> at which concn. in lymph - concn. in plasma is significant
	Lymph	Plasma	
Glucose	48.2 $\pm$ 11.0	70.6 $\pm$ 12.1	<0.01
Fructose	0.65 $\pm$ 0.43	1.16 $\pm$ 0.64	0.01
Lactose	12.5 $\pm$ 7.6	5.3 $\pm$ 5.1	<0.01
Acetone	0.46 $\pm$ 0.48	0.31 $\pm$ 0.31	0.1
Acetoacetic acid	0.19 $\pm$ 0.14	0.22 $\pm$ 0.12	0.4
Pyruvic acid	0.42 $\pm$ 0.07	0.71 $\pm$ 0.34	0.02
α -Oxoglutaric acid	0.17 $\pm$ 0.07	0.10 $\pm$ 0.05	<0.01
Citric acid	4.05 $\pm$ 1.67	2.63 $\pm$ 1.32	<0.01
Lactic acid	87.6 $\pm$ 15.2	7.0 $\pm$ 3.6	<0.01
Protein (g./100 ml.)	2.86 $\pm$ 1.00	8.41 $\pm$ 0.66	<0.01

The cow was always 'milked out' a short time before slaughter, as otherwise the lymph, which had been punctured from an udder abnormally tense with milk, was very turbid instead of being clear and contained the constituents of the milk in high concentrations. This shows that considerable reabsorption of milk by the lymphatics takes place under these conditions. Some reabsorption of readily diffusible milk constituents may occur under normal conditions. It is well known that urine and serum from pregnant and lactating women may contain lactose (Archer & Haram, 1948; Odin & Werner, 1949) and the higher concentration of lactose in lymph relative to plasma may similarly be explained. The higher concentration of citric acid and α -oxoglutaric acid in lymph may also be due to reabsorption from the milk, in which the average concentrations of citric acid and α -oxoglutaric acid are respectively 181 (Schwarz & Mumm, 1953) and 0.24 mg./100 ml. (Harper & Huber, 1956). The occurrence of α -oxoglutaric acid and citric acid in the udder lymph in higher concentrations than in the mammary-vein plasma would appear to provide additional evidence of the occurrence of the Krebs cycle in the mammary gland, as first demonstrated by Turner (1951).

Many investigations have shown that the blood glucose is the principal precursor of the lactose of the milk (Dimant, Smith & Lardy, 1953; Kleiber *et al.* 1955). Peeters, Sierens, Tindal & Coussens (1955) have found that during perfusion of the isolated mammary gland of the cow considerable quantities of glucose and fructose disappeared from the blood. It is probable that this may be related to the lower concentrations of glucose and fructose found in lymph relative to the plasma. Shaw (1946) shows no significant difference in pyruvic acid between arterial and venous blood samples from the cow's mammary gland. Peeters,

Coussens & Sierens (1954) have found a decrease of the blood pyruvic acid on perfusion of the isolated cow mammary gland in the presence of fluoroacetate. The chemical methods employed by these workers for determination of pyruvic acid are, however, not highly specific. It is possible that pyruvic acid is utilized by the mammary gland and in this case the lower content of this acid in lymph compared with plasma may be explained in the same way as for glucose and fructose. In milk the concentration of pyruvic acid, like that of glucose and of fructose, is very low (0.008 mg./100 ml. according to Harper & Huber, 1956).

It is very striking that the concentration of lactic acid is about 12 times as high in lymph as in plasma. This is not explicable by a reabsorption from the milk since the concentration of lactic acid in milk is about 2 mg./100 ml., of the same order as that in plasma. On the other hand, it is known that mammary tissue produces lactic acid on incubation (Knott & Petersen, 1945) and it is possible that this diffuses into the lymph.

SUMMARY

1. Sugars, ketone bodies and organic acids were determined in udder lymph and plasma from the veins of the udder of cattle.

2. The concentrations of glucose and fructose are lower and that of lactose is higher in lymph than in plasma.

3. The free acetone content attains higher values in lymph than in plasma, although the concentration of acetoacetic acid is practically the same in the two fluids.

4. The α -oxoglutaric acid, citric acid and especially lactic acid contents attain significantly higher values in lymph than in plasma; the pyruvic acid content of lymph is, however, below that of plasma.

REFERENCES

- Archer, H. E. & Haram, B. J. (1948). *Lancet*, i, 558.
 Bach, S. J. & Hibbit, K. G. (1959). *Biochem. J.* **72**, 87.
 Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
 Barros, L. A. (1942). *Arch. Soc. Biol. Montevideo*, **10**, 308.
 Blosser, T. H., Niedermeier, R. P. & Smith, V. R. (1951). *J. Dairy Sci.* **34**, 75.
 Cavallini, D. & Mondovi, B. (1957). *Clin. chim. Acta*, **2**, 312.
 Dimant, E., Smith, V. R. & Lardy, H. A. (1953). *J. biol. Chem.* **201**, 85.
 Harper, W. J. & Huber, R. M. (1956). *J. Dairy Sci.* **39**, 1609.
 Heyndrickx, G. V. (1959). *Quart. J. exp. Physiol.* **44**, 264.
 Heyndrickx, G. V. & Peeters, G. (1958a). *Quart. J. exp. Physiol.* **43**, 174.
 Heyndrickx, G. V. & Peeters, G. (1958b). *Enzymologia*, **20**, 161.
 Honer, C. J. & Tuckey, S. L. (1953). *J. Dairy Sci.* **36**, 1233.
 Kleiber, M., Black, A. L., Brown, M. A., Baxter, C. F., Luick, J. R. & Stadtman, F. H. (1955). *Biochim. biophys. Acta*, **17**, 252.
 Knodt, C. B. & Petersen, W. E. (1945). *J. Dairy Sci.* **28**, 415.
 Mayes, P. A. & Robson, W. (1957). *Biochem. J.* **67**, 11.
 Natelson, S., Pincus, J. & Lugovoy, J. (1948). *J. biol. Chem.* **175**, 745.
 Nordmann, R., Marty, A., Tholozan, A. & Nordmann, J. (1957). *C.R. Acad. Sci., Paris*, **245**, 2414.
 Odin, L. & Werner, I. (1949). *Acta chem. scand.* **3**, 989.
 Osteux, R. & Laturaze, J. (1956). *Clin. chim. Acta*, **1**, 378.
 Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
 Peeters, G., Coussens, R. & Sierens, G. (1954). *Arch. int. Pharmacodyn.* **98**, 288.
 Peeters, G., Sierens, G., Tindal, J. & Coussens, R. (1955). *Arch. int. Pharmacodyn.* **102**, 470.
 Piper, C. V. & Bernardin, L. J. (1958). *Tech. Pap. Pulp Pap. Ind., N.Y.*, **41**, 16.
 Powell, R. C. & Shaw, J. C. (1942). *J. biol. Chem.* **146**, 207.
 Schwarz, G. & Mumm, H. (1953). *Kiel. Milchw. ForschBer.* **5**, 511.
 Shaw, J. C. (1946). *J. Dairy Sci.* **29**, 183.
 Snedecor, G. W. (1950). *Statistical Methods*. Ames, Iowa: State College Press.
 Ternier, C. (1951). *Biochem. J.* **50**, 145.
 Van den Berg, W. (1946). *Tijdschr. Diergeneesk.* **71**, 497.
 Virtanen, A. I., Miettinen, J. K. & Kunttu, H. (1953). *Acta chem. scand.* **7**, 38.
 Walker, P. G. (1954). *Biochem. J.* **58**, 699.
 Wallenfels, K., Bernt, E. & Limberg, G. (1953). *Angew. Chem.* **65**, 581.
 Yoffey, J. M. & Courtice, F. C. (1956). *Lymphatics, Lymph and Lymphoid Tissue*. London: Edward Arnold Ltd.

Biochem. J. (1960) **75**, 3

The Synthesis of Methionine from Homocysteine by Enzymic Extracts of *Escherichia coli*

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In earlier work from this Laboratory it was shown that washed suspensions of various strains of *Escherichia coli* synthesize methionine from homocysteine (Gibson, 1952; Gibson & Woods, 1952, 1960). With strains unable to synthesize serine evidence was obtained that this amino acid was the probable ultimate source of the C₁ fragment for the methylation. With strains requiring *p*-amino-benzoic acid for growth it was shown that this factor was essential for methionine formation and there was strong, though indirect, evidence that it must first be converted into some form of folic acid. The purpose of the present work was to explore the probable function of folic acid under the more closely defined conditions possible with cell-free enzymic extracts and to determine other cofactor requirements.

In exploratory experiments published briefly by

Cross & Woods (1954) it was found that enzymic extracts made by grinding the organisms with alumina synthesized methionine from homocysteine and serine in a reaction mixture containing also hexose diphosphate, pyridoxal phosphate, adenosine triphosphate and Mg²⁺ ions; preparations made by ultrasonic disintegration or by shaking with minute glass beads were less active. A heated extract of *E. coli* increased methionine synthesis by about half. It was later found (by Dr M. J. Cross and the present authors, unpublished work) that extracts of acetone-dried organisms were a more potent and reliable source of the enzyme systems concerned. The problem was therefore investigated in detail with this material. Part of this work has been the subject of a preliminary communication (Szulmajster & Woods, 1955).