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The Uptake of Glutamine and other Amino Acids from the Blood Stream by the Lactating Mammary Gland

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Work with isotopic tracers has demonstrated that the mammary gland takes glutamine and glutamic acid from the blood stream for the synthesis of casein (Barry, 1956). The results reported in this paper show that the concentrations of glutamine and glutamic acid in the mammary venous blood of lactating cows are markedly lower than in the arterial blood; in dry cows these arteriovenous differences were not found. Arteriovenous differences for serine and threonine are also reported.

The fall in concentration of many metabolites across the mammary gland is a small percentage of their total concentration in blood, and is therefore difficult to demonstrate convincingly. However, arteriovenous differences in free amino acids should be very marked, because their concentrations in blood plasma are low in comparison with their concentrations, as protein, in milk. In fact, the concentrations of the amino acids measured were up to 55% lower in mammary venous blood than in arterial blood.

EXPERIMENTAL

Sampling in blood

The cows were Shorthorns in the herd of the National Institute for Research in Dairying. The lactating cows were giving about 4 gallons of milk/day at the time of sampling. They were milked as usual at 7 a.m. and the blood samples were taken 4 hr. later. The dry cows were in the interval between successive lactations; their milk yields during lactation were similar to those of the other cows.

It has been emphasized that the cow should not be excited while the blood samples are taken, and it has been shown in particular that excitement can cause the mammary venous-blood concentration to change by as much as 6%, and so may cause errors of about this magnitude in arteriovenous differences, especially of non-diffusible compounds (Graham, Kay & McIntosh, 1936; Shaw & Petersen, 1939). The arteriovenous differences we have found are between 11 and 55% of the arterial concentrations, and therefore errors due to excitement are not so important as in most arteriovenous measurements. However, care was taken that the cows should not be excited.

The arterial samples were taken by puncture of the internal iliac artery through the rectal wall, as described by Graham *et al.* (1936). The samples from the jugular and subcutaneous abdominal ('mammary') veins were at first taken from plastic catheters previously inserted in the veins. It was later found that samples could be taken with no more excitement in the following way. One man pierced the subcutaneous abdominal vein with the needle of a hypodermic syringe, and a second pierced the jugular vein. They then waited until a third had pierced the internal iliac artery with a hypodermic needle attached to a rubber tube leading to a flask, and then drew 100 ml. samples of blood at about the same rate as the arterial blood flowed into the flask. The sampling was completed within a few minutes of first approaching the cow. In experiments in which the arterial sample was omitted, the venous samples were withdrawn simultaneously into hypodermic syringes.

The blood samples were mixed with potassium oxalate, immediately centrifuged, and the plasma was separated and frozen solid until use.

Determination of free amino acids

Blood plasma. Glutamine, glutamic acid, threonine and serine were determined by chromatography on columns of Dowex-50 resin, as described by Moore & Stein (1951), but at room temperature instead of 37.5°. The amino acids then emerge in the order: threonine and aspartic acid, serine, glutamine and asparagine, and glutamic acid. Moore & Stein recovered 96.7% of the glutamic acid added to the columns, and glutamine was completely lost. At our lower temperature the recovery of glutamic acid was quantitative, and that of glutamine was about 65% but was not constant. The glutamine concentration could be roughly calculated from the height of the glutamine and asparagine peak, but the glutamine of blood plasma was determined accurately from the difference in glutamic acid before and after hydrolysis of the free amino acids with dilute HCl.

The following procedure was used to test whether the threonine, serine and glutamic acid peaks obtained in the analysis of blood plasma contained any other compound reacting with ninhydrin. Sodium and citrate ions were removed from the combined fractions of each peak by passing them through a small column of Dowex-50 (H⁺ form), washing the column with water, and then transferring the resin to a centrifuge tube and eluting the amino acids by adding 2N-NH₃ until the supernatant remained just alkaline. The eluate was evaporated to dryness, and the residue subjected to paper chromatography with aqueous phenol. The serine and glutamic acid peaks both gave only one spot in the expected position. The threonine peaks gave traces of aspartic acid, but not enough to cause a significant error in the threonine determinations.

The method of analysis gave closely agreeing replicates from single samples of blood plasma, and quantitative recoveries of the amino acids from mixtures of known composition. The procedure was as follows. Four 10 ml. portions of each plasma sample were freed from protein by the method of Hamilton & Van Slyke (1943), by adding 50 ml. of 1% (w/v) picric acid to each and, after a few minutes, centrifuging. To remove picric acid, 40 ml. of each supernatant was passed through a column of Dowex-2 (Cl⁻ form; 2 cm. x 2 cm.) and the amino acids were washed through quantitatively with three 5 ml. portions of 0.02N-HCl (Stein & Moore, 1954). The solutions were then brought to

pH 3.5 and freeze-dried. To hydrolyse glutamine, two of the samples were dissolved in a little 2N-HCl and heated under reflux at 100° for 3 hr., and were then again freeze-dried. All four samples were then dissolved in a little water and eluted from columns of Dowex-50 (100 cm. x 0.8 cm.) at about 3.6 ml./hr. at room temperature, and the effluent was collected in 2 ml. fractions. Otherwise, the procedure was that described by Moore & Stein (1951). The fractions containing the required amino acids came after 50 ml. of buffer but before 120 ml. had emerged from the column. By this method glutamine, glutamic acid and threonine were determined in duplicate in each plasma sample, and serine in quadruplicate.

Milk. The free amino acids were determined by treating milk exactly as blood plasma.

RESULTS

In Table 1 are shown the concentrations of glutamine and other amino acids in the arterial, jugular and mammary venous blood plasma of two lactating cows. It is seen that while any differences in concentration between arterial and jugular plasma are small, the concentrations in the mammary venous plasma are considerably lower than those in the arterial plasma.

Blackwood & Stirling (1932) have shown that blood from the jugular vein differs slightly in composition from arterial blood, because the salivary glands remove water from the blood stream. Therefore, a small difference between the concentration of a compound in jugular and in mammary venous blood does not necessarily demonstrate its uptake by the mammary gland. However, the arterio-venous differences in free amino acids are so large that they can be determined with little error from their concentrations in jugular and mammary

Table 1. *Concentrations of free amino acids in samples of blood plasma taken simultaneously from the internal iliac artery, jugular vein and mammary vein of lactating cows*

A = mg. of amino acid/100 ml. of plasma from internal iliac artery; J = mg. of amino acid/100 ml. of plasma from jugular vein; M = mg. of amino acid/100 ml. of plasma from mammary vein.

	$\frac{A-M}{A} \times 100$				
	A	J	M	A-M	
Cow 1					
Glutamine	4.84	4.88	3.76	1.08	22.3
Glutamic acid	1.25	1.14	0.62	0.63	50.4
Threonine	0.78	0.80	0.35	0.43	55.1
Serine	0.61	0.61	0.32	0.29	47.5
Cow 2					
Glutamine	4.31	4.53	3.32	0.99	23.0
Glutamic acid	1.21	1.02	0.66	0.55	45.5
Threonine	0.67	0.60	0.38	0.29	43.2
Serine	0.79	0.75	0.42	0.37	46.8

venous plasma. The results in Table 2 again show that the concentrations of the same amino acids in the mammary venous plasma of three more lactating cows were markedly lower than in the jugular plasma.

From Table 3 it is evident that there were no pronounced differences between the amino acid concentrations in the jugular and mammary venous plasma of two dry cows.

These arteriovenous differences were measured to give information on the amino acids used by the mammary gland for protein synthesis, and they will do so only if the concentrations of free amino acids in milk are low. The same four free amino acids were determined in samples of cows' and goats' milk, and the concentration of each was found to be less than 1% of its concentration in milk in combination as protein.

Table 2. Concentrations of free amino acids in samples of blood plasma taken simultaneously from the jugular and mammary veins of lactating cows

J = mg. of amino acid/100 ml. of plasma from jugular vein; M = mg. of amino acid/100 ml. of plasma from mammary vein.

	J	M	$J - M$	$\frac{J - M}{J} \times 100$
Cow 3				
Glutamine	4.76	3.98	0.78	16.4
Glutamic acid	1.14	0.66	0.48	42.1
Threonine	0.92	0.51	0.41	44.5
Serine	0.88	0.57	0.31	35.3
Cow 4				
Glutamine	4.73	3.74	0.99	20.9
Glutamic acid	1.14	0.87	0.27	23.7
Threonine	0.99	0.52	0.47	47.5
Serine	1.00	0.89	0.11	11.0
Cow 5				
Glutamine	5.11	4.28	0.83	16.2
Glutamic acid	1.56	0.88	0.68	43.6
Threonine	0.85	0.54	0.31	36.5
Serine	0.89	0.58	0.31	34.8

Table 3. Concentrations of free amino acids in samples of blood plasma taken simultaneously from the jugular and mammary veins of dry cows

J = mg. of amino acid/100 ml. of plasma from jugular vein; M = mg. of amino acid/100 ml. of plasma from mammary vein.

	Cow 6		Cow 7	
	J	M	J	M
Glutamine	4.31	4.44	4.99	4.73
Glutamic acid	1.47	1.36	1.40	1.57
Threonine	0.75	0.89	0.96	0.98
Serine	0.67	0.72	0.83	0.81

DISCUSSION

The results show that when blood flows through the mammary gland of a lactating cow, the glutamine and glutamic acid concentrations fall considerably. It is impossible to calculate accurately the arteriovenous difference which will be found for the precursor of a component of milk (Folley, 1949), but a rough calculation can be made. Cows' milk contains about 3% of casein (Davis & Macdonald, 1953), and when 100 g. of casein is hydrolysed it gives 22.4 g. of glutamic acid (Gordon, Semmett, Cable & Morris, 1949), about half of which is derived from glutamine (Barry, 1956). Cows' milk therefore contains about 336 mg. of both protein glutamine and glutamic acid per 100 ml. (ignoring the 0.5% of whey proteins in milk). Evidence from a number of experiments suggests that about 400 vol. of blood plasma flow through the mammary gland of a cow for each volume of milk secreted (Folley, 1949). Therefore, if the glutamine and glutamic acid of casein come from the glutamine and glutamic acid of plasma respectively, there should be an arteriovenous difference for each of about 0.8 mg./100 ml. of plasma. This calculation is very rough, but shows that the arteriovenous differences which were found were of the correct order of magnitude.

Casein yields 4.9% of threonine and 6.3% of serine (Gordon *et al.* 1949), and the arteriovenous differences of their precursors should therefore be about 0.37 and 0.47 mg./100 ml. of plasma respectively. The measured arteriovenous differences for threonine therefore confirm an earlier conclusion that all the essential amino acids of casein come from the free amino acids of plasma (Barry, 1952); and those for serine suggest that the serine of casein is derived at least partly from the free serine of plasma.

SUMMARY

1. Arteriovenous differences for the free glutamine, glutamic acid, threonine and serine of blood plasma have been determined across the mammary glands of both lactating and dry cows. In lactating cows the concentrations were up to 55% lower in the mammary venous blood than in the arterial; no marked differences were found in dry cows.

2. The results confirm the conclusions based on experiments with isotopic tracers that the glutamine and glutamic acid residues of casein come from the free glutamine and glutamic acid of blood, and that the essential amino acids of casein come from the free essential amino acids of blood. It also seems that the serine of casein comes at least partly from the free serine of blood.

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Metabolism of Polycyclic Compounds

10. ESTIMATION OF METABOLITES OF NAPHTHALENE BY PAPER CHROMATOGRAPHY*

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The examination by paper chromatography of the urine of rats dosed with naphthalene has shown that most of the metabolites of naphthalene could be detected in ultraviolet light. An advantage of this method is that urines can be examined without any previous chemical treatment. As quantitative elution from the paper chromatograms was difficult, the relationship between the spot area and amount of substances on paper chromatograms (Fisher, Parsons & Morrison, 1948) was investigated. Several authors have used this relationship to determine fatty acids (Reid & Lederer, 1951; Bryant & Overell, 1953), amino acids (Ohtsu & Mizuno, 1952) and indolyl-3-acetic acid (Bennet-Clark, Tambiah & Kefford, 1952). A method depending on spot areas similar to that used by Reid & Lederer (1951) for the estimation of fatty acids has been developed for the estimation of metabolites of naphthalene.

EXPERIMENTAL

Materials

1-Naphthol and 2-naphthol (A.R., British Drug Houses Ltd.) were each recrystallized from water before use. 1:2-Dihydronaphthalene-1:2-diol (m.p. 126°) was isolated from the urine of rats dosed with naphthalene by the method of Booth & Boyland (1949). 1-Naphthylsulphuric acid (potassium salt) was prepared by the method of Burkhardt & Lapworth (1926). 1-Naphthylglucosiduronic acid was

isolated from the urine of rats dosed with 1-naphthol by the method of Berenbom & Young (1951).

1:2-Dihydro-2-hydroxy-1-naphthylglucosiduronic acid. A powder containing 80% of the barium salt of 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid was isolated from the urine of rats dosed with naphthalene. During the isolation of the triacetylmethyl ester of 1:2-dihydro-1-naphthylglucosiduronic acid Boyland & Solomon (1955) eluted 1:2-dihydro-2-hydroxynaphthylglucosiduronic acid from a charcoal column containing adsorbed metabolites of naphthalene with 50% aqueous acetone. The concentrated eluate was purified on a cellulose-powder partition column from which it was eluted with isopropanol-water (4:1). The fractions containing 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid were evaporated to low volume under reduced pressure, neutralized with Ba(OH)₂ solution and crystallized from 95% aqueous propan-2-ol. After a few days at 4° a solid separated which on recrystallization from 95% aqueous propan-2-ol yielded a white solid. Measurement of the light-absorption at 264 mμ. indicated that this powder consisted of the barium salt of 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid in 80% purity (based on ϵ_{max} value of 1:2-dihydronaphthalene-1:2-diol component). Some of the powder was incubated at 37° with 10 Sigma units/ml. of bacterial glucuronidase (Sigma) at pH 6.0 for 5 hr.; the aqueous solution was extracted with benzene and the extract dried (Na₂SO₄). Evaporation of the benzene to small volume and crystallization from a benzene-light petroleum (b.p. 60–80°) yielded crystals of (–)-1:2-dihydronaphthalene-1:2-diol, m.p. 126°, $[\alpha]_D^{25}$ –156° in ethanol (c, 0.5). The powder gave a strong naphtha-resorcinol reaction and a precipitate of BaSO₄ with dilute H₂SO₄. Paper chromatography (solvent I, Table 1) showed that no other known metabolites of naphthalene were present.

* Part 9: Booth, Boyland & Manson (1955).