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THE MAMMARY BLOOD FLOW IN THE COW AS MEASURED BY THE ANTIPYRINE ABSORPTION METHOD[1])

By

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In a previous study it has been shown (*Rasmussen* 1963) that the mammary blood flow in goats can be determined by measuring the disappearance of antipyrine by absorption from the udder after intramammary injection, and the arterio-venous difference of antipyrine across the gland. The blood flow of the mammary gland of lactating goats was found to vary between 20—90 ml/min. per 100 g tissue and in dry goats from 16—46 ml/min. per 100 g. The antipyrine absorption method has later been compared to the thermodilution method and direct blood flow measurement in both conscious and anaesthetized goats as well as in perfused glands (*Rasmussen & Linzell* 1964). In most cases the antipyrine absorption method gave results equal to those obtained by the other methods, in a few experiments, however, higher figures were obtained from the antipyrine method. In the present work the antipyrine absorption method has been adapted for mammary blood flow determination in cows, and the following is a record of a series of experiments on anaesthetized and conscious cows.

MATERIAL AND METHODS

The experimental animals were four red Danish cows (R.D.M.) and five Jersey cows. The cows yielded between 1 and 20 liters of milk per day. Two anaesthetized cows and two other cows

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were slaughtered after the experiments, and the udders were weighed. In the other cows the gland weights were valued from measurements of height and circumference. The vascular system was dissected in two of the slaughtered animals, especially the venous valvular sufficiency and anastomoses were investigated.

Determination of antipyrine in blood plasma and milk was performed by the method of *Brodie, Axelrod, Soberman & Levy* (1949).

The calculations of the blood flow were carried out as previously described for experiments on goats (*Rasmussen* 1963).

RESULTS

Absorption of Antipyrine from the Mammary Gland.

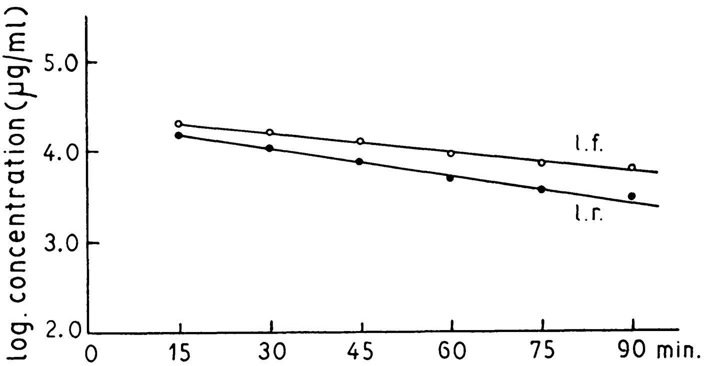
27 observations were made in seven cows in the standing position. The cows were milked and 3 g of antipyrine (in a few experiments 1 g) dissolved in 30 ml distilled water was injected into each quarter through the teat. After massage of the gland to distribute the antipyrine solution, 2—3 ml milk samples were drawn by milking every fifteen minutes for 1 to 1½ hour. After the last sampling an intravenous injection of 20 I.U. oxytocin was given, and the glands were emptied for the residual milk.

By analyses of the milk samples for antipyrine and recording the analytical figures in the semilogarithmic coordinate system rectilinear curves were always obtained. Fig. 1 shows the results from the two experiments on two glands on the same cow. It will be noticed that the slope of the curves is not the same for the two glands. This observation has been made in all the experiments, and the variations might be due to differences in the area of epithelium through which diffusion to the vascular system can take place (vide *Rasmussen* 1962, 1964, 1965).

The rectilinear half life curve means that the absorption of antipyrine proceeds as a first order reaction following the formula:

$$2.303 \log \left(\frac{N_t}{N_0} \right) = -\lambda t.$$

Therefore, when N_0 is known as the amount of antipyrine injected, and N_t as the amount withdrawn by emptying the gland, λ can be calculated. Then by means of N_0 and λ it is possible to calculate N_t at any time between injection and emptying.



F i g. 1. Decrease in concentration of antipyrine in milk after intramammary application in the left front gland and the left rear gland.
Ordinate: log. concentration μg/ml.
Abscissa: Time in minutes after administration.

Experiments on the Laterally Recumbent, Anaesthetized Cow.

Two cows were anaesthetized with chlorale hydrate and placed in lateral recumbency on the right side. Cannulae were inserted into the left jugular and the left subcutaneous abdominal vein. The left external pudic artery and vein were also cannulated superficial to the external orifice of the inguinal canal. The cannulae were closed tightly by metal stylets rinsed in heparine-saline (50 I.U. of heparine per ml).

At the beginning of the experiment 1 g antipyrine in 30 ml distilled water was injected into each teat, and the gland massaged. Fifteen min. later simultaneous samples of jugular, subcutaneous abdominal and pudic venous as well as pudic arterial blood were collected, and this was repeated every five minutes for a further 25—30 minutes. After the last sampling 20 I.U. oxytocin was injected intravenously, and the milk glands emptied by milking.

In Fig. 2 the concentrations of antipyrine in the blood samples are shown. It will be seen that the concentrations in the external pudic artery and the jugular vein are identical. The determination of the antipyrine concentration in jugular venous blood therefore reflects the concentration in the arterial in-flow to the udder.

The antipyrine concentration in blood from the subcutaneous abdominal vein and the external pudic vein were not identical but in both cases higher than the concentration in the jugular blood. This finding shows that in the lateral recumbent cow

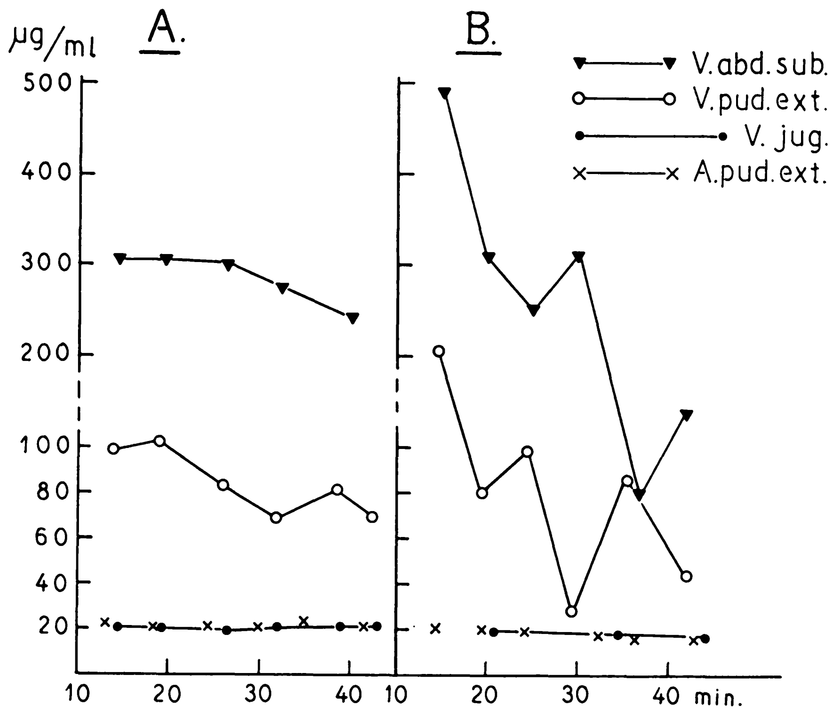


Fig. 2. Concentrations of antipyrine in blood from the subcutaneous abdominal vein, the external pudic vein, the jugular vein and the external pudic artery in two anaesthetized cows (*A*, *B*) in lateral recumbency.

Ordinate: Concentration in µg/ml.
Abscissa: Time in minutes after administration.

venous blood returns from the udder via both the subcutaneous abdominal and the external pudic veins.

As it is not known how the venous return under these circumstances is divided between the two veins on each side, it is not possible to calculate the blood flow by means of the antipyrine absorption method in lying cows.

Determination of Mammary Blood Flow in the Standing, Unanaesthetized Cow.

In the experiments on goats in the standing position the venous flow from the udder seems ordinarily to occur through the subcutaneous abdominal veins (*Linzell* 1960 a, *Rasmussen* 1963). This can be controlled by catheterization of both the

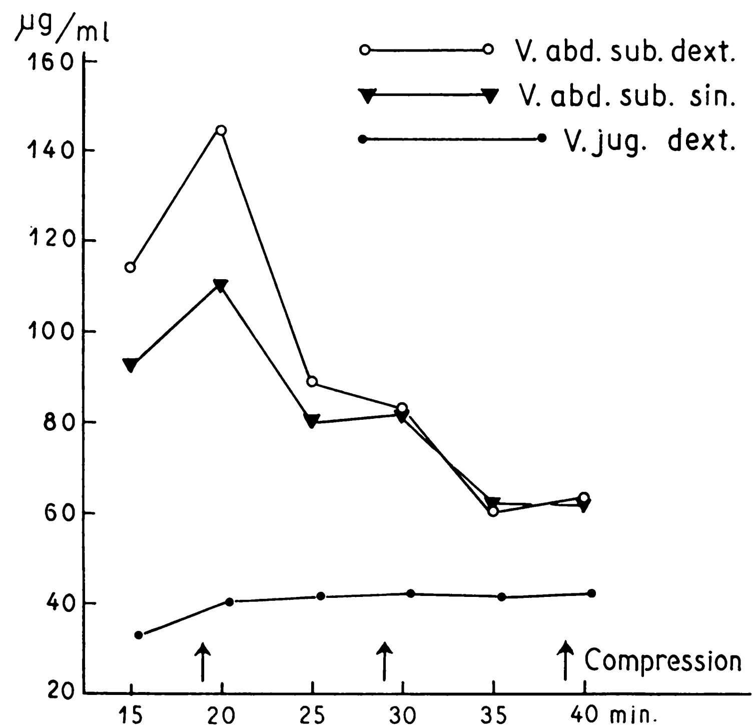


Fig. 3. Concentration of antipyrine in blood from the right and left subcutaneous abdominal veins and the right jugular vein in an unanaesthetized cow in standing position. The marks indicate manual compression of the external pudic veins.

Ordinate: Concentration in µg/ml.
Abscissa: Time in minutes after administration.

external pudic vein and the subcutaneous abdominal vein, a procedure which as for the external pudic vein unfortunately did not succeed as regularly in cows as in goats. It was therefore decided to exclude any possible flow through the external pudic veins in the standing cow by manual compression of the external pudic veins close to the external orifice of the inguinal canals, where the veins are rather easily located. Thereafter the experiment was carried out as follows: The right jugular and both subcutaneous abdominal veins were cannulated. The cow was milked, and 3 g antipyrine in 30 ml distilled water injected via the teat canal into all four glands. After massage of the quarters and a pause of about 15 minutes, blood samples were simultaneously drawn. This was repeated every five minutes in such a

Table 1. Calculation of the average blood flow through the left and right glands during 10 min. periods in a conscious cow (No. 1913).

Period No.	Lenght of period min.	Calculated amount of antipyrine in the gland Initial mg	Final mg	Absorbed antipyrine mg	Mean differences between concentrations of antipyrine in subc. abd.v. and jugul.v. μg/ml	Blood flow ml/min.
Left glands						
1 u.c.	10	2611	1505	1106	45	2460
3 u.c.	10	1505	913	592	28	2110
2 c.	10	1979	1174	805	52	1550
4 c.	10	1174	705	469	27	1740
Right glands						
1 u.c.	10	2441	1330	1111	59	1880
3 u.c.	10	1330	739	591	30	1970
2 c	10	1813	991	822	66	1250
4 c.	10	991	555	436	28	1560

u.c. = unclamped external pudic vein.
c. = clamped external pudic vein.

way that every other blood sample was taken during the last fifteen sec. of the one minute clamping of the external pudic veins. After about an hour the udder quarters were emptied separately by milking after intravenous injection of 20 I.U. of oxytocin.

The blood antipyrine levels from one experiment are shown in Fig. 3. The arrows indicate sampling while the external pudic veins were clamped. An increase in the concentration of antipyrine in the blood from the subcutaneous abdominal veins during manual compression of the pudic veins was noted and might be explained in one of the following two ways. It could indicate that a mammary venous outflow with a high concentration of antipyrine leaves the mammary gland through the external pudic veins. It could also indicate that blood from external pudic veins with a concentration of antipyrine equal to the concentration in the jugular vein flows back through the cranial mammary veins and diminishes the antipyrine content in the venous outflow in the subcutaneous abdominal vein. When there are two main routes for the venous outflow from the mammary glands it seems adequate only to use antipyrine concentration curves drawn after analyses of samples from the subcutaneous abdominal veins during clamping of the external pudic veins for the calculations

Table 2. Calculation of the blood flow through the left and right glands during one min. periods in a conscious cow (No. 1913).

Period No.	Calculated amount of antipyrine in the gland Initial mg	Calculated amount of antipyrine in the gland Final mg	Absorbed antipyrine mg	Mean difference between concentrations of antipyrine in subc. abd. v. and jug. v. µg/ml	Blood flow ml/min.
Left glands					
1 u.c.	2711	2567	144	53	2720
3 u.c.	1590	1510	80	36	2220
5 u.c.	946	893	53	19	2790
2 c.	2072	1966	106	68	1560
4 c.	1225	1164	61	36	1690
6 c.	735	697	38	18	2110
Right glands					
1 u.c.	2499	2355	144	74	1950
3 u.c.	1371	1291	80	43	1860
5 u.c.	754	710	44	17	2590
2 c.	1850	1741	109	95	1150
4 c.	1016	957	59	37	1600
6 c.	559	527	32	18	1780

u.c. = unclamped external pudic vein.
c. = clamped external pudic vein.

of the differences between the concentrations in blood from the subcutaneous abdominal veins and jugular vein to get representative differences between the antipyrine concentration in the mammary in- and outflow. The significance of this reflection appears from the blood flow calculations shown in Table 1. The amount of antipyrine absorbed in 10 min. periods, as found from the half life curves as described before, is divided by the difference in antipyrine concentrations between the subcutaneous abdominal vein and the jugular vein in periods when the pudic veins are clamped or not respectively. It is seen that the clamping results in lower figures for the blood flow. As the weight of the two left glands was 5 kg and the two rights 4 kg, the blood flow without clamping was 42—49 ml/min./100 g tissue for the left half, and 47—49 ml/min./100 g for the right. During clamping the figures were left 31—35 and right 31—39 ml/min./100 g.

When the results from the same experiment instead of average blood flow in 10 min. periods are calculated for one minute periods, the blood flow figures come out as in Table 2. Besides

Table 3. Blood flow through the udder-halves in seven conscious cows with clamped external pudic veins.

Cow No.	Udder-half weight kg	Daily milk yield per udder-half l	Length of period min.	Absorbed anti-pyrine mg	Mean difference between concentrations of anti-pyrine in subc. abd. v. and jug. v. μg/ml	Blood flow ml/min total	Blood flow ml/min per 100 g
1	2.1	0.5	5.5	253	90	510	24
			5	160	53	600	29
			5	115	35	660	31
			5	75	33	460	22
			5	57	21	540	26
1913	5.0	2.0	10	805	52	1550	31
			10	469	27	1740	35
	4.0	2.0	10	824	66	1250	31
			10	436	28	1560	39
1912	3.5	2.3	10	874	89	980	28
			10	502	49	1020	28
	4.0	2.7	10	861	54	1590	40
			10	517	29	1780	45
2	6.5	9.0	4.5	260	13	4440	68
			5.5	220	8	5000	77
			5.0	120	5	4800	74
			5.0	87	4	4350	67
68	9.0	10.0	5.0	309	12	5150	57
			4.5	227	9	5610	62
			4.5	262	14	4160	46
			4.5	225	14	3570	40
80	9.0	9.0	5.0	259	10	5180	58
			5.5	184	7	4780	53
84	11.0	9.0	5.0	315	8	7880	72
			4.5	262	7	8320	76
			5.5	366	6	11100	101
			4.0	216	5	10800	98

the influence of the clamping a somewhat greater variation is evident.

Table 3 summarizes the results obtained on seven cows during clamping of the external pudic vein calculated for the two udder-halves. The data presented show that the mammary blood flow in lactating cows varies from 22—101 ml/min./100 g glandular tissue.

Blood Flow Determination Using Two Glands.

It might be possible, and also convenient for simultaneous investigations of the mammary function, only to use two of the quarters for blood flow determinations. Therefore, in experiments on two Jersey cows an additional series of observations have been made.

1. *Antipyrine injected in two glands on one side.*

3 g antipyrine in 30 ml distilled water was injected into the right glands, and blood samples were drawn from the jugular vein and both subcutaneous abdominal veins. The results from one of the experiments are shown in Table 4. The concentration

Table 4. Concentrations of antipyrine in the jugular vein and the subcutaneous abdominal veins after injection of antipyrine in the two right glands.

Sample No.	Jugular vein μg/ml	Left subc. abd. vein μg/ml	Right subc. abd. vein μg/ml
1	48	63	343
2	45	82	183
3	45	62	163
4	46	58	102
5	45	53	106
6	44	50	71

of antipyrine in the left subcutaneous abdominal vein, draining the non-injected udder-half was somewhat higher than the concentration in the jugular vein, but much lower than the concentration in the blood in the subcutaneous abdominal vein from the injected glands. As the milk from the non-injected glands contained antipyrine in a concentration equal to that in the jugular blood this finding means, that venous anastomoses lead blood from the right side of the udder to the left subcutaneous abdominal vein. A reliable determination of the blood flow by this technique would therefore be difficult to obtain.

2. *Antipyrine injected in two front glands or two rear glands.*

After injection of 6 g antipyrine dissolved in 60 ml distilled wated in each of the two front glands or in other experiments in the rear glands, and collection of blood from one jugular vein and both subcutaneous abdominal veins the calculations of the average blood flow gave values as shown in Table 5. As for cow

Table 5. Blood flow through the udder-halves in two conscious cows with clamped external pudic veins after injection of antipyrine in all four, two front or two rear glands.

Antipyrine injected in		Four glands	Two front glands	Two rear glands
Cow No.	Udder-half	Blood flow ml/min.		
1912	left	1000	950	950
	right	1700	900	2000
1913	left	1650	1200	1900
	right	1400	1750	2750

no. 1912 the blood flow determination gave the same result for the left udder-half irrespective of whether the injection was in all four glands, the two front, or the two rear glands. On the right side, however, injection in the front glands resulted in a figure less than half of what was calculated from the other procedures. This flow calculated on the right side from the experiment on the two front glands was quite close to the flow found in the left udder-half. The discrepancy between the values calculated for the right side after injection in the front glands and in four glands or the two rear glands indicated a study of the blood vessels of the udder after slaughtering of the cow. It was found that the valves in the external pudic veins were sufficient as measured by water pressure. On the right side the external pudic vein which had a circumference of 16 mm was doubled by a vein with a circumference of 7 mm. This vein left the cranial mammary vein 2 cm anterior to the head branch. Besides the right perineal vein was twice as big as the left. This variation in the anatomy of the venous system on the right and left side might be responsible for blood flow from the right rear gland through these secondary veins which were not clamped during the experiment. After injection of antipyrine in the right rear gland the concentration of antipyrine in the blood flowing through these veins will be high and the blood samples drawn in the subcutaneous abdominal vein will not be representative. The concentration of antipyrine is too low and therefore the blood flow will be estimated too high.

As indicated in Table 5 there are greater variations in the blood flow estimations on the left side in cow no. 1913 than cow no. 1912. The blood flow through the right udder-half of cow no. 1913 calculated after injection the four glands and the front glands is of the same size as the flow through the left glands. After injection of antipyrine in the rear glands the flow through the right side is twice as high as the flow calculated earlier. By dissection of the udder veins of cow no. 1913 it was found that the valves in the external pudic veins were sufficient. Furthermore, the right subcutaneous abdominal vein was double branched and both the perineal veins and the caudal anastomoses were small, while the cranial anastomosis was large. This anastomosis arose from the left cranial mammary vein and passed through the left front gland. At the anterior border of this gland the vein divided into two equal branches, which joined the two subcutaneous abdominal veins. Through this anastomose a part of the blood from the left udder-half, specially the front gland, can flow to the right subcutaneous abdominal vein. This anastomose together with the double branched right subcutaneous abdominal vein might be responsible for the calculated high blood flow through the right udder-half when antipyrine is injected in the rear glands.

DISCUSSION

As in the goat the absorption of antipyrine from the udder in cows proceeds a first order reaction, and the principle of using antipyrine absorption for determination of the mammary blood flow in the cow is valid. Moreover, as in the goat, the antipyrine concentration in the jugular vein is equal to that in the external pudic artery, so the determination of the antipyrine in samples drawn from the jugular veins reflect the concentration of the test substance in the arterial mammary inflow.

The difficulties in using the method in cows are brought about by very great variations in the venous part of the circulatory system of the udder. As described by a.o. *Becker & Arnold* (1942), *Sweett & Matthews* (1949), *Ziegler & Mosimann* (1960) not only the anastomoses vary in number, position and size, but also the valvular pattern is different from one animal to another. The venous outflow therefore seems not to be as regular in the cow as it is found in the goat (*Linzell* 1960 a, *Rasmussen* 1963), and misinterpretations might result from determinations based on blood samples drawn exclusively from the subcutaneous abdominal veins. An error as flow through the external pudic veins might be corrected by manual compression of these vessels and the influence of variations in the anastomoses between the udder-halves can be diminished by injection of antipyrine solution into all four glands. By this procedure the whole venous system will contain a certain antipyrine concentration and dilution will not take place to a serious extent. By taking these precautions a blood flow of 22—101 ml/min./100 g gland tissue has been found in lactating cows, which is higher than the values found in the perfused udder-half (*Tindal* 1957) but just of the same order or a little higher than found in the goat (*Linzell* 1957, 1960 b, *Reynolds* 1964, *Rasmussen* 1963, *Rasmussen & Linzell* 1964).

From the two experimental animals, discussed in connection with the results given in Table 5, it is learned that even after injection in the four glands the method can give too high figures due to the shape of the venous system. However, it might be justified to conclude from these experiments that it is possible by preliminary testing to select cows suitable for physiological experiments where estimations of the blood flow are needed. If the procedure described — injection in all four glands, in the two front, in the rear glands, and in the two glands on each side — gives uniform results, the cow seems to be well-suited for such studies. Moreover, the uniformity of the results should allow the use of only two glands for blood flow determination and spare the two others for any other study of the milk secretion.

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SUMMARY

After intramammary application of 3 g antipyrine dissolved in 30 ml distilled water into each quarter the absorption of antipyrine from the udder proceeds as a first order reaction. As the injected amount is known as well as the amount of antipyrine milked out about 1 hour later can be determined, it is possible to calculate the amount absorbed at any time between injection and emptying.

It is shown, that the concentrations of antipyrine in the blood from the jugular vein and external pudic artery are identical after intramammary application of antipyrine. In experiments on lateral recumbent cows it is shown that the venous blood returns from the udder via both the subcutaneous abdominal and the external pudic veins. In the standing cows blood samples were drawn from the jugular and the subcutaneous abdominal veins. The blood samples from the subcutaneous abdominal veins were drawn during manual compression of the external pudic veins to get representative concentrations of antipyrine in the total venous blood from the udder. On account of the amount of antipyrine absorbed and the difference in antipyrine concentrations between the subcutaneous abdominal veins and the jugular vein the mammary blood flow in lactating cows was found to vary between 22—101 ml/min. per 100 g gland tissue.

The possibility of calculating the mammary blood flow after injection in two glands only — while the two remaining glands might be used for other studies — is shown and discussed. The influence of the individual differences in the venous anastomoses on the results is discussed, and a procedure is described to select cows suitable for experiments on mammary blood flow.

ZUSAMMENFASSUNG

Die mammäre Durchblutung beim Rind gemessen mit Hilfe der Antipyrin-Absorptionsmethode.

Nach intramammärer Applikation in jedes Euterviertel von 3 g Antipyrin gelöst in 30 ml dest. Wasser verläuft die Absorption von Antipyrin von dem Euter wie eine Reaktion ersten Ordens. Weil die injizierte Menge bekannt ist, und die restliche Menge, die beim Ausmelken etwa eine Stunde später entfernt wird, bestimmt werden kann, ist es möglich die absorbierte Antipyrinmenge zu berechnen.

Es wurde nachgewiesen, dass die Konzentration von Antipyrin im Blut der V. jugularis und der A. pudendae ext. nach intramammärer Applikation von Antipyrin identisch ist. Bei Versuchen mit liegenden Kühen konnte gezeigt werden, dass der venöse Ablauf vom Euter durch Vv. abdominales ext. und Vv. pudendae ext. erfolgt. Stehenden Kühen wurden Blutproben von V. jugularis und Vv. abdominales ext. entnommen. Die Blutproben von Vv. abdominales ext. wurden bei manueller Kompression von Vv. pudendae ext. entnommen um zu sichern, dass der Antipyringehalt im Blut der Vv. abdominales den totalen venösen Ablauf von den Milchdrüsen repräsentierte. Auf Grund der berechneten Antipyrinmenge, die von der Milchdrüse absorbiert wurde, und der gemessenen Antipyrinmenge im Blut von Vv. abdominales ext. und V. jugularis wurde die Durchblutungsgeschwindigkeit bei laktierenden Kühen zu 22—101 ml/Min. pro 100 g Milchdrüsengewebe berechnet.

Die Möglichkeit die mammäre Durchblutung nach intramammärer Applikation von Antipyrin in zwei Drüsen zu bestimmen, während die übrigen Drüsen für andere physiologische Studienzwecke benutzt werden, ist untersucht und diskutiert worden. Die Bedeutung der individuellen Unterschiede in den venösen Anastomosen für die Ergebnisse wird ebenfalls diskutiert und ein Verfahren zur Auslese geeigneter Versuchstiere für Bestimmungen der mammären Durchblutung wird erwähnt.

RESUMÉ

Den mammære blodgennemstrømning hos ko målt ved antipyrin absorptionsmetoden.

Efter intramammær applikation af 3 g antipyrin opløst i 30 ml destilleret vand i hver yverfjerdedel forløber absorptionen af antipyrin fra yveret som en reaktion af første orden. Da den injicerede mængde kendes, og den resterende, der fjernes ved udmalkning ca. 1 time senere, kan bestemmes, er det muligt at beregne den absorberede mængde antipyrin.

Det er vist, at koncentrationen af antipyrin i blod fra V. jugularis og A. pudenda ext. er identisk efter intramammær applikation af antipyrin. I forsøg på liggende køer er det vist, at det venøse afløb fra yveret foregår gennem Vv. abdominales ext. og Vv. pudendae ext. På stående køer er der udtaget blodprøver fra V. jugularis og Vv. abdominales externae. Blodprøverne fra Vv. abdominales externae er udtaget under manuel kompression af Vv. pudendae ext. for at sikre, at indholdet af antipyrin i blodet fra Vv. abdominales ext. er repræsentativt for det totale venøse afløb fra mælkekirtelen. På grundlag af den beregnede mængde af antipyrin absorberet fra mælkekirtelen og den målte koncentrationsdifferens af antipyrin i blod fra Vv. abdominales ext. og V. jugularis er blodgennemstrømningen hos lakterende køer beregnet til 22—101 ml/min. pr. 100 g mælkekirtelvæv.

Muligheden for at bestemme den mammære blodgennemstrømning efter intramammær applikation af antipyrin i to kirtler, mens de øvrige kirtler anvendes til andre fysiologiske studier, er undersøgt og diskuteret. Ligeledes diskuteres betydningen af de individuelle forskelle i venøse anastomoser for resultaterne, og der angives en fremgangsmåde til udpegning af egnede forsøgsdyr for måling af den mammære blodgennemstrømning.

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