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From the Department of Pharmacology and Toxicology, The Royal Veterinary and Agricultural College, Copenhagen.

# THE MAMMARY BLOOD FLOW IN THE GOAT AS MEASURED BY ANTIPYRINE ABSORPTION[1)]

By

*Folke Rasmussen*

Investigations of the absorption of various drugs from the mammary gland have demonstrated a rapid diffusion of antipyrine across the glandular epithelium (*Rasmussen* 1962). These studies also showed that the disappearance of antipyrine proceeds as a first order reaction. When the amount ($N_0$) of intramammary administered antipyrine is known, and the amount ($N_t$) in the milk withdrawn after a period of t minutes is measured, it is possible to calculate the slope ($\lambda$) of the absorption curve from the formula $2.303 \log \left(\frac{N_t}{N_0}\right) = -\lambda t$. From the slope of the curve and the initial amount $N_0$ it is possible to calculate N at any given time between the administration and the milking.

Because of the rapid absorption of antipyrine relatively large concentration differences should obtain between the blood flowing into and from the mammary gland. If the concentration of the drug in blood samples withdrawn from the afferent and efferent mammary vessels is measured it is possible to calculate the venous outflow from the arterio — venous difference and the amount of antipyrine absorbed from the gland.

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The following is a record of a series of preliminary experiments using this method on goats.

## EXPERIMENTAL MATERIAL AND METHODS

The experimental animals were five multiparous goats two of which were non-lactating and the rest were giving between 700 and 2000 ml of milk per day.

In the acute experiments the animals were anesthetized with sodium pentobarbital and placed in lateral recumbency. Cannulae were placed in the jugular and subcutaneous abdominal veins. The external pudic artery and vein were also cannulated superficial to the external orifice of the inguinal canal. In some of the experimental animals 3 days prior to the experiments plastic catheters were inserted into the external pudic vein and the subcutaneous abdominal vein through cannulae. The catheters were directed to the craniodorsal part of the udder and sutured to the abdominal skin. The cannulae and catheters were closed very tightly by metal stylets. To prevent clotting the catheters were rinsed at least once a day with heparin-saline (50 i.u. of heparin per ml) and this same solution was also injected into the catheters after each sampling. It was possible to withdraw blood samples through these catheters 4—10 days after the operation and to carry out the experiments on unanesthetized animals in standing position. Two to three days after the operation the daily milk yield was the same as before the operation.

At the beginning of the experiment a solution containing 1 g of antipyrine and 200 mg of urea dissolved in 30 ml of distilled water was administered into each teat and the gland massaged to distribute the solution. After 10—20 minutes blood samples were withdrawn at intervals of about 5 minutes. Immediately after withdrawal of the last blood sample 5 i.u. oxytocin was injected intravenously and the mammary glands were milked out completely.

The content of antipyrine in milk and blood plasma was measured by the precipitation method of *Brodie, Axelrod, Soberman & Levy* (1949). The blood concentration was calculated from the plasma content.

When the animal was killed the glands were weighed directly. Otherwise the gland weight was calculated by the water displacement method of *Linzell* (1960 b).

## RESULTS

*Antipyrine Concentrations in the External Pudic Artery and the Jugular Vein.*

Since the procedure might be simplified considerably if the antipyrine concentration in the external pudic artery was identical with the concentration in the jugular vein, preliminary experiments with simultaneous blood sampling from these two vessels were carried out. The results from four experiments are presented in Table 1. The concentrations agreed so satisfactorily that a future omission of catheterization of the external pudic artery was believed to be justified. The antipyrine concentration of the blood flowing into the udder could thus be measured in blood samples from the jugular vein.

Table 1. Concentrations of antipyrine in simultaneously drawn blood samples from the external pudic artery and the jugular vein.

| Experiment no. 1 | | | Experiment no. 2 | | | Experiment no. 3 | | | Experiment no. 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample no. | v. jugul. μg/ml | a. pud. ext. μg/ml | Sample no. | v. jugul. μg/ml | a. pud. ext. μg/ml | Sample no. | v. jugul. μg/ml | a. pud. ext. μg/ml | Sample no. | v. jugul. μg/ml | a. pud. ext. μg/ml |
| 1 | 62.6 | 63.0 | 1 | 75.0 | 75.0 | 1 | 68.8 | 76.0 | 1 | 80.0 | 83.0 |
| 2 | 61.6 | 61.6 | 2 | 70.0 | 71.4 | 2 | 73.4 | 74.2 | 2 | 66.4 | 70.6 |
| 3 | 66.0 | 61.6 | 3 | 68.4 | 69.8 | 3 | 72.2 | 70.0 | 3 | 56.0 | 61.0 |
| 4 | 61.6 | 58.0 | 4 | 66.6 | 68.2 | 4 | 68.8 | 68.0 | 4 | 47.0 | 46.0 |
| 5 | 52.0 | 53.5 | 5 | 72.0 | 68.2 | 5 | 66.4 | 59.4 | 5 | 41.0 | 40.0 |
| | | | 6 | 70.0 | 71.2 | 6 | 61.0 | 57.8 | 6 | 38.0 | 35.4 |
| | | | 7 | 67.8 | 67.8 | 7 | 56.0 | 55.4 | | | |

*Experiments on the Laterally Recumbent, Anesthetized Goat.*

This series consisted of 6 experiments on three goats with a total of 28 separate observations.

In all experiments the differences in the antipyrine concentrations of blood were found to be similar to those seen in Figure 1 which is a record of a single experiment. Blood samples were withdrawn simultaneously from the left jugular vein, the left external pudic vein, and the left subcutaneous abdominal vein. The antipyrine concentrations in blood from the external pudic and subcutaneous abdominal vein were not identical but these concentrations were always higher than those found in the jugular blood. This finding shows that in the lateral recumbent

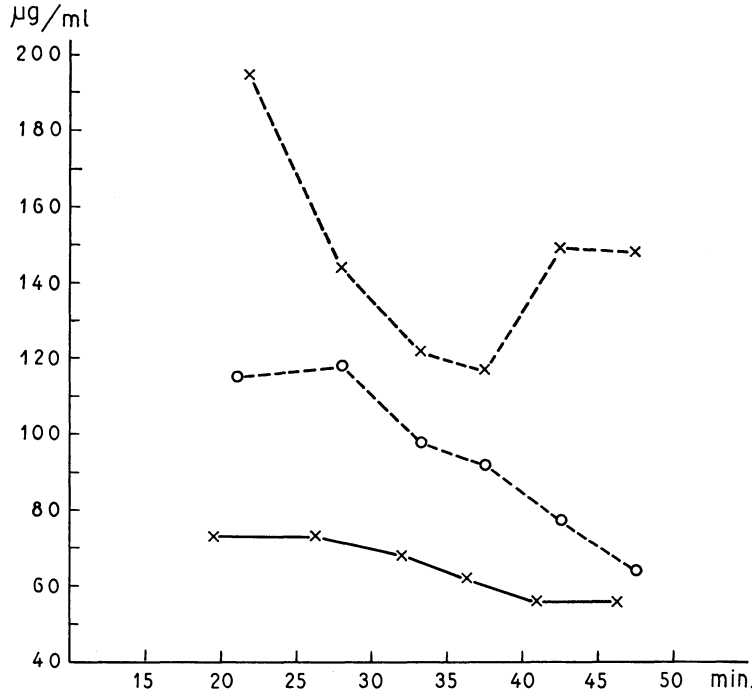


Fig. 1. Concentrations of antipyrine in blood from an anaesthetized goat in lateral recumbency. Goat No. 9.
x———x Left jugular vein.
x------x Left subcutaneous abdominal vein.
o------o Left external pudic vein.
Ordinate: Concentration in µg/ml.
Abscissa: Time in minutes after administration.

goat venous blood returns from the mammary gland via both the subcutaneous abdominal vein and the external pudic vein, which is in full agreement with the observations of *Linzell* (1960 a). However, as it is unknown how the venous return under these circumstances is divided between the two vessels measurement of the antipyrine absorption is not applicable for calculations of the mammary blood flow under these conditions.

*Experiments on the Standing, Unanesthetized Goat.*

In 21 observations from 5 experiments on 5 goats in the standing position it was found that the antipyrine concentrations in the jugular vein and the external pudic vein were identical,

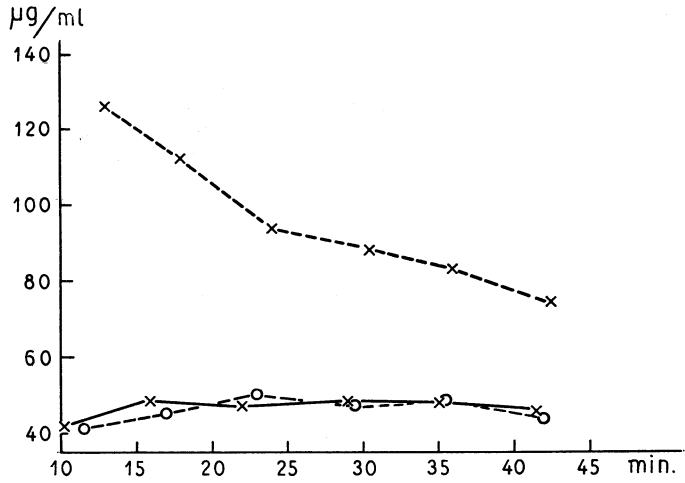


Fig. 2. Concentrations of antipyrine in blood from an unanesthetized goat in standing position. Same goat as in Fig. 1.
x———x Left jugular vein.
x------x Left subcutaneous abdominal vein.
o------o Left external pudic vein.
Ordinate: Concentration in µg/ml.
Abscissa: Time in minutes after administration.

while the concentration in blood samples withdrawn simultoneously from the subcutaneous abdominal vein was always higher. The venous return from the mammary gland of goats in the standing position thus seems, as shown by *Linzell* (1960 a), to occur exclusively through the subcutaneous abdominal vein. Consequently, it should be possible to calculate the mammary blood flow on the basis of antipyrine absorption from the mammary gland.

As an illustration of this all analytical results and calculations from one experiment are recorded in Figure 2 and Table 2. The experimental animal had a daily milk yield of 800 ml and an udder weight of 700 g. Plastic catheters were inserted, prior to the experiment, in the left external pudic vein and the left subcutaneous abdominal vein. At the beginning of the experiment a cannula with stylet was inserted in the left jugular vein. At 10:15 a.m. one g of antipyrine + 200 mg of urea in 30 ml distilled water was administered into each gland. Between 10:25 and 10:58 six blood samples were withdrawn from each vein at intervals of 5—7 minutes. Oxytocin (5 i.u.) was then injected intra-

Table 2. Calculation of the blood flow from one gland in a conscious goat.

| Period no. | Length of period min | Calculated amount of antipyrine in the gland | | Absorbed antipyrine mg | Mean difference between concentrations of antipyrine in abdom. subc. v. and jugul. v. μg/ml | Blood flow ml/min | |
|---|---|---|---|---|---|---|---|
| | | Initial mg | Final mg | | | total | per 100 g |
| 1 | 5.0 | 631 | 529 | 102 | 74 | 276 | 79 |
| 2 | 6.0 | 529 | 428 | 101 | 55 | 306 | 87 |
| 3 | 6.5 | 428 | 340 | 88 | 43 | 315 | 90 |
| 4 | 5.5 | 340 | 280 | 60 | 38 | 287 | 82 |
| 5 | 7.0 | 280 | 218 | 62 | 33 | 268 | 77 |

venously and at 11:03 each udder half was milked out separately. Antipyrine analyses of the milk (52 ml) from the left gland showed a concentration of 3570 μg/ml corresponding to a total of 186 mg. In the milk from the right gland the total amount was 180 mg.

In the before mentioned slope formula substitution of 186 mg for $N_t$, 1000 mg for $N_o$, and 48 minutes for t gave the value $\lambda = 0.0354$. The amount of antipyrine remaining in the mammary gland at the time each blood sample was taken could then be calculated from the same formula. This calculation gave following results: 10:28, 631 mg; 10:33, 529 mg; 10:39, 428 mg; 10:45, 340 mg; 10:51, 280 mg; and 10:58, 218 mg.

Figure 2 shows that the blood antipyrine concentrations in the jugular vein and the external pudic vein are very nearly identical, while the concentration in the subcutaneous abdominal vein is considerable higher.

The duration of each experimental period and the amount of antipyrine absorbed during the periods between blood samplings are shown in Table 2 together with the average differences between the antipyrine concentrations in the subcutaneous abdominal vein and in the jugular vein for each period. By means of these values the venous blood flow per minute was calculated, the flow being equal to the amount of antipyrine absorbed divided by the difference in blood concentration and the time in minutes. The results are given in the next to the last column of the table. The last column represents the venous flow per 100 g of mammary tissue. Similarly, Table 3 summarizes the rest of the results obtained in this series. It is evident that the blood flow varies from one period to another. The material presented shows that

Table 3. Blood flow from the left mammary gland in four conscious goats.

| Gland weight g | Daily milk yield ml | Period no. | Length of period min | Absorbed antipyrine mg | Mean difference between concentrations of antipyrine in abdom. subc. v. and jugul v. µg/ml | Blood flow ml/min | |
|---|---|---|---|---|---|---|---|
| | | | | | | total | per 100 g |
| 100 | dry | 1 | 5.5 | 171 | 1396 | 22 | 22 |
| | | 2 | 5.5 | 102 | 1065 | 17 | 17 |
| | | 3 | 7.5 | 96 | 789 | 16 | 16 |
| 125 | dry | 1 | 6.0 | 105 | 422 | 41 | 33 |
| | | 2 | 5.0 | 65 | 393 | 33 | 26 |
| | | 3 | 5.0 | 50 | 294 | 34 | 27 |
| | | 4 | 5.0 | 38 | 173 | 44 | 35 |
| | | 5 | 4.5 | 27 | 104 | 58 | 46 |
| 200 | 350 | 1 | 8.0 | 114 | 343 | 42 | 21 |
| | | 2 | 4.0 | 48 | 197 | 61 | 31 |
| | | 3 | 4.0 | 26 | 167 | 40 | 20 |
| | | 4 | 5.0 | 33 | 132 | 50 | 25 |
| | | 5 | 3.0 | 16 | 129 | 41 | 21 |
| 300 | 400 | 1 | 6.0 | 149 | 275 | 90 | 30 |
| | | 2 | 5.0 | 96 | 210 | 92 | 31 |
| | | 3 | 4.5 | 70 | 162 | 96 | 32 |

the blood flow of lactating goat udders varies from 20—90 ml/min. per 100 g glandular tissue and in dry, non-pregnant goat udders from 16—46 ml/min. per 100 g.

## DISCUSSION

The very rapid absorption of antipyrine from the mammary gland together with the fact that this drug is distributed evenly in body water (*Soberman, Brodie, Levy, Axelrod, Hollander & Steele* 1949) make it well suited for the investigation of the mammary blood flow.

The demonstrated occurrence of identical antipyrine concentrations in the jugular vein and the external pudic artery under the conditions of this experimental series is of great value as it allows the determination of the antipyrine concentration in the afferent mammary vessels without compromising the blood supply by withdrawal of samples from the artery.

The measurement of the antipyrine concentrations of blood samples simultaneously collected from the jugular, external pudic and subcutaneous abdominal veins (Figs. 1 and 2) show that the mammary venous outflow occurs through both the sub-

cutaneous abdominal vein and the external pudic vein when the goat is in lateral recumbancy. However, the venous return takes place solely through the milk vein when the animals is in the standing position. This is in full agreement with the results which *Linzell* (1960 a) obtained by radiograms and extensive anatomical studies.

The results obtained for the mammary blood flow of dry goats correspond perfectly with the results which *Linzell* (1960 b) obtained by the thermodilution method in conscious animals. However the results from the conscious lactating animals in the present study vary from 20—90 ml/min. per 100 g tissue, while the values in *Linzell*'s studies (1957, 1960 b) vary only from 20—70 ml/min. per 100 g. *Reynolds* (1962) arrived at similar results (20—41 ml/min. per 100 g) by the nitrous oxide diffusion method in anaesthetized goats. The minimum values obtained by the three different methods are thus identical, while the maximum values in part of the experimental periods of the present investigation are higher than those by *Linzell* (1960 b) and *Reynolds* (1962). The explanation for this variance may be that the perineal vein in the present experiments was patent, contrary to the method of *Linzell* (1960 b), so that blood from this vein may have passed through the external pudic vein to the milk vein. The abstract of *Reynolds* (1962) does not state the exact experimental procedure in this respect.

The possibility of errors arising from venous anastomoses between the udder halves was circumvented by the administration of antipyrine into both glands. Furthermore, pilot experiments showed that the administration of antipyrine into a single gland only resulted in concentration differences between the blood from the jugular vein and the ipsilateral milk vein. Part of the antipyrine absorbed from the mammary gland will be removed via the lymph, but since *Linzell* (1960 c) recorded maximal mammary lymph flows of only 0.04 ml/min. per 100 g of glandular tissue, this should have an insignificant effect on these blood flow measurements.

Finally it may be noted that a few preliminary investigations on unanesthetized cows in the standing position by means of the methods described here resulted in venous outflow values of 20—70 ml/min. per 100 g of mammary tissue.

## REFERENCES

*Brodie, B. B., J. Axelrod, R. Soberman & B. B. Levy:* The estimation of antipyrine in biological materials. J. Biol. Chem. 1949, *179*, 25—29.

*Linzell, J. L.:* The measurement of udder blood flow in the conscious goat. J. Physiol. 1957, *137*, 75 P.

*Linzell, J. L.:* Valvular incompetence in the venous drainage of the udder. J. Physiol. 1960 a, *153*, 481—491.

*Linzell, J. L.:* Mammary gland blood flow and oxygen, glucose and volatile fatty acid uptake in the conscious goat. J. Physiol. 1960 b, *153*, 492—509.

*Linzell, J. L.:* The flow and composition of mammary gland lymph. J. Physiol. 1960 c, *153*, 510—521.

*Rasmussen, F.:* Absorption of drugs from the mammary gland. 9th Nordic Veterinary Congress, 1962, Section G, No. 8.

*Reynolds, M.:* Mammary blood flow in lactating goats measured by nitrous oxide diffusion. Federation Proc. 1962, *21*, 102.

*Soberman, R., B. B. Brodie, B. B. Levy, J. Axelrod, V. Hollander & J. M. Steele:* The use of antipyrine in the measurement of total body water in man. J. Biol. Chem. 1949, *179*, 31—42.

## SUMMARY

The investigation has shown that the antipyrine concentrations in the external pudic artery and the jugular vein were identical after intramammary administration. In the standing unanesthetized goat the antipyrine concentrations in the jugular vein and the external pudic vein were identical, while the concentration in the subcutaneous abdominal vein was always higher. The blood from the mammary gland under these circumstances thus seems to flow exclusively through the subcutaneous abdominal vein, therefore the concentration difference between blood from the afferent and from the efferent mammary vessels can be determined from samples taken from the jugular and subcutaneous abdominal veins respectively. As the concomitantly absorbed amount of antipyrine can also be calculated, it is possible to compute the blood flow through the mammary gland. By use of this method it was shown that the blood flow of the udder of lactating goats varies between 20—90 ml/min. per 100 g mammary tissue and in dry goats from 16—46 ml/min. per 100 g tissue.

## ZUSAMMENFASSUNG

*Die Blutdurchströmung des Euters bei Ziegen, durch Absorption von Antipyrin gemessen.*

Die Versuche haben gezeigt, dass die Konzentration von Antipyrin in der A. pudenda ext, und in der V. jugularis nach intramammärer Applikation identisch war. Bei der stehenden, nicht anästhesierten Ziege war die Antipyrinkonzentration in der V. jugularis und V. pudenda ext. identisch, während sie in der V. abdominalis ext. stets höher war. Der Blutabfluss aus der Milchdrüse scheint somit beim stehenden

multiparen Tier ausschliesslich auf dem Wege der V. abdominalis ext. zu geschehen, und deshalb lässt sich die Konzentrationsdifferenz für Blut, das zur Drüse und von derselben fliesst, mit Hilfe von Blutproben aus der V. jugularis beziehungsweise aus der V. abdominalis externa bestimmen. Da man gleichzeitig die absorbierte Antipyrinmenge berechnen kann, ist es möglich, den venösen Abfluss aus der Milchdrüse zu bestimmen. Mit der beschriebenen Methode wurde geseigt, dass die Blutdurchströmung bei laktierenden Ziegen von 20—90 ml/Min. pro 100 g Milchdrüsengewebe und bei unfruchtbaren Ziegen von 16—46 ml/Min. pro 100 g Gewebe variiert.

RESUMÉ

*Den mammære blodgennemstrømning hos geder målt ved absorption af antipyrin.*

Forsøgene har vist, at koncentrationen af antipyrin i A. pudenda ext. og V. jugularis var identisk efter intramammær applikation. På stående, ikke anæsteseret ged var koncentrationen af antipyrin i V. jugularis og V. pudenda ext. identisk, mens den i V. abdominalis ext. altid var højere. Blodafløbet fra mælkekirtlen synes således på det stående multipare dyr udelukkende at foregå via V. abdominalis ext., og derfor kan koncentrationsdifferencen for blod, der løber til og fra kirtlen, bestemmes ved hjælp af blodprøver fra henholdsvis V. jugularis og V. abdominalis externa. Da man samtidig kan beregne den absorberede antipyrinmængde, er det muligt at bestemme det venøse afløb fra mælkekirtlen. Med den beskrevne metode er det vist, at blodgennemstrømningen hos lakterende geder varierer fra 20—90 ml/min per 100 g mælkekirtelvæv og hos golde geder fra 16—46 ml/min per 100 g væv.

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