J. Physiol. (1973), **233**, *pp.* 93–109
With 4 text-figures
Printed in Great Britain

THE TIME COURSE OF CARDIOVASCULAR CHANGES IN LACTATION IN THE RAT

By ANN HANWELL and J. L. LINZELL

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received 15 February 1973*)

SUMMARY

1. Cardiovascular changes in lactating rats have been traced from the first day post-partum to the end of the third week of lactation. The pattern of changes showed three phases.

2. Between days 1 and 5 of lactation there were sharp rises in both cardiac output and in the blood flow/g tissue for most organs, but little change in the distribution of the cardiac output.

3. Between days 5 and 15 of lactation cardiac output remained steady. The blood flow to tissues actively involved in the body's response to lactation (mammary glands, liver, gastrointestinal tract) also remained at high steady levels, but the blood flow to other tissues declined due to a redistribution of the cardiac output away from them and towards the growing mammary glands and splanchnic organs.

4. Between days 15 and 22 of lactation there were further rises in both cardiac output and in the blood flow/g tissue for most organs.

5. It is suggested that the increases in organ blood flows that occurred in the first few days after parturition (days 1–5) and at the end of lactation (days 15–22) were largely dependent on increases in cardiac output and may represent the maternal response to rapidly rising demands from the young at these times.

INTRODUCTION

Since lactating rats with large litters can produce large volumes of milk up to 45–78 g/day (Brody & Nisbet, 1938; Weihe, 1968; Hanwell & Linzell, 1972*a*), it is reasonable to suppose that lactation makes great demands upon the metabolism of the mother. Although a considerable amount of information has amassed on the cytological and biochemical changes that occur in the mammary gland during lactation (see Munford, 1964; Baldwin & Milligan, 1966), little is known as yet of the ways in which the rest of the body responds to the demands of milk production.

Recently, Chatwin, Linzell & Setchell (1969) showed that there are profound cardiovascular differences between lactating and non-lactating rats. The cardiac output is higher in the lactating rat and, in addition, certain tissues – the mammary glands, liver and gastrointestinal tract – receive a greater proportion of this output; the blood flow to these tissues is correspondingly increased. These results agree well with earlier reports of the dependence of milk secretion upon a high rate of mammary blood flow in goats (Linzell, 1960; Reynolds, 1965*a*) and of increased food consumption and growth of the liver and gastrointestinal tract in lactating animals (Cotes & Cross, 1954; Kennedy, Pearce & Parrott, 1958; Fell, Smith & Campbell, 1963).

Chatwin *et al.* (1969) also compared animals on the first and twelfth day of lactation and these results suggested that the cardiovascular differences between lactating and non-lactating animals develop in part during lactation. The present work represents a more detailed study of these changes, and an attempt is made to interpret the pattern of changes in relation to the increasing demands of the rapidly growing litter.

METHODS

Principles. Tissue blood flows were estimated by combining Fegler's (1954) thermodilution method for measuring cardiac output with Sapirstein's (1956, 1958) indicator fractionation method for determining the distribution of the cardiac output to the different tissues of the body (Chatwin *et al.* 1969). The principle of the indicator fractionation method is that, following rapid injection into the right atrium, an indicator is uniformly distributed in the arterial blood and is delivered to the tissues in proportion to their blood supply. If the indicator is a freely diffusible substance, it passes rapidly from the circulation into the tissues. Provided that it is extracted at an equal rate by all tissues, the proportion of the indicator within each organ, soon after injection, represents that organ's share of the cardiac output. Therefore if the circulation is stopped at this time, and the tissues quickly removed, the distribution of the cardiac output can be determined by measuring the amount of indicator in each organ. Equality of extraction is said to be demonstrated if the amount of indicator in each organ rises rapidly to a level that is maintained for several circulation times (Sapirstein, 1958).

It was decided, for several reasons, that ^{86}RbCl would be a more suitable indicator than the $^{3}H_2O$ used by Chatwin *et al.* (1969). Firstly, the analysis of tissue content of ^{86}Rb is considerably more rapid and less laborious than that of $^{3}H_2O$. Furthermore, Chatwin *et al.* (1969) reported that recovery of $^{3}H_2O$ was rather low (72 %) and it is probable that this represented some loss of indicator by evaporation and, unless corrected for, would lead to an underestimate of blood flow, especially in small organs. Finally, although $^{3}H_2O$ is taken up by all organs, its usefulness is limited because Chatwin *et al.* (1969) found that, in rats, several tissues (heart, kidneys, uterus and carcass) contained significantly less $^{3}H_2O$ when the animals were killed at 20 sec after injecting the isotope than when they were killed at 10 sec. In the present work the mean recovery of ^{86}Rb in twenty rats was $99{\cdot}3 \pm 2{\cdot}2$ % (S.E. of mean) of the amount injected. Furthermore, ^{86}Rb was shown to be a reliable indicator of blood flow for the mammary glands, liver, gastrointestinal tract, skin, carcass,

kidneys and uterus in both non-lactating and lactating rats by (i) establishing that the distribution of the isotope remained constant in different organs from 10 sec until at least 60 sec following I.V. injection, and (ii) showing that the distribution of the cardiac output recorded with ^{86}RbCl was very similar to that determined by an intracardiac injection of ^{169}Yb-labelled 25 μm microspheres (Minnesota Mining and Manufacturing Co.). ^{86}Rb is not an ideal indicator however, in that, owing to the 'blood–brain barrier', very little of the isotope is taken up by the brain and therefore brain blood flow is greatly underestimated (Sapirstein, 1958). Furthermore, we found that ^{86}Rb also understimates the fraction of the cardiac output delivered to the heart as determined with labelled microspheres, thus confirming the report of Mendell & Hollenberg (1971). However, data for the heart are given because the changes might reflect relative changes in coronary blood flow, if not absolute values of flow.

Animals. Wistar strain rats were housed under controlled conditions of temperature (20° C) and lighting (14 hr light, 10 hr dark). Water and M.R.C. Diet 41.B (Oxoid) were available *ad libitum*. Virgin females were placed in individual cages for pairing with males, and littered on average 22 days following the detection of a vaginal plug. The day when the litter was first observed was named day 1 of lactation. The litter was either removed altogether between 12 and 24 hr following parturition (non-lactating animals) or its size was adjusted to twelve pups within 1–2 days (lactating animals). In our rat colony the number of pups born per litter averages $11{\cdot}9 \pm 0{\cdot}15$ (S.E. of mean, 345 observations).

Ten groups of animals were used to study the development of cardiovascular changes during lactation and the rate at which these changes decay following parturition and only a brief period of suckling. Lactating animals were studied on days 1, 3, 5, 10, 15 and 22 of lactation and non-lactating parturient animals on days 3, 5, 10 and 15. There were six animals in each group except day-1 lactating (twelve) and day-15 non-lactating (seven). Twelve virgin animals were also studied.

In other animals, to determine the rate of growth of the young, the litter was weighed daily between 9.00 and 10.00 a.m. until day 22 of lactation.

Conduct of experiments. The animals were anaesthetized with sodium pentobarbitone (Abbott's Nembutal, 50 mg/kg body wt. I.P.). A polyvinyl catheter (0·8 mm o.d.) was inserted 3–3·5 cm into the right jugular vein to end in the right atrium and a 2 kΩ thermistor bead mounted at the tip of a stainless-steel tube (0·82 mm o.d.) was inserted approximately 3 cm into the left carotid artery to end in the arch of the aorta. A second 2 kΩ thermistor in the venous catheter near the rat recorded the temperature of injected solutions. Cardiac output was measured by injecting rapidly 0·2 ml. 0·9 % saline at room temperature into the right atrium and recording the passage of cooled blood from the left heart on a potentiometric recorder (Hanwell & Linzell, 1972*b*).

Since ^{86}Rb is incompletely extracted by the tissues (Sapirstein, 1958) some of the isotope remains in the blood at killing. Consequently the measured tissue contents represent less than their share of the cardiac output (see Delaney & Grim, 1964). Therefore a procedure was included to determine blood volume and thus to calculate the amount of isotope remaining in the blood. Each animal received 0·2 ml. I.V. of 0·25 % saline solution of Evan's Blue. Approximately 15 min and four to five determinations of cardiac output later, 10–90 μc ^{86}RbCl (Radiochemical Centre, Amersham) in 0·2–0·4 ml. isotonic saline were rapidly injected through the venous catheter, and the animal was killed 40 sec later by an I.V. injection of 0·5 ml. Nembutal. The organs were rapidly removed, blotted free of excess blood and placed in tared pots which were then sealed, reweighed and put aside for analysis. Spilled blood from the heart was quickly collected in a heparinized syringe for the determination of packed cell volume, plasma volume (Little & Williams, 1964) and isotope content. Standard solutions of ^{86}RbCl were prepared by injecting a duplicate

quantity of isotope through the same catheter into a 250 ml. volumetric flask and diluting to volume with water.

Analytical procedure. Weighed pieces of tissue were roughly chopped and the contents of each tared pot made up to constant volume (3 ml.) with water. The radioactivity was determined in a sodium iodide crystal, well-type scintillation counter. Large organs, e.g. carcass and skin, were minced and allowed to equilibrate with a known volume of water for several hours before taking aliquots in duplicate for counting. The digesta and faeces were removed from the stomach and intestines before these organs were weighed, and the isotope content of the digesta was measured and added to that of the tissue. Total hepatic blood flow was estimated by adding the isotope content of the liver (hepatic arterial flow) to that of the gastrointestinal tract and spleen (portal flow). The amount of isotope in the tissue was corrected for the portion of ^{86}Rb injectate that remained in the blood (usually 8–11 %); this was estimated from the blood volume and the concentration of ^{86}Rb in the blood.

RESULTS

The combination of Fegler's and Sapirstein's techniques provides the following data: cardiac output and the proportion of the cardiac output received by each organ. From these data, and from the weight of the different tissues, the blood flow to each organ (ml./min and ml./min.g tissue) may be calculated. However, it is useful to consider the distribution of the cardiac output to the different organs (fractional flow) together with their blood flows, as the former removes the cardiac output as a variable and thus gives an indication of the extent of local adjustment of blood flow in active tissues and of any compensatory changes in less active tissues. It also demonstrates the way in which growth of tissues affects the distribution of the circulation.

Fig. 1 illustrates changes in the cardiac output during lactation, while Figs. 2 and 3 illustrate changes in the blood flow (ml./min.100 g tissue), fractional flow (percentage distribution of the cardiac output) and weight (percentage of body weight) of different organs. The results are complicated and inter-related but three phases of lactation are distinguishable. The pattern is most clearly seen in the cardiac output (Fig. 1) and is characterized by two periods of increase (days 1–5 and days 15–22) and a stable intervening period (days 5–15). These three phases will be considered separately.

Day 1. On the first day of lactation cardiac output and organ blood flows were very variable and it was for this reason that the group size was doubled. This variation seemed to have at least two possible sources. First, the animals were used at widely different times during the first 24 hr post-partum (1·5–23·5 hr) and secondly, the number of pups per litter could not be controlled so that litter size also varied greatly (nine to eighteen pups). However, there was no obvious relationship between the cardiac output and the time following parturition (r = 0·108), whereas the

cardiac output was significantly related to the number of pups alive on day 1 ($r = 0{\cdot}610$, $P < 0{\cdot}05$). These results could be due to the number of pups carried *in utero* through pregnancy or to the number suckling immediately post-partum. However, it is probable that either delivery or suckling was involved since Linzell (1969) found no significant correlation between the cardiac output and the number of foetuses in rats on days 21 and 22 of pregnancy.

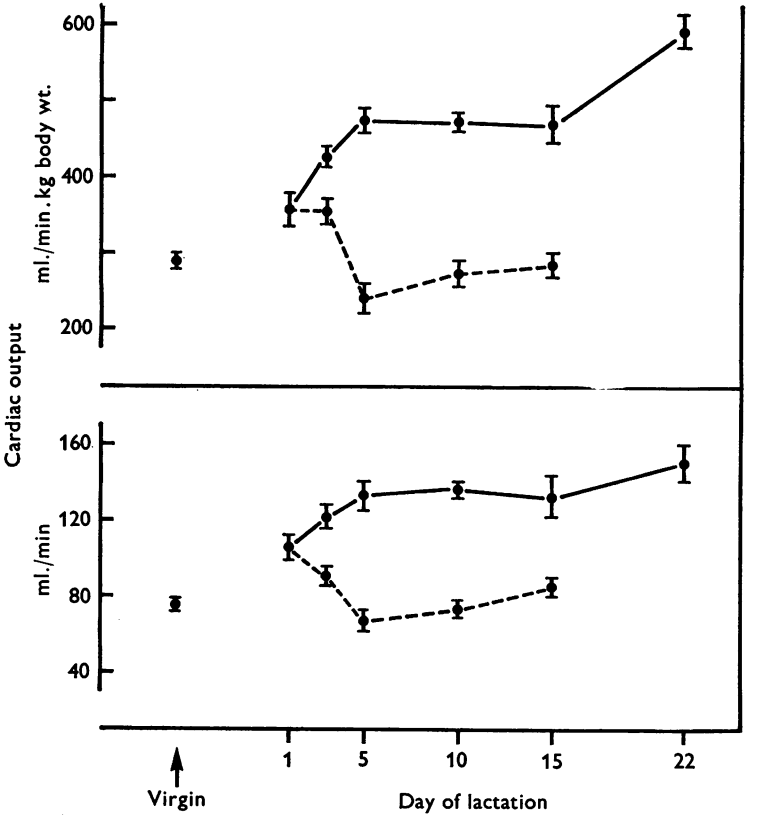

Fig. 1. Post-partum changes in the cardiac output of rats. The continuous lines represent lactating animals and the dashed lines represent non-lactating animals. Number of animals in each group given in text. Vertical bars represent 1 S.E. of mean each side of the mean.

For comparison with other stages, five animals with either small or large litters were rejected from this group so that it then consisted of seven animals that were nursing 12–14 pups each. Cardiac output in these animals was 24% higher than in virgin rats ($P < 0{\cdot}01$) (Fig. 1). The mammary glands and uterus had grown enormously during pregnancy (Figs. 2 and 3) and were now taking large proportions of this output. Furthermore,

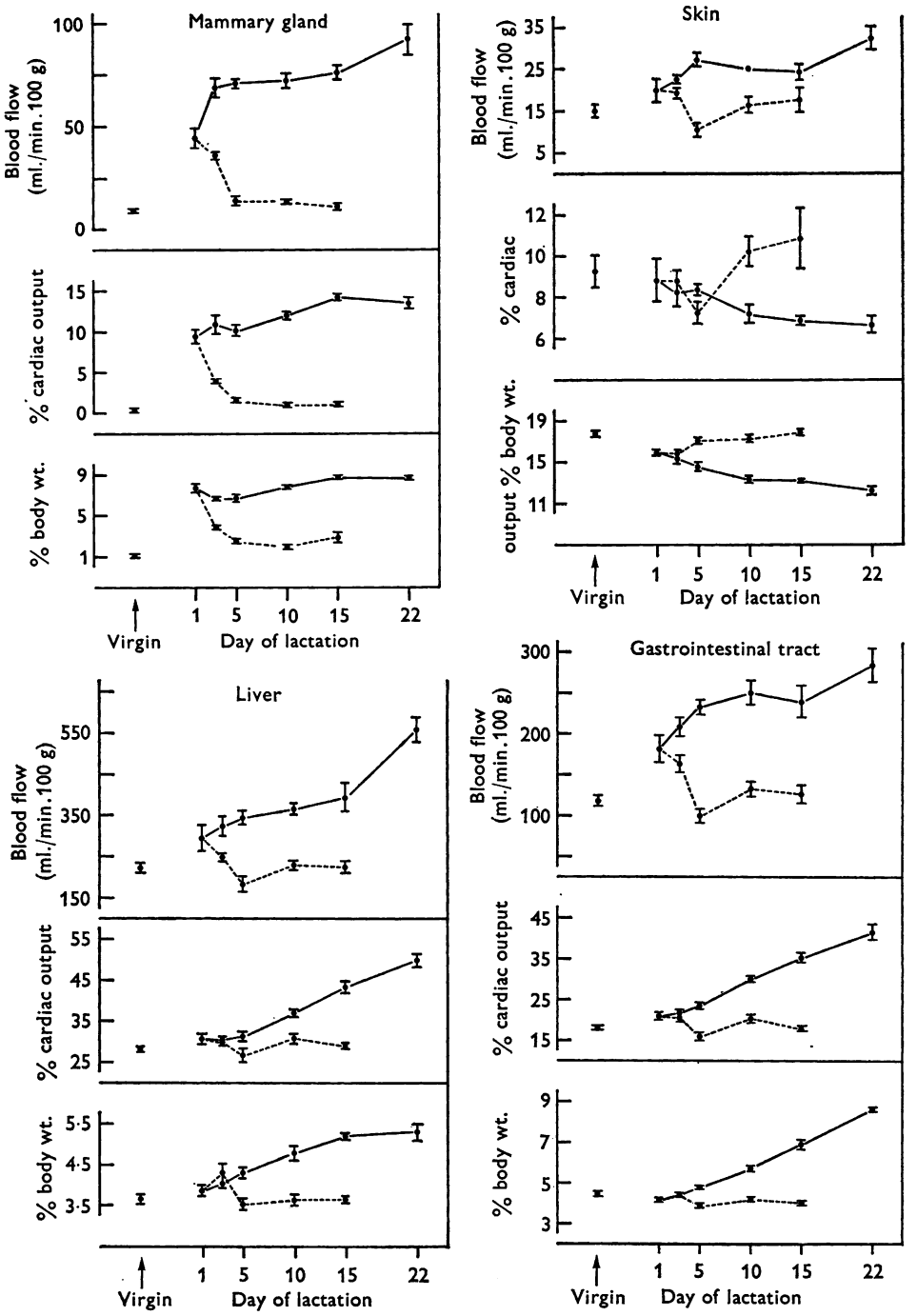

Fig. 2. Post-partum changes in the blood flow, fractional flow (% cardiac output) and weight of mammary glands, skin, liver and gastrointestinal tract. The continuous lines represent lactating animals and the dashed lines represent non-lactating animals. Number of animals in each group given in text. Vertical bars represent 1 S.E. of mean each side of the mean.

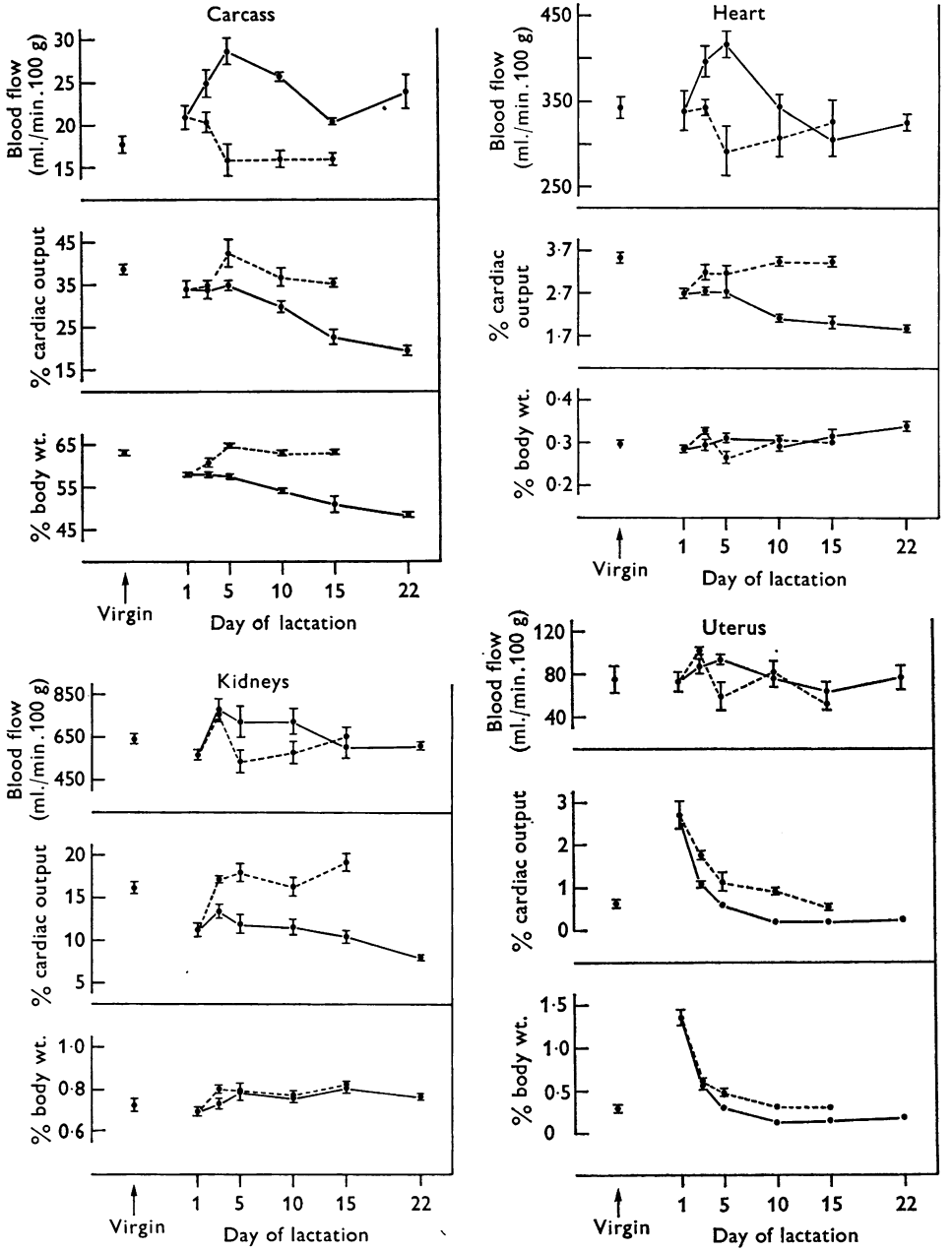

Fig. 3. Post-partum changes in the blood flow, fractional flow (% cardiac output) and weight of carcass, heart, kidneys and uterus. The continuous lines represent lactating animals and the dashed lines represent non-lactating animals. Number of animals in each group given in text. Vertical bars represent 1 S.E. of mean each side of the mean.

the blood flow/g mammary tissue was nearly five times higher than in virgin glands, indicating that the fully developed lobulo-alveolar tissue was considerably more vascular than the mammary fat pad of virgin animals. In addition, the blood flows to the liver and gastrointestinal tract were also raised and these organs received significantly increased proportions of the cardiac output (Fig. 2). This redistribution of the cardiac output was apparently achieved at the expense of the carcass, heart and kidneys as these tissues received significantly reduced fractions compared with virgin animals although, because of the higher cardiac output, the blood flow/g tissue was unchanged (Fig. 3).

Days 1–5. As lactation proceeded, the cardiac output increased further until on day 5 it was 33 % greater than on day 1 ($P < 0{\cdot}01$) (Fig. 1). There was little change in the distribution of the cardiac output during this time, except for a striking fall in the fraction taken by the involuting uterus. Therefore, the blood flows to most tissues followed a course comparable to that of the cardiac output; the flow to the skin, liver, gastrointestinal tract, carcass, heart and uterus increased steadily until day 5 of lactation so that, in all these tissues except the uterus, blood flow was now significantly greater than in virgin animals. In the mammary glands and kidneys the rise in blood flow was complete by day 3.

In animals whose young were removed on the first day of lactation the cardiac output did not rise as it did in lactating animals. It remained instead at a steady level for a day or two post-partum and then fell precipitously to low levels on day 5. The distribution of the cardiac output approached a condition similar to that in virgin animals within 2–4 days following parturition. The blood flows to most organs declined to slightly lower levels on day 3 (except the uterus and kidneys where blood flow increased) and then reflected the change in cardiac output by falling precipitously so that on day 5 they were generally rather lower than in virgin animals.

Days 5–15. Between day 5 and day 15 of lactation the cardiac output remained at a high steady level, 67 % greater than that in non-lactating animals (Fig. 1). During this period the tissues behaved in one of two ways: (1) in the mammary glands, liver, gastrointestinal tract and skin the blood flows remained at high and fairly steady levels (Fig. 2); (2) in the carcass, heart, uterus and kidneys the blood flows failed to maintain the high levels achieved on days 3 and 5 and fell progressively towards non-lactating and virgin levels despite the raised cardiac output (Fig. 3).

After day 5 of lactation the distribution of the cardiac output began to change and this became increasingly marked with advancing lactation (Figs. 2 and 3). The fractions of the cardiac output taken by the liver and gastrointestinal tract increased enormously so that on day 15 the gastro-

TABLE 1. Relative blood flow of tissues at different stages of lactation (blood flow/g tissue divided by cardiac output/g body)

	Day ... 1	3	5	10	15	22	Significance level. Day 1 *vs.* day 22
Mammary gland	1·22 ± 0·082	1·71 ± 0·11	1·54 ± 0·046	1·56 ± 0·057	1·64 ± 0·030	1·56 ± 0·075	$P < 0·02$
Skin	0·553 ± 0·056	0·534 ± 0·031	0·579 ± 0·024	0·535 ± 0·016	0·521 ± 0·018	0·546 ± 0·027	n.s.
Gastrointestinal tract	5·00 ± 0·24	4·87 ± 0·16	4·92 ± 0·18	5·29 ± 0·22	5·13 ± 0·25	4·83 ± 0·22	n.s.
Liver	8·05 ± 0·44	7·58 ± 0·32	7·30 ± 0·43	7·75 ± 0·22	8·35 ± 0·38	9·51 ± 0·57	n.s.
Carcass	0·585 ± 0·034	0·584 ± 0·037	0·605 ± 0·019	0·550 ± 0·025	0·442 ± 0·019	0·406 ± 0·026	$P < 0·01$
Heart	9·47 ± 0·35	9·02 ± 0·38	8·78 ± 0·28	7·16 ± 0·25	6·51 ± 0·23	5·55 ± 0·23	$P < 0·001$
Kidneys	16·2 ± 1·09	16·9 ± 0·99	14·9 ± 0·89	14·8 ± 0·95	12·9 ± 0·78	10·4 ± 0·31	$P < 0·001$
Uterus	2·00 ± 0·18	2·03 ± 0·13	1·98 ± 0·14	1·61 ± 0·17	1·36 ± 0·13	1·29 ± 0·15	$P < 0·02$
	(7)	(6)	(6)	(6)	(6)	(6)	

Mean ± S.E. of mean. Figures in parentheses indicate number of animals in each group.

intestinal tract received 35 % of the cardiac output, compared with 21 % on day 1 and 18 % in virgin animals (Fig. 2). These changes were associated with the growth of these tissues, and the redistribution of the cardiac output towards the gastrointestinal tract and liver allowed the blood flows per g. of tissue to remain at high and relatively stable levels. Similarly, the mammary glands steadily increased in weight between days 3 and 15, and took a correspondingly increased proportion of the cardiac output so that mammary blood flow/g tissue remained steady (Fig. 2). Conversely, progressively smaller fractions of the cardiac output were taken by the carcass, heart, kidneys and uterus in lactating animals, thus explaining the inability of these tissues to maintain a high level of blood flow after days 3 and 5, despite the raised cardiac output (Fig. 3). These findings suggest that the growth of the splanchnic organs and the mammary glands and their ability to maintain a high rate of blood flow were responsible for the redistribution of the cardiac output away from organs less actively involved in the body's response to lactation. The skin behaved like the mammary glands and splanchnic organs in that its blood flow remained at high and fairly steady levels.

The differences in the distribution of the cardiac output between lactating and non-lactating animals became highly significant and increased progressively as, with advancing lactation, the cardiac output was redistributed towards the growing splanchnic organs and mammary glands and away from the carcass, heart and kidneys (Figs. 2 and 3). In addition, the proportion received by the involuting uterus fell more markedly in lactating animals than in non-lactating animals (Fig. 3).

The present work confirmed the findings of Chatwin *et al.* (1969) that the skin and carcass lose weight in lactation (Figs. 2 and 3). It might be thought that the changes in relative weight (% body-weight) were apparent due to growth of the mammary glands and splanchnic organs, but it is significant that the absolute weights of skin and carcass showed the same marked changes. These findings may indicate mobilization of cutaneous and body fat depots in lactating animals.

Days 15–22. During this time there was a second large and significant increase in cardiac output ($P < 0{\cdot}01$) so that on day 22 it was 26 % greater than on day 15 and 65 % greater than on day 1 (Fig. 1). The rate of blood flow to all tissues reflected this rise in cardiac output so that blood flows either increased from the stable mid-lactating level (Fig. 2) or reversed their progressive fall (Fig. 3).

The mammary glands and liver did not grow further beyond day 15 but the gastrointestinal tract continued to grow and to take increasing proportions of the cardiac output at the expense of the carcass and kidneys. Furthermore, the carcass and skin continued to lose weight.

These findings are interesting in view of the fact that young rats are usually weaned towards the end of the third week of life and it is generally considered that, as the young begin to take solid food, their demands upon the mother wane. However, the patterns of changes in the cardiac output and in its distribution to the tissues between days 15 and 22 does not suggest any such decline in the demands of the young and it is probable that they may take more milk at this time (Weihe, 1968).

Relative blood flows. The changes in the distribution of the cardiac output described above appeared to be related both to changes in the weight of different organs and also to the ability of certain tissues to maintain a high rate of blood flow at the expense of other tissues. To examine this further, 'relative blood flows' (the ratio of blood flow per g tissue to mean blood flow per g for the whole body (see Waites & Setchell, 1966)) were calculated to determine which tissues received changed proportions of the cardiac output/g during the course of lactation.

The data (Table 1) indicate that only the mammary glands received an increased 'relative blood flow' during the first 5 days of lactation and that there were no significant changes in other tissues at this time. As lactation proceeded, the mammary glands, gastrointestinal tract and skin maintained their 'relative blood flows' at very stable levels. The 'flow' to the liver increased slightly as it received ever-increasing fractions of the cardiac output from the growing intestinal tract. However, 'relative blood flows' to the carcass, heart, uterus and kidneys suffered progressive reductions after day 5 so that by day 22 they were 30–40 % lower than on day 1. This means that these tissues received smaller proportions of the cardiac output/g in advanced lactation. It is probable that these progressive changes were related to the increasing demands that the mammary glands and splanchnic organs make upon the cardiac output at this time (Fig. 2).

Litter growth. The daily weight changes of an average litter of twelve pups are illustrated in Fig. 4*a*; each point represents the mean of nine to twelve litters. The eyes of the young opened between days 16 and 17 and they then began to leave the nest and were presumably able to start foraging for solid food soon after. However, observation showed that they continued to suckle the mother at least until day 22 and suggested that, if anything, suckling was more intensive at this time. Although young rats can be weaned at about 18 days of age it seems that, for a few days at least, they prefer the mother's milk to solid food.

Fig. 4 shows that the growth rate of the litter was approximately linear between days 7 and 20 (see also Cowie & Folley, 1947) and that this was closely paralleled, between days 5 and 15, by the growth of mammary tissue. The milk yield of the rat is known to increase progressively through lactation (Brody & Nisbet, 1938; Grosvenor & Turner, 1958; Weihe, 1968)

and since mammary blood flow/g tissue remains fairly steady between days 5 and 15 (Fig. 2), Fig. 4 suggests that the growth of mammary tissue is sufficient to accommodate the increasing demands of the young during this period. However, the mammary glands failed to grow further after day 15, and, since the young continued to grow at the same rate, it must be supposed that the milk yield/g tissue increased after this time. It is significant that both cardiac output and mammary blood flow also increased at this time. Furthermore, it is significant that cardiac output and

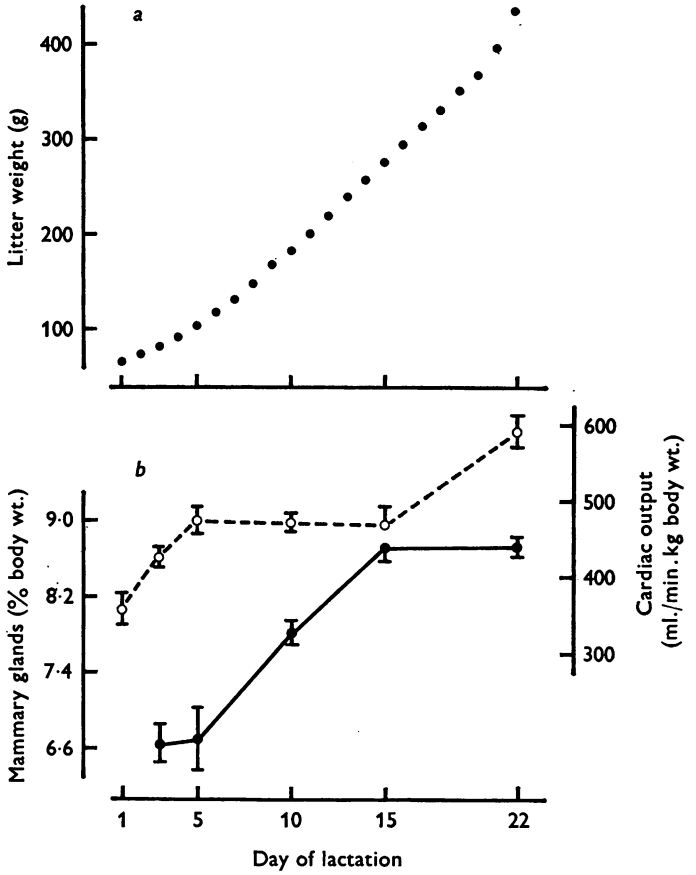

Fig. 4*a*, Changes in litter weight from birth to weaning. *b*, Changes in the cardiac output (upper curve) and weight of the mammary glands (lower curve) during lactation. Number of animals in each group given in text. Vertical bars represent 1 S.E. of mean each side of the mean. Note the parallel relationship between litter growth and mammary gland weight between days 5 and 15, and the stable cardiac output during this period. The mammary gland weight for day 1 was omitted because the fall in weight between days 1 and 3 of lactation (see Fig. 2) suggested a high proportion of retained milk and/or adipose tissue on day 1.

mammary blood flow increased early in lactation, between days 1 and 5, before the mammary glands started to grow. Both these periods of change (days 1–5, days 15–22) were also associated with increases in hepatic and gastrointestinal blood flow, probably related to an increased demand for milk precursors by the mammary tissue. During the period when the growth of the mammary glands matched that of the litter (days 5–15) the cardiac output and mammary, hepatic and gastrointestinal blood flows remained at fairly steady levels. These results suggest that changes in the cardiac output and tissue blood flows occur when the demands of the young exceed the present ability of the existing mass of mammary tissue to synthesize sufficient milk. These changes probably allow the mammary tissue to start working at a higher rate of milk production.

DISCUSSION

The present results confirm the conclusions of Chatwin *et al.* (1969) that marked cardiovascular changes occur in lactation in the rat but indicate that they may have underestimated the magnitude of many of the effects described here. In particular in the present work the blood flows to the liver and gastrointestinal tract are more than twice as great as in that of Chatwin *et al.* (1969). It is probable that ^{86}Rb is a more reliable indicator of tissue blood flow than the $^{3}H_2O$ used in the earlier study, partly because of the way in which it is rapidly taken up and retained in the intracellular space (like K) and partly because there is no danger of loss by evaporation post-mortem. Neverthless, both studies show that, compared with virgin animals under the same conditions, lactating rats have not only a significantly raised mammary blood flow but that blood flow to the liver and gastrointestinal tract and the cardiac output itself are also raised. In the present paper we investigated the time course of these and other changes during the course of lactation. An obvious criticism of both the present work and that of Chatwin *et al.* (1969) is that the experiments were made in anaesthetized animals, subjected to surgery. However, we have found that neither the cardiac output nor the distribution of the cardiac output are significantly different in anaesthetized rats from those in conscious unrestrained rats at the same stage of lactation (Hanwell & Linzell, 1972*d*, and unpublished). Therefore we feel confident in assuming that the present results reflect changes that actually occur in animals during the course of lactation.

Reynolds (1965*b*, 1969) proposed that the increase in udder blood flow in the goat in the first few days post-partum is directly associated with the reduced uterine blood flow occurring at this time. However, it would seem extremely unlikely that volumes of blood are shifted in any simple

way from one organ to another; any decrease in the vascular resistance of the mammary glands following parturition is more likely to be related to changes in the metabolic activity of the mammary tissue than to changes in the blood flow to the uterus. Moreover, Linzell (1969) studied the cardiovascular changes associated with lactogenesis in rats and found that, whilst uterine blood flow fell from 10% of the cardiac output to less than 1% within an hour of parturition, mammary blood flow rose from 4·5 to only 7·5%. More recently, Reynolds (1970) found that there was only a small increase in the proportion of the cardiac output delivered to the udder of the goat after parturition and that the increase in mammary blood flow/g tissue at this time was more closely associated with an increase in the cardiac output. The present results suggest that there is little change in the distribution of the cardiac output in the rat following parturition so that many tissues besides the mammary gland benefit with an increased blood flow as the cardiac output climbs steadily to high levels in the first few days of lactation. This explanation interprets the increase in cardiac output as the primary change, with the blood flows to the organs reflecting this increase. An alternative explanation is that the rise in cardiac output is secondary to increased venous return following a fall in peripheral vascular resistance. However, this must allow for the fact that the cardiac output increases without any apparent change in its distribution and, furthermore, must be of sufficient magnitude to account for a 70% difference in cardiac output between lactating and non-lactating animals.

After day 5 of lactation, only certain tissues (the mammary glands, liver, gastrointestinal tract and skin) maintain high and steady rates of blood flow. The blood flows to other tissues fall progressively towards non-lactating levels despite the cardiac output remaining raised. These changes are associated with a redistribution of the cardiac output away from these tissues and towards the growing gastrointestinal tract, liver and mammary glands. Thus these growing tissues are able to maintain high rates of relative blood flow throughout lactation, seemingly at the expense of other tissues less actively involved in the body's reaction to the demands of lactation. The mechanisms by which this is achieved are probably complex but the adjustment of blood flow to active secretory and absorptive tissues may be local and related to the production of vasodilator metabolites (see Haddy & Scott, 1968). The finding that skin blood flow is raised in lactation is interesting (see also Chatwin *et al.* 1969) and, in view of the fact that thermoregulatory mechanisms are not fully developed in young rats until approximately 18 days of age (see Hahn, 1956), it is possible that this represents an important means of increasing the transfer of heat from the mother to the pups. It is perhaps significant that the brood patch de-

veloped in birds during the incubation period to facilitate heat transfer from the parent bird to its eggs is also high vascularized and is prolactin-induced (see Bern & Nicoll, 1968).

The present study provides an opportunity for comparing the relative nutritive and metabolic demands of pregnancy and lactation. No pregnant animals were studied but a comparison of tissue weights and the distribution of the cardiac output on the day following parturition with those in virgin animals and those in animals in advanced lactation (Figs. 2 and 3) confirms that the demands of lactation are considerably greater than those of pregnancy. This is hardly surprising when it is remembered that at birth the litter weighs only 70 g but that at weaning, 3 weeks later, it has increased to approximately 400 g, about 50 % more than the weight of the mother herself. Blaxter (1971) compared the calorific value of new-born animals with the calorific value of the milk produced in a single day and concluded that the demands imposed on metabolism by lactation are considerable when compared with pregnancy, and that they increase as the size of the species decreases.

Although early workers claimed that the growth of mammary tissue due to cell division was completed during the first two thirds of gestation, more recent studies have shown that there are further increases in mammary gland cellularity and total DNA throughout pregnancy and also during the first half of lactation (see Munford, 1964). It is probable that many of the discrepancies in the literature concerning mammary growth during lactation are associated with differences in litter size; Tucker (1966) showed that the total mammary gland DNA of rats nursing large litters increased progressively between day 1 and day 16 of lactation, but that no increase in DNA was observed in rats nursing small litters. In the present work, the increase in weight of the mammary glands paralleled the growth curve of the litter between days 5 and 15, and it is suggested that, during this time, growth of the mammary tissue accommodated the demands of the young for milk.

It is further suggested that the increases in cardiac output and tissue blood flows in the first few days following parturition (days 1–5) and in the last week of lactation (days 15–22) are at times when the demands of the young temporarily outstrip the supply of milk; these changes would help to redress this situation by bringing increased amounts of metabolites and milk precursors to the secretory epithelium. We have shown elsewhere (Hanwell & Linzell, 1972*c*) that the high cardiac output in lactating rats is strongly related to the suckling stimulus so that it seems possible that the changes in cardiac output in early lactation and in late lactation are associated with increased stimulation of the teats due to vigorous suckling by the unsatisfied young at these times. This also offers an explanation for

the finding that the cardiac output on the first day of lactation is related to the number of pups in the litter (suckling intensity). The response of the cardiac output and tissue blood flows to increased demands from the young may be viewed as the short term solution to changes in demand, whereas tissue growth is a slower process and serves as the long term solution. There is some evidence (Hanwell & Linzell, 1972*c*) that suckling-induced release of anterior pituitary hormones (prolactin and/or growth hormone) may be responsible for the raised cardiac output of lactating rats. The cessation of mammary gland growth in late lactation at a time when the cardiac output is rising may indicate that these are regulated by different functions of the anterior pituitary or may represent differences in the response of the target organs.

We are grateful to the M.R.C. for the award of a Studentship to A.H.

REFERENCES

BALDWIN, R. L. & MILLIGAN, L. P. (1966). Enzymatic changes associated with the initiation and maintenance of lactation in the rat. *J. biol. Chem.* **241**, 2058–2066.

BERN, H. A. & NICOL, C. S. (1968). The comparative endocrinology of prolactin. *Recent Prog. Horm. Res.* **24**, 681–713.

BLAXTER, K. L. (1971). The comparative biology of lactation. In *Lactation*, ed. FALCONER, I. R., pp. 51–69. London: Butterworths.

BRODY, S. & NISBET, R. (1938). A comparison of the amounts and energetic efficiencies of milk production in rat and dairy cow. *Res. Bull. Mo. agric. Exp. Stn* **285**, 1–30.

CHATWIN, A. L., LINZELL, J. L. & SETCHELL, B. P. (1969). Cardiovascular changes during lactation in the rat. *J. Endocr.* **44**, 247–254.

COTES, P. M. & CROSS, B. A. (1954). The influence of suckling on food intake and growth of adult female rats. *J. Endocr.* **10**, 363–367.

COWIE, A. T. & FOLLEY, S. J. (1947). The measurement of lactational performance in the rat in studies of the endocrine control of lactation. *J. Endocr.* **5**, 9–13.

DELANEY, J. P. & GRIM, E. (1964). Canine gastric blood flow and its distribution. *Am. J. Physiol.* **207**, 1195–1202.

FEGLER, G. (1954). Measurement of cardiac output in anaesthetized animals by a thermo-dilution method. *Q. Jl exp. Physiol.* **39**, 153–164.

FELL, B. F., SMITH, K. A. & CAMPBELL, R. M. (1963). Hypertrophic and hyperplastic changes in the alimentary canal of the lactating rat. *J. Path. Bact.* **85**, 179–188.

GROSVENOR, C. E. & TURNER, C. W. (1958). Pituitary lactogenic hormone concentration and milk secretion in lactating rats. *Endocrinology* **63**, 535–539.

HADDY, F. J. & SCOTT, J. B. (1968). Metabolically linked vasoactive chemicals in local regulation of blood flow. *Physiol. Rev.* **48**, 688–707.

HAHN, P. (1956). Effect of environmental temperatures on the development of thermoregulatory mechanisms in infant rats. *Nature, Lond.* **178**, 96–97.

HANWELL, A. & LINZELL, J. L. (1972*a*). A simple technique for measuring the rate of milk secretion in the rat. *Comp. Biochem. Physiol.* **43A**, 259–270.

HANWELL, A. & LINZELL, J. L. (1972*b*). Validation of the thermodilution technique for the estimation of cardiac output in the rat. *Comp. Biochem. Physiol.* **41A**, 647–657.

HANWELL, A. & LINZELL, J. L. (1972*c*). Elevation of the cardiac output in the rat by prolactin and growth hormone. *J. Endocr.* **53**, lvii–lviii.

HANWELL, A. & LINZELL, J. L. (1972*d*). Determination of cardiac output and mammary blood flow in the conscious lactating rat. *J. Physiol.* **226**, 24–25*P*.

KENNEDY, G. C., PEARCE, W. M. & PARROTT, D. M. V. (1958). Liver growth in the lactating rat. *J. Endocr.* **17**, 158–160.

LINZELL, J. L. (1960). Mammary gland blood flow and oxygen, glucose and volatile fatty acid uptake in the conscious goat. *J. Physiol.* **153**, 492–509.

LINZELL, J. L. (1969). Cardiac output and organ blood flow in late pregnancy and early lactation in rats. In *Lactogenesis: the Initiation of Milk Secretion at Parturition*, ed. REYNOLDS, M. & FOLLEY, S. J., pp. 153–156. Philadelphia: University of Pennsylvania Press.

LITTLE, J. M. & WILLIAMS, C. (1964). Determination of Evan's Blue in plasma and plasma-dextran mixtures by protein precipitation and extraction. *Proc. Soc. exp. Biol. Med.* **115**, 564–567.

MENDRELL, P. L. & HOLLENBERG, N. K. (1971). Cardiac output distribution in the rat: comparison of rubidium and microsphere methods. *Am. J. Physiol.* **221**, 1617–1620.

MUNFORD, R. E. (1964). A review of anatomical and biochemical changes in the mammary gland with particular reference to quantitative methods of assessing mammary development. *Dairy Sci. Abstr.* **26**, 293–304.

REYNOLDS, M. (1965*a*). Udder blood flow in unanaesthetized, lactating goats measured with nitrous oxide. *Am. J. Physiol.* **209**, 669–672.

REYNOLDS, M. (1965*b*). Increases in udder blood flow associated with initiation of lactation. *Fedn Proc.* **24**, 451.

REYNOLDS, M. (1969). Relationship of mammary circulation and oxygen consumption to lactogenesis. In *Lactogenesis: the Initiation of Milk Secretion at Parturition*, ed. REYNOLDS, M. & FOLLEY, S. J., pp. 145–151. Philadelphia: University of Pennsylvania Press.

REYNOLDS, M. (1970). Proportion of cardiac output and of total body oxygen consumption which is used by the mammary glands. *Physiologist, Wash.* **13**, 292.

SAPIRSTEIN, L. A. (1956). Fractionation of the cardiac output of rats with isotopic potassium. *Circulation Res.* **4**, 689–692.

SAPIRSTEIN, L. A. (1958). Regional blood flow by fractional distribution of indicators. *Am. J. Physiol.* **193**, 161–168.

TUCKER, H. A. (1966). Regulation of mammary nucleic acid content by various suckling intensities. *Am. J. Physiol.* **210**, 1209–1214.

WAITES, G. M. H. & SETCHELL, B. P. (1966). Changes in blood flow and vascular permeability of the testis, epididymis and accessory reproductive organs of the rat after the administration of cadmium chloride. *J. Endocr.* **34**, 329–342.

WEIHE, VON W. H. (1968). Der Milchleistungstest bei Versuchstieren. *Z. Versuchstierkunde* **10**, 147–157.