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The Effect of Adrenalectomy on the Metabolism of the Mammary Glands of Lactating Rats

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The mammary glands of lactating rats extensively utilize glucose as an energy source and as a source of metabolic precursors for the synthesis of milk constituents. It is well established that the pentose phosphate pathway of glucose utilization is very active in milk-producing mammary-gland tissue (Glock & McLean, 1953; Abraham, Hirsch & Chaikoff, 1954; Peeters, Debackere & Sierens, 1957), and that there is a close correlation between lactational ability and the extent of operation of this pathway (see Glock & McLean, 1958; McLean, 1958*a*; Abraham & Chaikoff, 1959). It is probable that an intimate relation exists between the pentose phosphate pathway and the systems for the synthesis of lipids, mediated via NADP⁺ and NADPH, in mammary gland (McLean, 1960; see Folley & Greenbaum, 1960), as well as in liver (Siperstein & Fagan, 1958), since both the possible mechanisms for lipogenesis (the malonyl-CoA pathway and the reversed Lynen-Green system with crotonyl-CoA reductase) require NADPH (Wakil, 1962; Langdon, 1957). The presence of the malonyl-CoA pathway in the mammary gland has been reported (Ganguly, 1960; Abraham, Matthes & Chaikoff, 1961; Dils & Popják, 1962).

Adrenalectomy is known to cause a diminished milk yield from the mammary glands of lactating

rats (Cowie & Folley, 1948; see Mayer & Klein, 1949), and it was decided to study its effects on pathways of intermediary metabolism that are closely associated with the synthesis of the constituents of milk, in an endeavour to establish the nature of the primary block caused by the loss of adrenal hormones, and with particular reference to the relationships between the several pathways of glucose catabolism. It was hoped to find a preferential loss of activity of one of the pathways and to clarify the role of the nicotinamide nucleotides in the regulation of the intermediary metabolism of the mammary gland. Thus investigations have been made: into the relationships between the Embden-Meyerhof and the pentose phosphate pathways in the condition of diminished lactation produced by the loss of the adrenal hormones and induced at a time when both pathways are fully established in the mammary gland; into lipogenesis associated with these systems; and into the limitations that the availability of the nicotinamide nucleotides imposes on them. That the activities of the enzymes in the pentose phosphate pathway in mammary-gland tissue were decreased by adrenalectomy has been shown by Willmer (1960*b*), and it is shown below that the activity of the Embden-Meyerhof pathway is also decreased, but that lipogenesis from acetate is unaffected.

METHODS

Animals. Hooded Norwegian rats of body wt. 180–200 g., and undergoing their first lactation, were used in all experiments. Bilateral adrenalectomies, and sham experiments on the animals used as controls, were performed under ether anaesthesia on the fourth day of lactation, when the litters were restricted to six pups per rat. The animals were maintained with free access to a stock diet (MRC no. 41 B; Bruce & Parkes, 1946), and with water *ad libitum*, except that adrenalectomized rats were supplied with 1% (w/v) NaCl solution *ad libitum* after the operation. They were killed 4 days later, when the milk yield of the adrenalectomized rats was much diminished but not negligible. The 24 hr. weight increases of the six pups immediately before the mothers were killed were: 7–10 g. for controls; 2–3 g. for adrenalectomized animals. If an increase smaller or greater than these respectively occurred, the animal was discarded.

Reagents. Radioactive substrates were purchased from The Radiochemical Centre, Amersham, Bucks. Alcohol dehydrogenase and glucose 6-phosphate dehydrogenase were obtained from Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Preparation of tissue slices. After the rats had been killed, the abdominal mammary glands were dissected out and weighed in a chilled beaker. Slices were cut with a chilled hand microtome (Stadie & Riggs, 1944), and placed on filter paper moistened with the buffer solution used for the incubations, which had been cooled to 0°, and appropriate weights of slices were added to the incubation flasks. Experiments were carried out as described below.

Respiratory activity. Slices (300 ± 20 mg.) were placed in chilled Warburg flasks containing the following: 100 μ moles of sodium phosphate buffer, pH 7.4, 75 μ moles of KCl, 20 μ moles of $MgSO_4$, 3 μ moles of ATP, 10 μ moles of cytochrome *c* and 50 μ moles of substrate in the main compartment; and 0.2 ml. of 20% (w/v) KOH in the centre well. The final volume was 3.0 ml. Where no substrate was used, water was added instead to make up the volume. The substrate was glucose, pyruvate or succinate. Oxygen uptakes were measured for 45 min. at 37°, after an initial gassing with O_2 for 5 min. At the end of the incubation periods the slices from each set of flasks were pooled and homogenized at 0° in 15 ml. of 5% (w/v) trichloroacetic acid: the DNA content of the pellet obtained by centrifugation after standing for 30 min. at 0° was estimated by the Slater (1962) modification of the diphenylamine method.

Embden-Meyerhof and pentose phosphate pathways of carbohydrate metabolism. Slices (200 ± 15 mg.) were added to 50 ml. conical flasks possessing a centre well and containing, in the main compartment, 5 ml. of Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932) previously gassed with $O_2 + CO_2$ (95:5). Four types of incubation mixture were used as follows: the 5 ml. of bicarbonate buffer contained: (a) 0.50 μ C of [1- ^{14}C]glucose and 50 μ moles of unlabelled glucose; (b) 0.50 μ C of [6- ^{14}C]glucose and 50 μ moles of unlabelled glucose; (c) 0.50 μ C of [1- ^{14}C]glucose, 50 μ moles of unlabelled glucose and 1 μ mole of phenazine methosulphate; or (d) 0.50 μ C of [1- ^{14}C]acetate, 50 μ moles of unlabelled glucose and 50 μ moles of unlabelled acetate. The flasks were sealed with rubber serum caps and incubated at 37° for 90 min., the gas phase being $O_2 + CO_2$ (95:5). At the end of this time, 1 ml. of 20% (w/v)

NaOH was injected through the cap into the centre well of each flask, followed by 1.5 ml. of 2N-HCl into the main compartment. After a further incubation for 1½ hr., to allow the CO_2 to be absorbed, the NaOH was removed for determination of its $^{14}CO_2$ content, and the lipids in the slices were extracted for determination of the incorporation of ^{14}C . The DNA content of the mammary-gland tissue was estimated, as described above, with a separate sample of the gland.

Determination of the yield of $^{14}CO_2$. The NaOH in the centre well of each flask was quantitatively transferred to a centrifuge tube containing 2 ml. of 5% (w/v) $BaCl_2$ –0.5% NH_4Cl solution, and the precipitated $BaCO_3$ was washed twice with water and twice with acetone before being dried and weighed. After transfer to plastic planchets of 1 cm.² area, it was counted at infinite thickness by using an end-window Geiger-Müller tube EHM/2S (General Electric Co. Ltd.), of window weight 1.7 mg./cm.². Under the conditions employed this system had an efficiency of 3.2%. Each 1 μ C of substrate converted into $^{14}CO_2$ by the persulphate oxidation method of Abraham & Hassid (1957) gave 383×10^3 total counts (i.e. counts/min. at infinite thickness $\times$ mg. of $BaCO_3$ produced); similarly, all values reported below for $^{14}CO_2$ produced are expressed as counts/min. at infinite thickness $\times$ mg. of $BaCO_3$ collected.

Determination of ^{14}C in lipids. The slices from each flask were collected and washed and their total fats were extracted by the procedure of Bligh & Dyer (1959). The resultant chloroform solution was evaporated to dryness for determination of the weight of fat isolated. The fat was then redissolved in chloroform and portions were plated on plastic planchets of 2 cm.² area at infinite thickness. They were counted, after being weighed, as for the $^{14}CO_2$ estimations. The values in the Tables are expressed as specific activities, as total counts/100 mg. of slices and as total counts/whole gland.

Interactions of glucose and acetate metabolism. The procedures were the same as those described above, except that the flasks contained 5 ml. of Krebs-Ringer bicarbonate buffer with the following additions: (a) 0.50 μ C of [U- ^{14}C]glucose and 50 μ moles of unlabelled glucose; (b) 0.50 μ C of [U- ^{14}C]glucose and 50 μ moles of unlabelled glucose, 50 μ moles of unlabelled acetate; (c) 0.50 μ C of [1- ^{14}C]acetate and 50 μ moles of unlabelled acetate; (d) 0.50 μ C of [1- ^{14}C]acetate and 50 μ moles of unlabelled glucose and 50 μ moles of unlabelled acetate.

Pyruvate metabolism. Slices (120 ± 15 mg.) were added to flasks containing 5 ml. of Krebs-Ringer bicarbonate buffer, in which were 75 μ moles of unlabelled pyruvate, 50 μ moles of unlabelled glucose and 0.50 μ C of either [1- ^{14}C]pyruvate or [2- ^{14}C]pyruvate. The procedures were as described above, except that the incorporation into lipids of ^{14}C from [1- ^{14}C]pyruvate was not investigated.

Nicotinamide nucleotides in mammary-gland tissue. The nicotinamide nucleotides were measured by the method of Slater & Sawyer (1962) by using approx. 1.0 g. of mammary-gland tissue for each of the acid and alkaline extracts. The rates of reduction of the 2,6-dichlorophenol-indophenol were measured at 600 $m\mu$ on an Optica recording spectrophotometer.

Presentation of results. The results were usually expressed on the basis of 100 mg. or g. wet wt. of tissue, and no attempt was made to correct them for the weight of the retained milk present in the mammary-gland tissue ('milk error').

This correction is based on the lactose content of the tissue, by assuming a constant lactose concentration in this retained milk. No information is available about alterations in the composition of milk synthesized by the mammary gland under the influence of adrenalectomy, and a milk correction cannot therefore be applied.

Results were also calculated for the whole gland, as it is the whole gland that supplies the pups with milk, and alterations in milk yield, e.g. those produced by adrenalectomy, will reflect decreases in the total metabolism of the gland. The results are also expressed on the basis of incorporation of $^{14}\text{C}/100\ \mu\text{g.}$ of DNA phosphorus to facilitate comparison with other tissues.

RESULTS

Effect of adrenalectomy on respiratory activity. The loss of the adrenal hormones impairs the ability of the mammary glands of lactating rats to oxidize pyruvate, glucose and succinate, and the endogenous respiration is also lowered (Table 1). An overall decrease in metabolic activity is apparent, involving the various pathways of glucose catabolism and the tricarboxylic acid cycle. It is possible to define these effects more quantitatively by the use of radioactive substrates, and the results of such experiments are given below.

Effect of adrenalectomy on the pentose phosphate and Embden-Meyerhof pathways of carbohydrate metabolism: (a) Conversion of $[1-^{14}\text{C}]$ glucose, $[6-^{14}\text{C}]$ glucose and $[1-^{14}\text{C}]$ acetate into $^{14}\text{CO}_2$. Adrenalectomy has no effect on the oxidation of $[6-^{14}\text{C}]$ glucose to $^{14}\text{CO}_2$, but markedly decreases the

conversion of $[1-^{14}\text{C}]$ glucose into $^{14}\text{CO}_2$ (Table 2). In the presence of phenazine methosulphate, the formation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose is stimulated to a much larger degree with mammary-gland tissue from adrenalectomized rats than with tissue from sham-operated rats, as shown by the ratios in Table 2. Further, mammary glands from adrenalectomized rats liberate twice as much $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ acetate as do those from control animals.

The $^{14}\text{CO}_2$ produced from $[1-^{14}\text{C}]$ glucose/ $^{14}\text{CO}_2$ produced from $[6-^{14}\text{C}]$ glucose ratio is decreased by a factor of about 3 as a result of the loss of the adrenal hormones (Table 2), and this suggests that the activity of the pentose phosphate pathway is lowered to a greater degree by the loss of the adrenal hormones than that of the Embden-Meyerhof pathway.

(b) Incorporation of ^{14}C from $[1-^{14}\text{C}]$ glucose, $[6-^{14}\text{C}]$ glucose and $[1-^{14}\text{C}]$ acetate into the total lipids. Table 3 demonstrates that ^{14}C from both $[1-^{14}\text{C}]$ glucose and $[6-^{14}\text{C}]$ glucose is incorporated to a lesser extent into fats by mammary-gland tissue from adrenalectomized rats than by tissue from the control animals, whereas adrenalectomy has no effect on the rate of incorporation from $[1-^{14}\text{C}]$ acetate.

The ^{14}C incorporated from $[6-^{14}\text{C}]$ glucose— ^{14}C incorporated from $[1-^{14}\text{C}]$ glucose/ ^{14}C incorporated from $[6-^{14}\text{C}]$ glucose ratio is unaltered by the loss of the adrenal hormones, and so is the ^{14}C incorporated from $[1-^{14}\text{C}]$ glucose in the presence of phenazine methosulphate/ ^{14}C incorporated from $[1-^{14}\text{C}]$ glucose ratio (column 8 in Table 3).

Table 1. *Effect of adrenalectomy on the respiratory activity of mammary-gland tissue*

Mammary-gland slices (300 mg.) were incubated for 45 min. at 37° in the following mixture in Warburg flasks: 100 μmoles of sodium phosphate buffer, pH 7.4, 75 μmoles of KCl, 20 μmoles of MgSO_4 , 3 μmoles of ATP, 10 μmoles of cytochrome c and 50 μmoles substrate in the main compartment; and 0.2 ml. of 20% (w/v) KOH in the centre well. The final volume was 3.0 ml. The substrate was succinate, pyruvate or glucose, with water making up the volume where no substrate was added. The gas phase was O_2 . Values are given as means $\pm$ S.E.M. In parentheses are given Fisher's *P* values for the probability of difference between the mean values; N.S., not significantly different. Where substrate was present, individual values have been corrected for the corresponding 'no-substrate' oxygen uptake.

Substrate	Status of animal	No. of expts.	Oxygen uptake ($\mu\text{l./hr.}$)		
			Per 100 mg. wet wt. of tissue	Per 100 $\mu\text{g.}$ of DNA P	Per total gland
None	Sham-operated	7	93 ± 5	490 ± 23	4600 ± 300
	Adrenalectomized	7	82 ± 2 ($P = 0.071$)	360 ± 15 ($P < 0.001$)	2900 ± 300 ($P = 0.002$)
Succinate	Sham-operated	6	81 ± 2	440 ± 20	4000 ± 200
	Adrenalectomized	7	51 ± 4 ($P < 0.001$)	220 ± 15 ($P < 0.001$)	1800 ± 100 ($P < 0.001$)
Pyruvate	Sham-operated	7	35 ± 4	198 ± 24	2000 ± 300
	Adrenalectomized	7	29 ± 5 (N.S.)	121 ± 22 ($P = 0.040$)	1000 ± 200 ($P = 0.011$)
Glucose	Sham-operated	6	28 ± 3	160 ± 28	1500 ± 200
	Adrenalectomized	6	16 ± 4 ($P = 0.032$)	57 ± 20 ($P = 0.013$)	600 ± 200 ($P = 0.006$)

Table 2. *Effect of adrenalectomy on the ability of mammary-gland tissue to convert [1-¹⁴C]glucose, [6-¹⁴C]glucose and [1-¹⁴C]acetate into ¹⁴CO₂*

Mammary-gland slices (200 mg.) were incubated for 1½ hr. at 37° in 5.0 ml. of Krebs-Ringer bicarbonate buffer containing: (a) 0.50 μC of [1-¹⁴C]glucose and 50 μmoles of unlabelled glucose; (b) 0.50 μC of [6-¹⁴C]glucose and 50 μmoles of unlabelled glucose; (c) 0.50 μC of [1-¹⁴C]glucose, 50 μmoles of unlabelled glucose and 1 μmole of phenazine methosulphate; or (d) 0.50 μC of [1-¹⁴C]acetate, 50 μmoles of unlabelled glucose and 50 μmoles of unlabelled acetate. The gas phase was O₂ + CO₂ (95:5). Values are given as means ± S.E.M. In parentheses are given Fisher's *P* values for the probability of difference between the mean values; N.S., not significantly different.

		10 ⁻³ × ¹⁴ C incorporated into CO ₂ (counts/min.)			
Radioactive substrate	Status of animal	No. of expts.	Per 100 mg. wet wt. of tissue	Per 100 μg. of DNA <i>P</i>	Per total gland
(a) [1- ¹⁴ C]Glucose	Sham-operated	5	41.5 ± 4.1	193.9 ± 15.9	1628 ± 142
	Adrenalectomized	5	17.5 ± 1.9	61.9 ± 14.8*	611 ± 106
			(<i>P</i> < 0.001)	(<i>P</i> < 0.001)	(<i>P</i> < 0.001)
(b) [6- ¹⁴ C]Glucose	Sham-operated	5	5.0 ± 0.5	24.8 ± 4.5	209 ± 35
	Adrenalectomized	5	5.6 ± 0.5	17.3 ± 4.4*	192 ± 28
			(N.S.)	(N.S.)	(N.S.)
(c) [1- ¹⁴ C]Glucose + phenazine methosulphate	Sham-operated	5	86.2 ± 5.8	405.2 ± 24.2	3516 ± 447
	Adrenalectomized	5	59.9 ± 7.0	198.1 ± 11.7*	2024 ± 307
			(<i>P</i> = 0.020)	(<i>P</i> < 0.001)	(<i>P</i> = 0.023)
(d) [1- ¹⁴ C]Acetate	Sham-operated	6	4.2 ± 0.6	18.5 ± 2.4	138 ± 21
	Adrenalectomized	6	9.6 ± 0.9	44.5 ± 5.0	285 ± 40
			(<i>P</i> < 0.001)	(<i>P</i> < 0.001)	(<i>P</i> = 0.008)

* Mean of three values only.

Calculated ratios:
¹⁴CO₂ produced from [1-¹⁴C]glucose
¹⁴CO₂ produced from [6-¹⁴C]glucose
 Sham-operated 8.6 ± 1.3
 Adrenalectomized 3.1 ± 0.2
 (*P* = 0.003)

¹⁴CO₂ produced from [1-¹⁴C]glucose in the presence of phenazine methosulphate
¹⁴CO₂ produced from [1-¹⁴C]glucose
 Sham-operated 2.0 ± 0.2
 Adrenalectomized 3.2 ± 0.5
 (*P* = 0.050)

Interactions of glucose and acetate metabolism:
 (a) Conversion of [U-¹⁴C]glucose and [1-¹⁴C]acetate into ¹⁴CO₂. Table 4 shows that mammary-gland tissue from adrenalectomized animals oxidizes less glucose and more acetate than does the tissue from control animals. Unlabelled acetate has very little stimulating effect on ¹⁴CO₂ produced from [U-¹⁴C]-glucose by mammary-gland tissue from adrenalectomized animals, whereas a more pronounced, but not large, stimulation occurs with tissue from sham-operated animals. Unlabelled glucose causes a smaller depression of the formation of ¹⁴CO₂ from [1-¹⁴C]acetate by mammary-gland tissue from adrenalectomized animals than it does with tissue from control rats. These interactions are similar to those obtained by Hirsch, Baruch & Chaikoff (1954) for normal lactating rats.

(b) Incorporation of ¹⁴C from [U-¹⁴C]glucose and [1-¹⁴C]acetate into the total lipids. Mammary-gland tissue from control rats incorporates ¹⁴C from [U-¹⁴C]glucose into lipids much more readily than does tissue from adrenalectomized animals, and the difference is even more pronounced when unlabelled acetate is also present (Table 5). Tissue from neither type of animal incorporates [1-¹⁴C]-acetate at all well in the absence of glucose, and in the presence of glucose there is a trend towards a higher degree of incorporation into the lipids from the control animals. The effect shown in Table 5 is somewhat larger than that in Table 3. The small difference in these two Tables is, presumably, seasonal.

Effect of adrenalectomy on the metabolism of pyruvate. The loss of the adrenal hormones decreases the ability of mammary-gland tissue to oxidize [1-¹⁴C]pyruvate to ¹⁴CO₂, and also lowers its ability to convert [2-¹⁴C]pyruvate both into ¹⁴CO₂ and into ¹⁴C-labelled lipids (Tables 6 and 7).

Effect of adrenalectomy on the nicotinamide nucleotide content of mammary gland. In terms of μg./g. wet wt. of tissue, adrenalectomy has no effect on the NAD⁺, NADH, NADP⁺ and NADPH concentrations in mammary-gland tissue (Table 8). However, some decreases in the total amounts present in the whole gland are apparent, although these are not of high significance statistically. Comparison of the values in Table 8 for normal animals with those obtained by McLean (1958*b*) shows that the method of estimation used in the present work gives slightly different results from the one that she describes, which involves nicotinamide nucleotide-linked cytochrome *c* reductase, the present results being approximately two-thirds of those reported by McLean (1958*b*). The differences probably arise from the use of different strains of rat, which T. F. Slater (personal communication) has shown may cause considerable variations in the determinations of nucleotides,

Table 3. *Effect of adrenalectomy on the ability of mammary-gland tissue to incorporate ^{14}C from $[1\text{-}^{14}\text{C}]\text{glucose}$, $[6\text{-}^{14}\text{C}]\text{glucose}$ and $[1\text{-}^{14}\text{C}]\text{acetate}$ into the total lipids*

Radioactive substrate	Status of animal	No. of expts.	$10^{-3} \times ^{14}\text{C}$ incorporated into lipids (counts/min.)				Calculated ratios:
			Per g. of fat isolated	Per 100 mg. wet wt. of tissue	Per 100 μg . of DNA P	Per total gland	
(a) $[1\text{-}^{14}\text{C}]\text{Glucose}$	Sham-operated	5	174 $\pm$ 31	2.6 $\pm$ 0.3	12.3 $\pm$ 1.6	97.7 $\pm$ 17.1	$\frac{^{14}\text{C} \text{ incorporated from } [6\text{-}^{14}\text{C}]\text{glucose}}{^{14}\text{C} \text{ incorporated from } [1\text{-}^{14}\text{C}]\text{glucose}}$ Sham-operated 0.47 $\pm$ 0.03 Adrenalectomized 0.46 $\pm$ 0.03 (N.S.)
	Adrenalectomized	5	52 $\pm$ 10 ($P = 0.004$)	0.8 $\pm$ 0.2 ($P = 0.001$)	2.9 $\pm$ 0.8* ($P = 0.005$)	29.4 $\pm$ 8.2 ($P = 0.008$)	
(b) $[6\text{-}^{14}\text{C}]\text{Glucose}$	Sham-operated	5	340 $\pm$ 53	4.9 $\pm$ 0.8	23.3 $\pm$ 3.9	191.9 $\pm$ 22.7	$\frac{^{14}\text{C} \text{ incorporated from } [1\text{-}^{14}\text{C}]\text{glucose in the presence of phenazine methosulphate}}{^{14}\text{C} \text{ incorporated from } [1\text{-}^{14}\text{C}]\text{glucose}}$ Sham-operated 0.39 $\pm$ 0.07 Adrenalectomized 0.39 $\pm$ 0.07 (N.S.)
	Adrenalectomized	5	102 $\pm$ 24 ($P = 0.003$)	1.5 $\pm$ 0.3 ($P = 0.004$)	5.2 $\pm$ 1.1* ($P = 0.014$)	53.9 $\pm$ 14.3 ($P = 0.001$)	
(c) $[1\text{-}^{14}\text{C}]\text{Glucose} + \text{phenazine methosulphate}$	Sham-operated	5	74 $\pm$ 12	1.1 $\pm$ 0.2	5.4 $\pm$ 1.3	45.3 $\pm$ 9.3	
	Adrenalectomized	5	24 $\pm$ 6 ($P = 0.005$)	0.3 $\pm$ 0.1 ($P = 0.003$)	1.0 $\pm$ 0.1* ($P = 0.036$)	12.3 $\pm$ 4.1 ($P = 0.001$)	
(d) $[1\text{-}^{14}\text{C}]\text{Acetate}$	Sham-operated	6	193 $\pm$ 34	2.3 $\pm$ 0.3	10.0 $\pm$ 1.9	75.9 $\pm$ 10.1	
	Adrenalectomized	6	232 $\pm$ 24 (N.S.)	2.5 $\pm$ 0.1 (N.S.)	10.5 $\pm$ 1.2 (N.S.)	70.7 $\pm$ 9.0 (N.S.)	

* Mean of three values only.

even when only a single method of determination is used. Values in Table 8 for the $[NAD^+]/[NADH]$ ratios are in reasonable agreement with those of McLean (1958b), but the values for the $[NADPH]/[NADP^+]$ ratios are lower than those that she

reported by a factor of about 4, which is probably due to the difficulties inherent in estimating the very low amounts of $NADP^+$ present in mammary-gland tissue, and small changes of which would dramatically change the ratio.

Table 4. *Effect of adrenalectomy on the production of $^{14}CO_2$ by mammary-gland tissue from $[U-^{14}C]$ glucose in the presence and absence of unlabelled acetate, and from $[1-^{14}C]$ acetate in the presence and absence of unlabelled glucose*

Mammary-gland slices (200 mg.) were incubated for $1\frac{1}{2}$ hr. at 37° in 5.0 ml. of Krebs-Ringer bicarbonate buffer containing: (a) $0.50\ \mu C$ of $[U-^{14}C]$ glucose and 50 μ moles of unlabelled glucose; (b) $0.50\ \mu C$ of $[U-^{14}C]$ glucose, 50 μ moles of unlabelled glucose and 50 μ moles of unlabelled acetate; (c) $0.50\ \mu C$ of $[1-^{14}C]$ acetate and 50 μ moles of unlabelled acetate; or (d) $0.50\ \mu C$ of $[1-^{14}C]$ acetate, 50 μ moles of unlabelled acetate and 50 μ moles of unlabelled glucose. The gas phase was $O_2 + CO_2$ (95:5). Values are given as means $\pm$ s.e.m. In parentheses are Fisher's *P* values for the probability of difference between the mean values; N.S., not significantly different.

Radioactive substrate	Status of animal	No. of expts.	$10^{-3} \times ^{14}C$ incorporated into CO_2 (counts/min.)		
			Per 100 mg. wet wt. of tissue	Per 100 $\mu g.$ of DNA P	Per total gland
(a) $[U-^{14}C]$ Glucose	Sham-operated	6	29.4 ± 4.6	163.2 ± 29.1	1176 ± 224
	Adrenalectomized	6	17.3 ± 3.9 (<i>P</i> = 0.073)	85.8 ± 24.0 (<i>P</i> = 0.062)	515 ± 136 (<i>P</i> = 0.031)
(b) $[U-^{14}C]$ Glucose + acetate	Sham-operated	8	41.3 ± 4.0	268.9 ± 29.5	1502 ± 152
	Adrenalectomized	8	22.5 ± 1.3 (<i>P</i> < 0.001)	118.4 ± 6.9 (<i>P</i> < 0.001)	625 ± 44 (<i>P</i> < 0.001)
(c) $[1-^{14}C]$ Acetate	Sham-operated	6	11.5 ± 1.2	63.1 ± 6.5	450 ± 56
	Adrenalectomized	5	15.9 ± 1.1 (<i>P</i> = 0.024)	77.6 ± 8.2 (N.S.)	461 ± 49 (N.S.)
(d) $[1-^{14}C]$ Acetate + glucose	Sham-operated	8	5.2 ± 0.5	34.4 ± 3.4	189 ± 15
	Adrenalectomized	8	11.6 ± 1.1 (<i>P</i> < 0.001)	62.0 ± 7.5 (<i>P</i> = 0.004)	329 ± 43 (<i>P</i> < 0.001)

Table 5. *Effect of adrenalectomy on the incorporation of ^{14}C into total lipids by mammary-gland tissue from $[U-^{14}C]$ glucose in the presence and absence of unlabelled acetate, and from $[1-^{14}C]$ acetate in the presence and absence of unlabelled glucose*

Mammary-gland slices (200 mg.) were incubated for $1\frac{1}{2}$ hr. at 37° in 5.0 ml. of Krebs-Ringer bicarbonate buffer containing: (a) $0.50\ \mu C$ of $[U-^{14}C]$ glucose and 50 μ moles of unlabelled glucose; (b) $0.50\ \mu C$ of $[U-^{14}C]$ glucose, 50 μ moles of unlabelled glucose and 50 μ moles of unlabelled acetate; (c) $0.50\ \mu C$ of $[1-^{14}C]$ acetate and 50 μ moles of unlabelled acetate; or (d) $0.50\ \mu C$ of $[1-^{14}C]$ acetate, 50 μ moles of unlabelled acetate and 50 μ moles of unlabelled glucose. The gas phase was $O_2 + CO_2$ (95:5). Values are given as means $\pm$ s.e.m. In parentheses are given Fisher's *P* values for the probability of difference between the mean values; N.S., not significantly different. No s.e.m. or *P* values are given for the experiments in which only $[1-^{14}C]$ acetate was used, as the incorporations were so small.

Radioactive substrate	Status of animal	No. of expts.	Per g. of fat isolated	$10^{-3} \times ^{14}C$ incorporated into lipids (counts/min.)		
				Per 100 mg. of wet wt. of tissue	Per 100 $\mu g.$ of DNA P	Per total gland
$[U-^{14}C]$ Glucose	Sham-operated	6	88 ± 16	1.47 ± 0.27	8.0 ± 1.5	58.7 ± 12.5
	Adrenalectomized	6	51 ± 17 (N.S.)	0.73 ± 0.27 (<i>P</i> = 0.081)	3.9 ± 1.4 (<i>P</i> = 0.073)	22.8 ± 8.9 (<i>P</i> = 0.041)
$[U-^{14}C]$ Glucose + acetate	Sham-operated	8	257 ± 42	2.75 ± 0.45	18.4 ± 3.2	99.9 ± 15.6
	Adrenalectomized	8	88 ± 10 (<i>P</i> = 0.002)	0.99 ± 0.07 (<i>P</i> = 0.002)	5.3 ± 0.5 (<i>P</i> = 0.001)	27.6 ± 2.3 (<i>P</i> < 0.001)
$[1-^{14}C]$ Acetate	Sham-operated	6	3	0.05	0.2	1.8
	Adrenalectomized	6	8	0.10	0.5	3.2
$[1-^{14}C]$ Acetate + glucose	Sham-operated	8	262 ± 40	2.83 ± 0.39	19.1 ± 3.1	103.6 ± 14.9
	Adrenalectomized	8	198 ± 13 (N.S.)	2.10 ± 0.22 (N.S.)	11.3 ± 1.5 (<i>P</i> = 0.037)	59.7 ± 8.0 (<i>P</i> = 0.021)

DISCUSSION

That adrenalectomy diminishes the ability of the mammary glands of lactating rats to oxidize glucose is shown by both the respiratory and iso-

topic studies. It is unlikely that this decline can be accounted for in terms of the anorexia that follows this operation (Folley & French, 1949), as other authors have shown, on a variety of tissues, that the anorexia is not very marked at the short time-

Table 6. *Effect of adrenalectomy on the ability of mammary-gland tissue to convert [1-¹⁴C]pyruvate and [2-¹⁴C]pyruvate into ¹⁴CO₂*

Mammary-gland slices (120 mg.) were incubated for 1 hr. at 37° in 5.0 ml. of Krebs-Ringer bicarbonate buffer containing: (a) 0.50 μ C of [1-¹⁴C]pyruvate, 50 μ moles of unlabelled glucose and 75 μ moles of unlabelled pyruvate; or (b) 0.50 μ C of [2-¹⁴C]pyruvate, 50 μ moles of unlabelled glucose and 75 μ moles of unlabelled pyruvate. The gas phase was O₂ + CO₂ (95:5). Values are given as means $\pm$ s.e.m. In parentheses are given Fisher's *P* values for the probability of difference between the mean values.

Radioactive substrate	Status of animal	No. of expts.	10 ⁻³ $\times$ ¹⁴ C incorporated into CO ₂ (counts/min.)		
			Per 100 mg. wet wt. of tissue	Per 100 μ g. of DNA P	Per total gland
(a) [1- ¹⁴ C]Pyruvate	Sham-operated	6	104.6 $\pm$ 3.9	718.6 $\pm$ 33.5	3809 $\pm$ 256
	Adrenalectomized	6	69.9 $\pm$ 3.9 (<i>P</i> < 0.001)	390.2 $\pm$ 39.9 (<i>P</i> < 0.001)	1959 $\pm$ 191 (<i>P</i> < 0.001)
(b) [2- ¹⁴ C]Pyruvate	Sham-operated	8	20.7 $\pm$ 1.3	136.7 $\pm$ 10.2	752 $\pm$ 45
	Adrenalectomized	8	16.6 $\pm$ 0.9 (<i>P</i> = 0.017)	89.8 $\pm$ 8.7 (<i>P</i> = 0.004)	468 $\pm$ 43 (<i>P</i> < 0.001)

Table 7. *Effect of adrenalectomy on the ability of mammary-gland tissue to incorporate ¹⁴C from [2-¹⁴C]pyruvate into the total lipids*

Mammary-gland slices (120 mg.) were incubated for 1 hr. at 37° in 5.0 ml. of Krebs-Ringer bicarbonate buffer containing: 0.50 μ C of [2-¹⁴C]pyruvate, 50 μ moles of unlabelled glucose and 75 μ moles of unlabelled pyruvate. The gas phase was O₂ + CO₂ (95:5). Values are given as means $\pm$ s.e.m. In parentheses are given Fisher's *P* values for the significance of difference of the mean values; N.S., not significantly different.

Radioactive substrate	Status of animal	No. of expts.	10 ⁻³ $\times$ ¹⁴ C incorporated into lipids (counts/min.)			
			Per g. of fat isolated	Per 100 mg. of wet wt. of tissue	Per 100 μ g. of DNA P	Per total gland
[2- ¹⁴ C]Pyruvate	Sham-operated	8	174 $\pm$ 32	1.99 $\pm$ 0.34	13.1 $\pm$ 2.7	71.7 $\pm$ 6.9
	Adrenalectomized	8	136 $\pm$ 27 (N.S.)	1.59 $\pm$ 0.35 (N.S.)	9.0 $\pm$ 2.2 (N.S.)	49.0 $\pm$ 9.0 (<i>P</i> = 0.066)

Table 8. *Effect of adrenalectomy on the nicotinamide nucleotide content of mammary-gland tissue*

Values are given as means $\pm$ s.e.m. In parentheses are given Fisher's *P* values for the probability of difference between the mean values; N.S., not significantly different.

Nucleotide	Status of animal	No. of expts.	Nucleotide content (μ g.)		Calculated ratios:
			Per g. wet wt of tissue	Per total gland	
NAD ⁺	Sham-operated	5	224 $\pm$ 26	1068 $\pm$ 66	$\frac{[\text{NAD}^+]}{[\text{NADH}]}$
	Adrenalectomized	7	254 $\pm$ 28 (N.S.)	752 $\pm$ 98 (<i>P</i> = 0.006)	
NADH	Sham-operated	5	50 $\pm$ 10	212 $\pm$ 34	$\frac{[\text{NADPH}]}{[\text{NADP}^+]}$
	Adrenalectomized	7	46 $\pm$ 5 (N.S.)	140 $\pm$ 22 (<i>P</i> = 0.087)	
NADP ⁺	Sham-operated	5	12 $\pm$ 2	52 $\pm$ 10	Sham-operated
	Adrenalectomized	7	14 $\pm$ 2 (N.S.)	40 $\pm$ 6 (N.S.)	
NADPH	Sham-operated	5	44 $\pm$ 8	188 $\pm$ 32	Adrenalectomized
	Adrenalectomized	7	36 $\pm$ 7 (N.S.)	108 $\pm$ 24 (<i>P</i> = 0.073)	

intervals used in the present work (Carr & Beck, 1937; Tipton, 1941; Willmer, 1960*a, c*; Strickland, 1963).

The major systems for glucose utilization in mammary-gland tissue are the synthesis of lactose, the Embden-Meyerhof pathway and the pentose phosphate pathway. Other pathways do not seem to play any significant role (Abraham & Chaikoff, 1959). The ($^{14}\text{CO}_2$ produced from $[1\text{-}^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6\text{-}^{14}\text{C}]\text{glucose}$) ratio has been proposed as a measure of the extent of operation of the pentose phosphate relative to the Embden-Meyerhof pathway (Bloom & Stetten, 1953), and many workers have applied this to mammary gland. Abraham *et al.* (1954) found a ratio of 16 with mammary-gland slices from 18-day-lactating rats, and Glock, McLean & Whitehead (1956) found that the ratio increased progressively from 1 in late pregnancy to 11 at 8 days of lactation and 16 at 10–18 days. The increasing values of the ($^{14}\text{CO}_2$ produced from $[1\text{-}^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6\text{-}^{14}\text{C}]\text{glucose}$) ratio parallel the increasing activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (McLean, 1958*a*), and the increased production of milk indicating that the ratio is a valid, if only approximate, guide to the degree of activity of the pentose phosphate pathway.

Adrenalectomy causes a fall in the ($^{14}\text{CO}_2$ produced from $[1\text{-}^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6\text{-}^{14}\text{C}]\text{glucose}$) ratio (Table 2) and thus decreases the activity of, at least, the first enzymes of the pentose phosphate pathway relative to the Embden-Meyerhof pathway, in agreement with Willmer (1960*b*), who found a decline in the activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the gland under similar conditions. In contrast with these observations are the many reports that adrenalectomy has little effect on the same enzymes in liver (Huggins & Fung-O Yao, 1959; Weber, Banerjee & Bronstein, 1961; Ashmore, 1959; Freedland & Barnes, 1963).

It is not possible to make a quantitative assessment of the decrease in activity of the pentose phosphate pathway from the ($^{14}\text{CO}_2$ produced from $[1\text{-}^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6\text{-}^{14}\text{C}]\text{glucose}$) ratio in view of the cogent criticisms by Katz & Wood (1960), who have proposed that a better estimate could be obtained by comparing the ^{14}C incorporated into fatty acids from $[6\text{-}^{14}\text{C}]\text{glucose}$ with that from $[1\text{-}^{14}\text{C}]\text{glucose}$.

On the basis that nearly all the ^{14}C incorporated into the total lipids is located in the fatty acid constituents, Chaikoff and his colleagues (Abraham *et al.* 1954; Abraham, Cady & Chaikoff, 1957; Abraham & Chaikoff, 1959) proposed that the (^{14}C incorporated from $[6\text{-}^{14}\text{C}]\text{glucose}$) – (^{14}C incor-

porated from $[1\text{-}^{14}\text{C}]\text{glucose}$)/(^{14}C incorporated from $[6\text{-}^{14}\text{C}]\text{glucose}$) ratio represents the fraction of glucose converted into fatty acids by the pentose phosphate pathway. By this method they have calculated that 40–70 % of the glucose catabolism of normal lactating-rat mammary-gland tissue is by way of the pentose phosphate pathway (Abraham *et al.* 1954; Abraham & Chaikoff, 1959) and have found similar values in the condition of diminished lactation caused by hypophysectomy (Abraham, Cady & Chaikoff, 1960).

By applying a similar calculation to the results in Table 3, which shows that a greater degree of incorporation of ^{14}C from $[6\text{-}^{14}\text{C}]\text{glucose}$ into the lipids occurs, values of the ratio are obtained which show that approx. 50 % of the glucose is metabolized by each of the two pathways (column 8 in Table 3), a value in reasonable agreement with those reported by Chaikoff and his colleagues. As reported for hypophysectomized rats by Abraham *et al.* (1960), the decreased lactation resulting from adrenalectomy does not alter the distribution of glucose catabolism between the two major pathways. This finding is not surprising since three glucose molecules must be oxidized via the pentose phosphate pathway to produce one triose molecule for incorporation into lipid; it is clear that the ratio will not be very sensitive to variations in this pathway.

Katz & Wood (1960) have calculated, on the basis of a model system, that values obtained as above would be maximal, and it was calculated from the results in Table 3 by using their method of calculation that the pentose phosphate pathway in mammary-gland tissue from both adrenalectomized and sham-operated animals may be responsible for only about 25 % of the catabolism of glucose. We have attempted to confirm this calculation by using the incorporation of ^{14}C from $[U\text{-}^{14}\text{C}]\text{glucose}$ as proposed by Katz & Wood (1960), but the (^{14}C incorporated from $[6\text{-}^{14}\text{C}]\text{glucose}$) – (^{14}C incorporated from $[U\text{-}^{14}\text{C}]\text{glucose}$)/(^{14}C incorporated from $[U\text{-}^{14}\text{C}]\text{glucose}$) ratio has a value of 1.6 for sham-operated animals and 0.8 for adrenalectomized animals. As one of these values is greater than unity, it seems that the results obtained under our conditions are not suitable for evaluation in this way.

The incorporation of ^{14}C from $[1\text{-}^{14}\text{C}]\text{glucose}$ into lipids is decreased by adrenalectomy. Since the incorporation of ^{14}C from $[1\text{-}^{14}\text{C}]\text{glucose}$ into lipid occurs almost exclusively via the Embden-Meyerhof pathway, it follows that the decrease of this incorporation in adrenalectomized animals is due to a decreased activity of this pathway. Fat synthesis from $[1\text{-}^{14}\text{C}]\text{acetate}$ remains unimpaired. The results in Table 3 on lipogenesis from acetate probably reflect a true picture of conditions *in vivo*,

as there is some evidence that glucose does not appreciably dilute the acetate pool, i.e. changes in the rate of glucose metabolism when the concentration exceeds $5 \mu\text{moles/ml.}$ do not affect acetate incorporation by variations in isotope dilution at the acetyl-CoA stage (Abraham, Fitch & Chaikoff, 1961).

The above results, then, suggest a metabolic block between glucose and the formation of acetyl-CoA which was responsible for the diminished conversion of glucose into lipids. This is probably at the level of the decarboxylation of pyruvate, as the yields of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ pyruvate and the decreased incorporation of ^{14}C from $[2-^{14}\text{C}]$ pyruvate into $^{14}\text{CO}_2$ and lipids indicate. Metabolic blocks at other points along the Embden-Meyerhof pathway are not eliminated, however; loss of the adrenal hormones has been reported both to decrease the activities of phosphoglucumutase and phosphoglucose isomerase in liver (Willmer, 1960*a*) and to leave them unchanged (Weber *et al.* 1961).

The 60 % decrease caused by adrenalectomy in the yield of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]$ glucose is a further indication of a fall in the activities of all the pathways that convert glucose into carbon dioxide. The loss of the adrenal hormones would also seem to lower the susceptibility of the mammary gland to alterations in its environment, shown by the differing effects of added glucose or acetate on the metabolism of each other with tissue from either adrenalectomized or sham-operated animals. Thus, for both normal and adrenalectomized rats, unlabelled acetate increases the rate of production of both $^{14}\text{CO}_2$ and ^{14}C -labelled lipid from $[\text{U-}^{14}\text{C}]$ glucose, and unlabelled glucose decreases the yield of $^{14}\text{CO}_2$ and increases the yield of ^{14}C -labelled lipid from $[1-^{14}\text{C}]$ acetate. The magnitude of these effects is, however, much less in mammary-gland tissue from adrenalectomized animals. The connecting link between glucose and acetate metabolism is the role played by NADP, first as a hydrogen acceptor from the pentose phosphate pathway of glucose catabolism and secondly as a hydrogen donor, in its reduced form, in the synthesis of fatty acids from acetate. This interdependence in the mammary gland has been extensively studied by Abraham & Chaikoff (1959). The stimulating effect of acetate on the production of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]$ glucose can readily be explained in terms of the reoxidation of NADPH, stimulating oxidation via the pentose phosphate pathway, and the effect of glucose in stimulating lipogenesis from acetate could be explained in terms of the formation of NADPH via the same pathway to provide the reductive potential required for lipogenesis. The provision of NADPH would also tend to divert acetyl-CoA into lipogenesis rather than into an oxidative pathway.

Adrenalectomy considerably decreases the conversion of glucose into acetyl-CoA, as shown by the results in Table 6 on the decarboxylation of pyruvate, so that the slight response of mammary-gland tissue from adrenalectomized animals to added acetate or glucose where glucose metabolism or acetate metabolism respectively is concerned is not unexpected.

McLean (1960) used phenazine methosulphate in her studies of the metabolism of mammary gland, and suggested that this hydrogen acceptor could be used to assess the potential activity of the pentose phosphate pathway under conditions where its activity might be limited by the availability of NADP^+ . In the present experiments phenazine methosulphate caused a greater increase in the production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose by slices from the adrenalectomized animals than from the controls, indicating that the pentose phosphate pathway is more limited by the availability of NADP^+ in adrenalectomized animals than in the controls. Since the regeneration of NADP^+ is brought about by lipogenesis it might be thought that the greater increase in the production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose in the presence of phenazine methosulphate in adrenalectomized animals could be ascribed to a decrease in the rate of fatty acid synthesis in these animals. However, the incorporation of acetate into lipids is as efficient in the mammary glands of adrenalectomized rats as it is in those from the controls, and it would appear that the enzymic machinery of lipogenesis is unimpaired; it follows, therefore, from the above results, that the most significant lesion is in the formation of acetyl-CoA from pyruvate. Impairment of the activity of this step would diminish lipogenesis by decreasing the availability of substrate and this, in turn, would decrease the activity of the pentose phosphate pathway by decreasing the rate of reoxidation of NADPH. In similar studies Fain & Wilhelmi (1962) reported that adrenalectomy does not affect the incorporation of ^3H from ^3H -labelled water into fats, although they did not investigate mammary gland. On the other hand, Perry & Bowen (1956*a, b*) concluded that there was a slight decrease in lipogenesis in rat liver as a consequence of adrenalectomy.

The results on the nicotinamide nucleotide contents of mammary-gland tissue presented above show that adrenalectomy has no effect, either on the concentrations ($\mu\text{g./g.}$) present or on the relative amounts of the oxidized and reduced forms present. If there are nucleotide limitations caused by adrenalectomy, as suggested above for NADP, and which affect glucose metabolism, these must be at the intracellular level and therefore not detectable in the studies on whole cells described above. The amounts present in the whole gland, however,

are considerably decreased by adrenalectomy, and as it is the whole gland that is the physiological unit supplying the pups with milk this is perhaps a more reliable estimate of the influence of the loss of the adrenal hormones on mammary-gland tissue.

Thus the overall picture emerges that, on the basis of total activity in the whole mammary gland, adrenalectomy lowers every metabolic parameter tested by a factor of up to 3, relative to the mammary glands of the controls, except that the conversion of acetate into carbon dioxide remains higher. If, as seems likely, it is the absence of the adrenal-cortical steroids that gives rise to the effects of adrenalectomy (Cowie, 1952; Cowie & Tindal, 1955; Anderson & Turner, 1962), then the suggestion that hormones may regulate cellular metabolism by directing the flow of substrates along the various pathways that utilize them (Ashmore, Cahill & Hastings, 1960) is attractive to explain some of the effects described above, particularly if the hormones modify the pattern of distribution of the various coenzymes at the sites where they are needed. It is also possible that the loss of these hormones adversely affects the permeability of the cell membranes of mammary-gland tissue to glucose and other metabolites (see Hechter & Lester, 1960; Gallagher, 1960), and that this is, in part, responsible for the results described above, but the nature of the present experiments permits no definite conclusion to be drawn along these lines.

SUMMARY

1. The effect of adrenalectomy on the metabolism of glucose by the mammary gland was studied with the aid of differentially ^{14}C -labelled glucose and pyruvate, and with ^{14}C -labelled acetate.

2. Both the Embden-Meyerhof and the pentose phosphate pathways of glucose degradation were decreased in activity by the removal of the adrenal glands. The addition of phenazine methosulphate to mammary-gland slices oxidizing $[1-^{14}\text{C}]$ glucose restored the incorporation of ^{14}C into $^{14}\text{CO}_2$ towards control levels, indicating that the observed decrease in the activity of the pentose phosphate pathway could be attributed, at least in part, to a limitation imposed by a lack of available NADP^+ .

3. The incorporation of ^{14}C from $[1-^{14}\text{C}]$ acetate into $^{14}\text{CO}_2$ and ^{14}C -labelled lipids indicated that the pathways of lipogenesis and the tricarboxylic acid cycle were relatively unimpaired by adrenalectomy.

4. By using $[1-^{14}\text{C}]$ pyruvate and $[2-^{14}\text{C}]$ pyruvate it was shown that a metabolic block exists in the mammary glands of adrenalectomized rats at the level of the decarboxylation of pyruvate.

5. Measurements were made of the nicotinamide nucleotide content of the mammary gland. The concentration and the ratio of oxidized to

reduced forms remained unchanged by the operation, although there was a loss of all forms when the results were considered on the whole-gland basis.

6. The results are discussed in relation to the mutually regulatory effects of the two pathways of glucose catabolism and lipogenesis, and in relation to the nature of the primary lesion in the mammary glands of adrenalectomized rats which leads to the observed changes.

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The Effect of Reserpine on the Metabolism of Glucose by Mammary Glands from Rats after Weaning

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Removal of the pups from a lactating animal leads to involution of the mammary glands. The involutionary process is characterized by an engorgement of the gland with retained milk, followed, after some 15 hr., by changes in the metabolic activity that eventually result in the destruction of alveolar tissue, a diminution in the size of the gland and the cessation of milk secretion (Cowie & Folley, 1961). The sequence of events leading to these morphological and biochemical changes is not known, nor has the lesion that initiates the process been defined. Factors that might be involved are the removal of the suckling stimulus (Selye & McKeown, 1934; Williams, 1945; Benson & Folley, 1957), or a disruption of the alveolar cells or lysosomal particles by the high back-pressure of the accumulated milk in the early stages of involution (Slater, Greenbaum & Wang, 1963).

There have been a number of studies of the changes in the activities of enzymes in involution, and the pattern that has emerged is one of a general decrease in the activities of the enzymes involved in respiration (Folley & French, 1949; Greenbaum & Slater, 1957), in glucose catabolism (Glock & McLean, 1953) and in amino acid metabolism (Greenbaum & Greenwood, 1954). On the other hand, the activities of the lysosomal enzymes increase during the early stages of involution (Greenbaum & Greenwood, 1954; Slater, 1961; Slater *et al.* 1963), as does the activity of 5'-

nucleotidase (Wang, 1962). It seems therefore that involution causes decreases in the activities of those enzymes involved in the energy metabolism and synthetic processes of the gland while it causes increases above the lactational level in the activities of the hydrolases.

The administration of the tranquillizing drug reserpine to weaned rats prevents many of the degenerative changes seen in the mammary glands of untreated weaned animals. Morphological studies on the glands of reserpine-treated rats (Benson, 1958, 1960) show a remarkable degree of maintenance of the alveolar structure of the mammary-gland tissue for up to 9 days after removal of the pups, at times when untreated glands show extensive regression of the tissue structure. What is not clear from these studies is whether the glands maintain their biochemical, as well as their anatomical, integrity under the influence of reserpine. The present investigations were into some aspects of glucose metabolism in the mammary glands of reserpine-treated animals to establish to what degree the histological picture found by Benson (1958, 1960) reflects a maintenance of biochemical function.

METHODS

Animals. All the animals used were hooded Norwegian rats undergoing their first lactation, and of body wt. 220–240 g. The number of pups in the litters was restricted