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Biochem. J. (1964) **91**, 317

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

to eight at birth. A standard diet (MRC no. 41 B; Bruce & Parkes, 1946) and water were supplied *ad libitum*. Weaning took place on the sixteenth to the eighteenth day of lactation, when the administration of reserpine was begun; this consisted of a daily dose of 75 μ g. of the commercial preparation Serpasil (Ciba Ltd., Horsham, Sussex) for 3 days, injected subcutaneously. After this time the animals were killed by cervical dislocation and their abdominal mammary glands excised.

Reagents. [1^{14}C]Glucose, [6^{14}C]glucose and [1^{14}C]acetate 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.

Experimental procedures. The preparation of tissue slices, the conditions of incubation, the procedures for the measurement of the radioactivity of $^{14}\text{CO}_2$ and ^{14}C -labelled lipids formed from the labelled substrates, and the determination of the nicotinamide nucleotides were as described by Greenbaum & Darby (1964).

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 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 the retained milk. No information is available about alterations in the composition of milk in mammary glands during involution or under the influence of reserpine, and a milk correction cannot therefore be applied.

Results were also calculated for the whole gland, as it is the whole gland that supplies pups with milk, and changes in the total metabolism of the gland will reflect alterations in its potential milk-producing capacity.

RESULTS

If reserpine maintains the morphological integrity of the involuting mammary gland, the weight of tissue taken from rats treated with reserpine should be greater than that taken from rats undergoing normal involution. Some indication that this is so emerges from the values obtained in the present work for the total gland weights, namely 4.8 ± 0.45 g. for control animals (six rats) and 6.8 ± 0.56 g. for reserpine-treated animals (six rats). Correction for the retained milk in the glands is not possible for either set of animals (see above), and the difference in total gland weight makes a comparison of metabolic activities expressed on a 100 mg. or g. wet wt. of tissue basis less meaningful than a comparison of activities for the total mammary-gland tissue. The latter was therefore adopted.

Reserpine significantly increases the production of $^{14}\text{CO}_2$ by involuting mammary-gland tissue from both [1^{14}C]glucose and from [6^{14}C]glucose when expressed as $^{14}\text{CO}_2$ produced by the entire gland. The effect is so large that it is still significant for [6^{14}C]glucose expressed on the basis of counts/100 mg. wet wt. of tissue and almost so for [1^{14}C]glucose (Table 1). The addition of phenazine metho-

Table 1. *Effect of reserpine on the ability of involuting mammary-gland tissue to convert [1^{14}C]glucose, [6^{14}C]glucose, and [1^{14}C]acetate into $^{14}\text{CO}_2$*

Radioactive substrate	Treatment	No. of expts.	$10^{-3} \times ^{14}\text{C}$ incorporated into CO_2 (counts/min.)		Calculated ratios:
			Per 100 mg. wet wt. of tissue	Per total gland	
(a) [1^{14}C]Glucose	None	6	4.2 ± 0.4	192 ± 20	$^{14}\text{CO}_2$ produced from [1^{14}C]glucose $^{14}\text{CO}_2$ produced from [6^{14}C]glucose Control Reserpine-treated
	Reserpine	6	5.2 ± 0.4 ($P = 0.062$)	362 ± 26 ($P < 0.001$)	
(b) [6^{14}C]Glucose	None	6	1.2 ± 0.1	52 ± 5	$^{14}\text{CO}_2$ produced from [1^{14}C]glucose in the presence of phenazine methosulphate $^{14}\text{CO}_2$ produced from [1^{14}C]glucose Control Reserpine-treated
	Reserpine	5	2.0 ± 0.2 ($P = 0.005$)	128 ± 15 ($P < 0.001$)	
(c) [1^{14}C]Glucose + phenazine methosulphate	None	6	36.8 ± 3.4	1705 ± 245	$^{14}\text{CO}_2$ produced from [1^{14}C]glucose in the presence of phenazine methosulphate $^{14}\text{CO}_2$ produced from [1^{14}C]glucose Control Reserpine-treated
	Reserpine	6	42.8 ± 5.4 (N.S.)	3053 ± 397 ($P = 0.016$)	
(d) [1^{14}C]Acetate	None	6	5.3 ± 0.5	230 ± 20	$^{14}\text{CO}_2$ produced from [1^{14}C]glucose Control Reserpine-treated
	Reserpine	6	5.0 ± 0.6 (N.S.)	343 ± 37 ($P = 0.031$)	

Animals were weaned for 3 days before killing; the treatment with reserpine consisted of one injection of 75 μ g. of reserpine daily during this period. 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 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 mol of phenazine methosulphate; or (d) 0.50 μ C of [1^{14}C]acetate, 50 μ moles of unlabelled acetate. The gas phase was $\text{O}_2 + \text{CO}_2$ (95:5). Values are expressed 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.

Table 2. *Effect of reserpine on the ability of involuting mammary-gland tissue to incorporate ^{14}C from $[1-^{14}\text{C}]\text{glucose}$, $[6-^{14}\text{C}]\text{glucose}$ and $[1-^{14}\text{C}]\text{acetate}$ into the total lipids*

Animals were weaned for 3 days before being killed; the treatment with reserpine consisted of one injection of 75 μg . of reserpine daily during this time. Mammary-gland slices (200 mg.) were incubated for 1½ hr. at 37° in 5.0 ml. of Krebs–Ringer bicarbonate buffer solution containing: (a) 0.50 μC of $[1-^{14}\text{C}]\text{glucose}$ and 50 μmoles of unlabelled glucose; (b) 0.50 μC of $[6-^{14}\text{C}]\text{glucose}$ and 50 μmoles of unlabelled glucose; (c) 0.50 μC of $[1-^{14}\text{C}]\text{glucose}$, 50 μmoles of unlabelled glucose and 1 μmole of phenazine methosulphate; or (d) 0.50 μC of $[1-^{14}\text{C}]\text{acetate}$, 50 μmoles of unlabelled acetate and 50 μmoles of unlabelled glucose. The gas phase was $\text{O}_2 + \text{CO}_2$ (95:5). Values are expressed as means $\pm$ s.e.m. N.S., not significantly different. No values are shown for the incubations with phenazine methosulphate, as the incorporations obtained were too low to measure.

Radioactive substrate	Treatment	No. of expts.	$10^{-3} \times ^{14}\text{C}$ incorporated into lipids (counts/min.)		
			Per g. of fat isolated	Per 100 mg. wet wt. of tissue	Per total gland
(a) $[1-^{14}\text{C}]\text{Glucose}$	None	6	11 $\pm$ 3	0.16 $\pm$ 0.02	7.4 $\pm$ 1.1
	Reserpine	6	12 $\pm$ 2 (N.S.)	0.16 $\pm$ 0.04 (N.S.)	10.1 $\pm$ 2.4 (N.S.)
(b) $[6-^{14}\text{C}]\text{Glucose}$	None	6	9 $\pm$ 2	0.13 $\pm$ 0.02	6.0 $\pm$ 1.4
	Reserpine	6	10 $\pm$ 2 (N.S.)	0.13 $\pm$ 0.04 (N.S.)	8.1 $\pm$ 2.0 (N.S.)
(d) $[1-^{14}\text{C}]\text{Acetate}$	None	6	5 $\pm$ 2	0.08 $\pm$ 0.01	3.6 $\pm$ 0.7
	Reserpine	6	7 $\pm$ 2 (N.S.)	0.08 $\pm$ 0.02 (N.S.)	4.9 $\pm$ 1.1 (N.S.)

sulphate stimulates the production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$ equally with mammary-gland slices from both types of rat. The oxidation of $[1-^{14}\text{C}]\text{acetate}$ is also significantly increased when considered on the basis of the whole gland but is not affected when calculated per 100 mg. wet wt. of tissue. The ($^{14}\text{CO}_2$ produced from $[1-^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6-^{14}\text{C}]\text{glucose}$) ratio is decreased by the administration of the drug: this might indicate a decrease in the activity of the pentose phosphate pathway relative to the Embden–Meyerhof pathway (but see the Discussion section).

Comparison of the results in Table 1 with those available for normal lactating animals (Greenbaum & Darby, 1964) indicates that the yields of $^{14}\text{CO}_2$ from both $[1-^{14}\text{C}]\text{glucose}$ and $[6-^{14}\text{C}]\text{glucose}$ are considerably decreased by the involutory processes, which is in agreement with the results of McLean (1958*a*; see Glock & McLean, 1958), and the low ($^{14}\text{CO}_2$ produced from $[1-^{14}\text{C}]\text{glucose}$)/($^{14}\text{CO}_2$ produced from $[6-^{14}\text{C}]\text{glucose}$) ratios also agree with their published results. Phenazine methosulphate stimulates the production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$ to a much greater extent with mammary-gland tissue from weaned rats than with that from lactating rats. The yields of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{acetate}$ is also much greater in tissue from weaned rats if the results are expressed on the basis of the whole gland, although they are virtually unchanged when considered on the basis of 100 mg. wet wt. of tissue.

The incorporation of ^{14}C from $[1-^{14}\text{C}]\text{glucose}$, $[6-^{14}\text{C}]\text{glucose}$ and $[1-^{14}\text{C}]\text{acetate}$ into the total

lipids of mammary-gland tissue from involuting glands is negligible when compared with the incorporation found for lactating tissue (Greenbaum & Darby, 1964), and reserpine does not affect this low rate of incorporation (Table 2). In the presence of phenazine methosulphate no incorporation of ^{14}C could be detected.

In view of the possible decrease in the activity of the pentose phosphate pathway caused by the administration of reserpine (see the ratios in column 6 in Table 1), the nicotinamide nucleotide contents of the involuting glands were also investigated. Comparison of results in Table 3 with the published results of McLean (1958*b*) and Greenbaum & Darby (1964) for normal lactating rats shows that the involutory process is accompanied by a decrease in the total gland contents of NAD^+ , NADH and NADPH , while the NADP^+ content remains relatively unchanged. The values, with the exception of NADH , are also in good agreement with those of McLean (1958*b*) for involuting mammary gland. The drug reserpine has no effect on the NAD^+ and NADP^+ contents, but causes a twofold increase in the NADH and NADPH contents.

DISCUSSION

The most obvious effects of involution on the mammary gland are the virtual disappearance of lipogenesis, a decrease in the activity of both pathways of glucose utilization and a fall in the amount of nicotinamide nucleotide available, particularly the reduced nicotinamide nucleotide. Treatment of the animal with reserpine during the period of

Table 3. *Effect of reserpine on the nicotinamide nucleotide content of involuting mammary-gland tissue*

Animals were weaned for 3 days before being killed; the treatment with reserpine consisted of one injection of 75 μ g. of reserpine daily during this time. 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	Treatment	No. of expts.	Nucleotide content (μ g.)			Calculated ratios:
			Per g. wet wt. of tissue	Per 100 μ g. of DNA P	Per total gland	
NAD ⁺	None	7	87.2 $\pm$ 12.6	100.3 $\pm$ 9.3	458 $\pm$ 75	$\left\{ \begin{array}{l} \frac{[\text{NAD}^+]}{[\text{NADH}]} \\ \text{Control} \quad 9.1 \pm 2.7 \\ \text{Reserpine-treated} \quad 3.2 \pm 0.5 \\ (P = 0.071) \end{array} \right.$
	Reserpine	6	44.4 $\pm$ 2.6 (<i>P</i> = 0.010)	43.6 $\pm$ 4.5 <i>P</i> < 0.001	425 $\pm$ 55 (N.S.)	
NADH	None	7	12.2 $\pm$ 1.9	15.0 $\pm$ 2.0	63 $\pm$ 9	$\left\{ \begin{array}{l} \frac{[\text{NADPH}]}{[\text{NADP}^+]} \\ \text{Control} \quad 2.1 \pm 0.4 \\ \text{Reserpine-treated} \quad 4.2 \pm 0.6 \\ (P = 0.014) \end{array} \right.$
	Reserpine	6	15.0 $\pm$ 1.8 (N.S.)	14.7 $\pm$ 2.0 (N.S.)	143 $\pm$ 20 (<i>P</i> = 0.003)	
NADP ⁺	None	7	4.0 $\pm$ 0.7	4.8 $\pm$ 0.9	21 $\pm$ 4	$\left\{ \begin{array}{l} \frac{[\text{NADPH}]}{[\text{NADP}^+]} \\ \text{Control} \quad 2.1 \pm 0.4 \\ \text{Reserpine-treated} \quad 4.2 \pm 0.6 \\ (P = 0.014) \end{array} \right.$
	Reserpine	6	2.2 $\pm$ 0.3 (<i>P</i> = 0.050)	2.1 $\pm$ 0.3 (<i>P</i> = 0.008)	20 $\pm$ 3 (N.S.)	
NADPH	None	7	6.9 $\pm$ 1.1	8.3 $\pm$ 3.9	36 $\pm$ 6	$\left\{ \begin{array}{l} \frac{[\text{NADPH}]}{[\text{NADP}^+]} \\ \text{Control} \quad 2.1 \pm 0.4 \\ \text{Reserpine-treated} \quad 4.2 \pm 0.6 \\ (P = 0.014) \end{array} \right.$
	Reserpine	6	8.3 $\pm$ 0.4 (N.S.)	8.2 $\pm$ 0.8 (N.S.)	76 $\pm$ 6 (<i>P</i> = 0.001)	

involution has no effect on the loss of lipogenetic ability, but considerably retards the rate of disappearance of the pentose phosphate and Embden-Meyerhof pathways and shifts the steady state between the oxidized and reduced forms of the nicotinamide nucleotides in favour of the reduced forms.

If the results are considered on the basis of the activity of the total gland, it is clear that 3 days of involution decreases the conversion of [1-¹⁴C]-glucose into ¹⁴CO₂ by about eightfold, whereas the conversion of [6-¹⁴C]glucose into ¹⁴CO₂ is reduced by fourfold, relative to the values reported by Greenbaum & Darby (1964) for mammary-gland tissue from normal rats that had been lactating for 8 days. It may be deduced that the activity of the pentose phosphate pathway for glucose degradation is considerably lowered by the involutionary process, relative to the activity of the Embden-Meyerhof pathway. In view of the criticisms of Wood & Katz (1958) and Katz & Wood (1960), it is not desirable to attempt to assess quantitatively the contribution of the pentose phosphate pathway to glucose catabolism in the involuting gland from the results presented above. The (¹⁴C produced from [1-¹⁴C]glucose)/(¹⁴CO₂ produced from [6-¹⁴C]glucose) ratio is, however, so widely different in lactation and involution (19 on the eighteenth day of lactation, 4 on the third day of involution) that it may be concluded that the activity of the pentose phosphate pathway falls more rapidly after the onset of involution than does that of the Embden-Meyerhof pathway. This loss of activity of the pentose phosphate pathway in involution is in agreement with the enzymic and isotopic studies by McLean (1958a).

The decrease in the activity of the pentose phosphate pathway is not entirely due to the loss of enzymic material. The addition of phenazine methosulphate to slices of mammary-gland tissue from weaned rats raises the rate of production of ¹⁴CO₂ from [1-¹⁴C]glucose towards the level found with slices from fully lactating glands in the presence of the same substrate. McLean (1960) used phenazine methosulphate as a hydrogen acceptor to circumvent a limitation in the supply of NADP⁺, thus releasing the full potential activity of the pathway. The effect of this acceptor in restoring the production of ¹⁴CO₂ from [1-¹⁴C]glucose towards the level found for the lactating gland, in the present work, may be interpreted as showing that the apparent loss of activity of the pentose phosphate pathway is only partially due to an actual loss of enzyme, and that other factors are involved (see below). The restoration in activity as a result of the addition of phenazine methosulphate is greater for the reserpine-treated rats (to about 80 % of the value found for 18-day-lactating rats) than it is for the normal involuting gland (to about 50 % of the value found for 18-day-lactating rats). One result of treatment with reserpine, therefore, is that it tends to preserve this part of the enzymic machinery.

The association of a high potential activity of the pentose phosphate pathway with a low activity in the absence of phenazine methosulphate (Table 1) is best explained in terms of the observed failure of lipogenesis (Table 2). The two processes are intimately linked by way of NADP, and a failure of fat synthesis would result in a lack of NADP⁺ owing to the accumulation of NADPH. There is a small difference in the rate of lipogenesis between the

mammary-gland tissue from reserpine-treated animals and that of the weaned control animals (Table 2), and this is, apparently, sufficient to bring about a stimulation of the oxidation of [$1\text{-}^{14}\text{C}$]glucose in the glands of the reserpine-treated animals (Table 1). However, only about one-tenth of the potential activity for the oxidation of [$1\text{-}^{14}\text{C}$]glucose (Greenbaum & Darby, 1964) is observed in the involuting gland of either type of rat.

On the basis of the above considerations, changes might be expected in the relative amounts of the oxidized and reduced forms of the nicotinamide nucleotides in the involuting mammary-gland tissue, and the accumulation of these in the reduced forms is not surprising in view of the very low rate of fatty acid synthesis. The very close association between the activity of the pentose phosphate pathway and lipogenesis in the mammary gland has been amply demonstrated (Glock & McLean, 1953; Glock, McLean & Whitehead, 1956; Abraham & Chaikoff, 1959; McLean, 1960). The failure of lipogenesis would result in a decline in the reoxidation rate of the NADPH generated in the pentose phosphate pathway, and the accumulation of the reduced form. The higher content of these forms in the reserpine-treated animals is also readily explained by a higher activity of the pentose phosphate pathway in these animals, accompanying a smaller rise in lipogenesis.

The oxidation of acetate by the tricarboxylic acid cycle is probably unimpaired after 3 days of involution, although the Embden-Meyerhof pathway for glucose degradation is lowered in activity, as judged by the yields of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]acetate and [$6\text{-}^{14}\text{C}$]glucose, which may be compared with the values reported by Greenbaum & Darby (1964) for mammary glands from normal 8-day-lactating animals. It seems that involuting mammary-gland tissue possesses a reasonably active Embden-Meyerhof pathway, a dormant (but potentially active) pentose phosphate pathway and an active tricarboxylic acid cycle. Reserpine affects only the decline in activity of the two former pathways, and this only to a minor degree.

Fat synthesis is virtually non-existent. A partial explanation for this may be found in the decreased activity of the two pathways of glucose utilization and the unimpaired activity of the tricarboxylic acid cycle. It may be suggested that the lowered rate of glucose catabolism leads to a diminished rate of acetyl-CoA formation. At low concentrations of acetyl-CoA, competition in the involuting gland for the available substrate between lipogenesis and the tricarboxylic acid cycle could be heavily weighted in favour of the latter system. This would decrease lipogenesis which would, in turn, lead to a further depression in the rate of glucose catabolism owing to a lack of NADP^+ .

From the above considerations, it would appear that the involuting gland still possesses a considerable capacity for the oxidation of glucose, and the nicotinamide nucleotide complement necessary to achieve this. The low rate of oxidation of glucose found can therefore be ascribed to a failure of the systems leading to the regeneration of the oxidized forms of the nicotinamide nucleotides, i.e. lipogenesis. It seems probable, then, that the primary lesion in the involuting gland is the failure of lipogenesis. The consequences of this can readily be seen in terms of the accumulation of NADH and NADPH, both of which are the primary hydrogen donors in the fat-synthesis pathways (Wakil, 1962; Ganguly, 1960; Matthes, Abraham & Chaikoff, 1963). The nature of the block in lipogenesis is not yet resolved.

It is apparent that, although reserpine tends to maintain the integrity of some of the enzyme systems (when the results are calculated on the basis of total enzyme in the mammary glands), it does little to maintain their actual enzymic activity. There is little to distinguish the normal involuting gland from the gland from a reserpine-treated animal on a biochemical basis.

SUMMARY

1. Studies were made of the metabolism *in vitro* of glucose and acetate by the mammary glands of lactating rats after removal of the young, and of the pretreatment of such rats with reserpine. Differentially ^{14}C -labelled glucose and acetate were used for this purpose.

2. The activities of both the Embden-Meyerhof and pentose phosphate pathways of glucose catabolism in the mammary glands of weaned rats were about one-tenth of those found in the mammary glands of lactating rats. Treatment with reserpine maintained these activities nearer lactational levels.

3. Phenazine methosulphate restored the rate of production of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glucose by the involuting mammary-gland slices to about 50 % of the rate found for mammary-gland slices from normal lactating rats. Pretreatment with reserpine accentuated the stimulatory effect of phenazine methosulphate, the rate of production of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glucose being about 80 % of that found for lactating animals.

4. Reserpine had virtually no effect on the exceedingly low rate of lipogenesis found for the mammary glands of the weaned animals.

5. The nicotinamide nucleotides of involuting mammary gland were maintained in the reduced form by treatment with reserpine.

6. The results are discussed in terms of the interdependence of glucose catabolism and lipogenesis, mediated by the nicotinamide nucleotides.

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Biosynthesis of Polynucleotides

5. THE EFFECT OF ADENINE ON THE UTILIZATION OF GLUCOSE FOR RIBONUCLEIC ACID SYNTHESIS IN *CANDIDA UTILIS**

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During the previous work on the formation of RNA in *Candida utilis* (Barker, Hignett, Jackson & Wadsworth, 1961) it was found that addition of adenine to cultures growing exponentially in a synthetic medium containing glucose as the sole carbon source reduced the incorporation of isotopic carbon from [¹⁴C]glucose into the ribosyl residues of RNA. A preliminary report of some of the experiments has been published (Barker, Hignett, Nicholson & Thompson, 1962). The present paper records in detail the conditions under which the effect is observed.

MATERIALS AND METHODS

Analytical methods. Ribose was determined by the method of Albaum & Umbreit (1946), glucose by the iodometric method of Somogyi (1952), and phosphorus by the method of Fiske & Subbarow (1925). Concentrations of nucleotide solutions were measured spectroscopically,

appropriate molecular extinction coefficients being used (Beaven, Holiday & Johnson, 1955). Radioactivities of nucleotides and ribose were determined with an end-window counter as previously described (Barker *et al.* 1961). Radioactivity of BaCO₃ was measured with the same end-window counter and layers of BaCO₃ containing not less than 20 mg./cm.² on planchets having an area of 1 cm.². Specific activities of BaCO₃ were determined by comparison of experimental samples with a standard planchet.

Preparation of mixed ion-exchange resin column. Amberlite IR-45 (Rohm and Haas Co., Philadelphia, U.S.A.) and Zeo-Karb 225 (The Permutit Co., London) (both 200 mesh) were washed with hot ethanol to remove coloured material and were converted respectively into the OH⁻ and H⁺ forms (after repeated cycling through salt forms). The resins were mixed in quantities inversely proportional to their capacities, and a slurry of the mixture was introduced into a column (10 cm. × 1 cm.) and the liquid removed rapidly by application of pressure to avoid separation of the resins during settling.

Growth of yeast. The organisms used were *Candida utilis* strain IMI 23311, obtained from the Commonwealth Mycological Institute, and strain NCYC 321 supplied by

* Part 4: Barker, Hignett, Jackson & Wadsworth (1961).