

## Interactions of Insulin, Corticosterone and Prolactin in Promoting Milk-Fat Synthesis by Mammary Explants from Pregnant Rabbits

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1. The rate of fatty acid synthesis by mammary explants from rabbits pregnant for 16 days or from rabbits pseudopregnant for 11 days was stimulated up to 15-fold by culturing for 2–4 days with prolactin. This treatment initiated the predominant synthesis of C<sub>8:0</sub> and C<sub>10:0</sub> fatty acids, which are characteristic of rabbit milk. 2. Inclusion of insulin in the culture medium increased the rate of synthesis of these medium-chain fatty acids. By contrast the inclusion of corticosterone led to the predominant synthesis of long-chain fatty acids. When explants were cultured for 2–4 days with insulin, corticosterone and prolactin, the rate of fatty acid synthesis increased up to 42-fold, but both medium- and long-chain fatty acids were synthesized. 3. These results show that the stimulus to mammary-gland lipogenesis and the initiation of synthesis of medium-chain fatty acids observed between days 16 and 23 of pregnancy in the rabbit can be simulated *in vitro* by prolactin alone. 4. When mammary explants from rabbits pregnant for 23 days were cultured for 2 days with insulin, corticosterone and prolactin, the rate of fatty acid synthesis increased fivefold, but there was a preferential synthesis of long-chain fatty acids. Culture with prolactin alone had little effect on the rate or pattern of fatty acids synthesized. 5. The results are compared with findings *in vivo* on the control of lipogenesis in the rabbit mammary gland, and are contrasted with the known effects of hormones *in vitro* on the mammary gland of the mid-pregnant mouse.

Rabbit milk triglyceride contains up to 70% of octanoic (C<sub>8:0</sub>) and decanoic (C<sub>10:0</sub>) acids (Smith *et al.*, 1968). These acids are synthesized within the mammary glands of lactating rabbits (Popják *et al.*, 1953; Carey & Dils, 1972), and are the major fatty acids synthesized by slices from this tissue (Strong & Dils, 1972a). We have found (Strong & Dils, 1972b) that mammary tissue from rabbits pregnant for 16 days synthesized predominantly long-chain (C<sub>14</sub>–C<sub>18</sub>) fatty acids. By day 23 of pregnancy, the rate of fatty acid synthesis had increased up to 12-fold and C<sub>8:0</sub> and C<sub>10:0</sub> acids were being synthesized in the same proportions as in the lactating gland. At the end of pregnancy, a second increase in the rate of lipogenesis occurred although the pattern of fatty acids synthesized did not change.

When mammary explants from pseudopregnant rabbits were cultured with insulin, corticosterone and prolactin, there was a striking increase both in the overall rate of fatty acid synthesis and in the proportions of C<sub>8:0</sub> and C<sub>10:0</sub> acids synthesized (Strong *et al.*, 1972). In the present paper, we describe the effects of combinations of these hormones on the rate and pattern of fatty acids synthesized by mammary explants from rabbits on days 16 and 23 of

pregnancy and by mammary explants from pseudopregnant rabbits.

A preliminary report has been given of some of the results (Dils *et al.*, 1972).

### Materials and Methods

#### Animals

Each experiment was done with mammary tissue from a single rabbit. Four rabbits pregnant for 16 days and two rabbits pregnant for 23 days were used. Pregnancy was timed from the day of mating. Two other rabbits were used on the eleventh day of pseudopregnancy induced by intravenous injection of 50 i.u. of human chorionic gonadotrophin. Rabbits were previously unmated New Zealand White females aged 9–12 months. Other details have been published previously (Strong *et al.*, 1972).

#### Methods

Details of the preparation, culture and incubation of lobulo-alveolar explants and of the analysis of synthesized <sup>14</sup>C-labelled fatty acids have been given (Strong *et al.*, 1972). Groups of six explants were

cultured on 1 ml of medium 199 containing combinations of insulin (5 µg/ml), corticosterone (1 µg/ml) and prolactin (1 µg/ml) as indicated. Unless otherwise stated, more than 75% of the epithelial cells remained viable at the end of the culture periods. The criteria used to assess viability have been described (Strong *et al.*, 1972). After culture, explants were incubated for 1 h at 37°C in 1.0 ml of Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) containing 0.1 mM-sodium [ $^{14}\text{C}$ ]acetate (2 µCi) plus 10 mM-glucose as substrate. The variation observed in the rate of acetate incorporation into fatty acids between groups of explants from duplicate cultures was usually  $\pm 10\%$  of the mean value.

## Results

### *Fatty acid synthesis by mammary explants from rabbits pregnant for 16 days*

Mammary explants from rabbits on day 16 of pregnancy synthesized predominantly long-chain ( $\text{C}_{12}$ – $\text{C}_{18}$ ) fatty acids from  $^{14}\text{C}$ -labelled acetate and glucose (Table 1).

When explants were cultured for 2 days with insulin, there was little change in the rate of fatty acid synthesis, though there was a decrease in the proportions of  $\text{C}_{8:0}$  and  $\text{C}_{18}$  acids formed. By contrast, culture for 2 days with prolactin stimulated fatty acid synthesis eightfold, and led to a striking increase in synthesis of the medium-chain fatty acids which are characteristic of rabbit milk. In this experiment, the overall rate of fatty acid synthesis and the proportions of medium-chain fatty acids synthesized increased further when the culture period was extended to 4 days. In a second experiment, however, survival of mammary explants was poor after culture for 4 days in prolactin alone. Insulin in the presence of prolactin increased the overall rate of fatty acid synthesis by explants cultured for 2 or 4 days, and tended to improve tissue viability over the 4-day culture period. It did not, however, alter the pattern of fatty acids synthesized.

Culture with corticosterone and prolactin for 4 days stimulated the overall rate of fatty acid synthesis to the same extent as culture with insulin and prolactin. The proportion of medium-chain fatty acids formed remained rather low, and synthesis of  $\text{C}_{8:0}$  and  $\text{C}_{10:0}$  acids was stimulated to different extents. Similarly, culture for 2 days with insulin and corticosterone followed by culture for 2 or 4 days with insulin and prolactin or with prolactin alone led to higher overall rates of synthesis, but a lower proportion of  $\text{C}_{8:0}$  acid synthesized, than did culture for 4 days with insulin and prolactin. Hence corticosterone antagonized the effect of prolactin in stimulating the synthesis of medium-chain fatty acids. Nevertheless, explants appeared to be more responsive to prolactin

(in terms of the rate of synthesis) after they had been cultured with insulin and corticosterone.

The highest overall rates of fatty acid synthesis occurred after culture with all three hormones, but the proportion of medium-chain fatty acids formed was higher when corticosterone was present only during the first 2 days of culture.

In summary, only prolactin was required to effect the synthesis of medium-chain fatty acids which are characteristic for rabbit milk. Though corticosterone was needed to achieve maximum rates of synthesis, it led to the preferential synthesis of long-chain fatty acids.

### *Fatty acid synthesis by mammary explants from rabbits pregnant for 23 days*

We have shown (Strong & Dils, 1972*b*) that by day 23 of pregnancy the proportions of  $\text{C}_{8:0}$  and  $\text{C}_{10:0}$  acids synthesized by rabbit mammary gland were those synthesized by the gland in lactation, though the overall rate of synthesis was considerably lower. The gestation period for these rabbits was 31–32 days. Between day 28 of pregnancy and day 2 *post partum* the overall rate of fatty acid synthesis increased about tenfold, approaching the rate observed in full lactation. The pattern of fatty acids synthesized was not affected (Strong & Dils, 1972*b*). The response to insulin, corticosterone and prolactin of mammary explants from rabbits on day 23 of pregnancy was therefore examined (Table 2).

When explants were cultured for 2 days with prolactin, the overall rate of synthesis of medium-chain fatty acids was maintained, but there was a marked decrease in the proportion of  $\text{C}_{8:0}$  acids compared with  $\text{C}_{10:0}$  acids formed. Culture with insulin and corticosterone decreased both the overall rate of synthesis and the proportion of medium-chain fatty acids formed. With all three hormones, there was a fourfold increase in the overall rate of fatty acid synthesis. Compared with cultures containing only prolactin, the rates of synthesis of  $\text{C}_{8:0}$ ,  $\text{C}_{10:0}$ ,  $\text{C}_{12:0}$ ,  $\text{C}_{14:0}$  and  $\text{C}_{16}$  acids increased 2.7-, 3.7-, 5.9-, 9.8- and 10.8-fold respectively, showing a preferential synthesis of long-chain fatty acids.

### *Fatty acid synthesis by mammary explants from rabbits pseudopregnant for 11 days*

Most studies *in vitro* on the response of rabbit mammary gland to hormones have used tissue from pseudopregnant rabbits (Bolton & Bolton, 1970; Falconer & Fiddler, 1970; Bolton, 1971; Fiddler *et al.*, 1971; Delouis & Denamur, 1972). This tissue readily responds to insulin, corticosterone and prolactin by increasing the overall rate of fatty acid synthesis and the proportion of medium-chain fatty acids synthesized (Strong *et al.*, 1972). We have therefore compared its response with that of mammary

Table 1. *Effects of insulin, corticosterone and prolactin on fatty acid synthesis by mammary explants from rabbits pregnant for 16 days*

Mammary explants prepared from two rabbits pregnant for 16 days were cultured with each of the combinations of hormones shown except for insulin alone where only one of these rabbits was used (I = insulin; C = corticosterone; P = prolactin). With one of the rabbits, survival of explants was poor after culture for 4 days with prolactin alone and the results are not included. Two further rabbits pregnant for 16 days were also used to prepare explants which were not cultured, or were cultured for 2 days with prolactin or with insulin plus corticosterone or with all three hormones. Duplicate groups of five explants from each animal were then incubated for 1 h at 37°C in Krebs-Henseleit buffer containing 0.1 mm-sodium [ $^{14}$ C]acetate (2  $\mu$ Ci) plus 1.0 mm-glucoose. The values in parentheses indicate the total number of incubations performed for each hormone treatment; results are means  $\pm$  S.E.M.

Time in culture (days)	Hormones present	Rate of fatty acid synthesis (nmol of acetate incorporated/h per five explants)	Percentage distribution of radioactivity in fatty acids						
			C <sub>8:0</sub>	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub> + C <sub>16:1</sub>	C <sub>18:0</sub> + C <sub>18:1</sub>	
0	- (8)	0.09 $\pm$ 0.02	4.9 $\pm$ 1.4	8.5 $\pm$ 1.0	13.2 $\pm$ 1.7	24.1 $\pm$ 1.6	34.8 $\pm$ 1.5	14.5 $\pm$ 1.4	
2	I (2)	0.15 $\pm$ 0.06	0.0 $\pm$ 0.0	5.0 $\pm$ 3.8	17.7 $\pm$ 0.5	33.0 $\pm$ 3.8	40.9 $\pm$ 2.9	3.4 $\pm$ 3.4	
2	P (8)	0.72 $\pm$ 0.09	19.2 $\pm$ 2.1	41.1 $\pm$ 2.6	21.7 $\pm$ 1.0	10.2 $\pm$ 0.8	7.2 $\pm$ 1.2	0.6 $\pm$ 0.2	
4	P (2)	1.05 $\pm$ 0.10	25.5 $\pm$ 1.5	51.0 $\pm$ 2.3	15.8 $\pm$ 2.4	5.1 $\pm$ 1.0	2.6 $\pm$ 0.4	0.0 $\pm$ 0.0	
2	I+P (4)	1.28 $\pm$ 0.25	10.8 $\pm$ 2.0	36.8 $\pm$ 2.0	23.5 $\pm$ 1.7	16.2 $\pm$ 1.8	12.3 $\pm$ 0.6	0.4 $\pm$ 0.4	
4	I+P (4)	1.26 $\pm$ 0.14	25.9 $\pm$ 2.6	46.6 $\pm$ 0.9	14.8 $\pm$ 1.4	6.8 $\pm$ 1.1	5.5 $\pm$ 0.6	0.4 $\pm$ 0.3	
2	C+P (4)	0.93 $\pm$ 0.41	4.5 $\pm$ 1.0	19.2 $\pm$ 2.6	20.8 $\pm$ 3.6	24.3 $\pm$ 3.3	30.7 $\pm$ 3.5	0.5 $\pm$ 0.5	
4	C+P (4)	1.55 $\pm$ 0.40	5.4 $\pm$ 0.5	20.4 $\pm$ 0.3	18.5 $\pm$ 0.3	21.7 $\pm$ 1.3	32.9 $\pm$ 1.0	1.1 $\pm$ 0.4	
2	I+C (8)	0.30 $\pm$ 0.06	2.5 $\pm$ 1.2	5.9 $\pm$ 2.4	12.2 $\pm$ 0.9	24.0 $\pm$ 0.9	42.5 $\pm$ 3.0	12.9 $\pm$ 1.9	
2	I+C, P (4)	2.36 $\pm$ 0.44	9.2 $\pm$ 2.1	27.8 $\pm$ 3.7	20.1 $\pm$ 0.6	19.6 $\pm$ 1.6	22.6 $\pm$ 4.0	0.7 $\pm$ 0.2	
2	I+C, P (4)	2.05 $\pm$ 0.19	18.0 $\pm$ 1.0	38.1 $\pm$ 2.4	18.4 $\pm$ 0.6	13.6 $\pm$ 0.9	11.1 $\pm$ 1.8	0.4 $\pm$ 0.4	
2	I+C, I+P (4)	3.08 $\pm$ 0.23	6.7 $\pm$ 0.3	25.0 $\pm$ 0.8	20.8 $\pm$ 0.8	21.0 $\pm$ 1.3	25.9 $\pm$ 1.8	0.7 $\pm$ 0.3	
2	I+C, I+P (4)	0.91 $\pm$ 0.29	11.6 $\pm$ 1.0	40.0 $\pm$ 0.6	19.3 $\pm$ 0.9	15.5 $\pm$ 0.5	13.6 $\pm$ 1.0	0.0 $\pm$ 0.0	
2	I+C+P (8)	2.74 $\pm$ 0.36	5.0 $\pm$ 1.1	19.7 $\pm$ 2.7	18.0 $\pm$ 1.2	23.9 $\pm$ 1.2	31.8 $\pm$ 3.8	1.6 $\pm$ 0.5	
4	I+C+P (4)	3.81 $\pm$ 0.54	4.6 $\pm$ 0.5	18.8 $\pm$ 1.4	17.6 $\pm$ 0.9	23.3 $\pm$ 1.5	33.6 $\pm$ 1.5	2.1 $\pm$ 0.3	
6	I+C+P (4)	2.71 $\pm$ 0.16	6.7 $\pm$ 1.1	23.0 $\pm$ 2.0	17.6 $\pm$ 0.5	22.6 $\pm$ 0.9	28.0 $\pm$ 2.7	2.1 $\pm$ 1.0	

Table 2. Effects of insulin, corticosterone and prolactin on fatty acid synthesis by mammary explants from rabbits pregnant for 23 days

Mammary explants prepared from two rabbits pregnant for 23 days were cultured with each of the combinations of hormones shown (I = insulin; C = corticosterone; P = prolactin). Duplicate groups of five explants from each animal were then incubated for 1 h at 37°C in Krebs-Henseleit buffer containing 0.1 mm-sodium [ $U-^{14}C$ ]-acetate (2  $\mu$ Ci) plus 1.0 mm-glucose. The mean values  $\pm$ S.E.M. of the four incubations are given.

Time in culture (days)	Hormones present	Rate of fatty acid synthesis (nmol of acetate incorporated/h per five explants)	Percentage distribution of radioactivity in fatty acids						
			C <sub>8:0</sub>	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub> + C <sub>16:1</sub>	C <sub>18:0</sub> + C <sub>18:1</sub>	
0	-	0.85 $\pm$ 0.08	61.6 $\pm$ 2.3	26.3 $\pm$ 2.0	5.4 $\pm$ 0.8	3.6 $\pm$ 0.8	2.6 $\pm$ 0.5	1.0 $\pm$ 0.6	
2	P	0.95 $\pm$ 0.29	37.3 $\pm$ 1.1	40.8 $\pm$ 3.1	11.5 $\pm$ 0.9	5.0 $\pm$ 1.4	4.8 $\pm$ 1.7	0.6 $\pm$ 0.3	
2	I+C	0.38 $\pm$ 0.08	14.3 $\pm$ 6.0	28.8 $\pm$ 11.7	13.8 $\pm$ 2.9	13.4 $\pm$ 4.7	21.4 $\pm$ 11.2	8.3 $\pm$ 4.5	
2	I+C+P	3.87 $\pm$ 0.46	21.3 $\pm$ 0.7	36.8 $\pm$ 4.3	15.9 $\pm$ 1.3	12.2 $\pm$ 1.3	12.8 $\pm$ 3.7	1.0 $\pm$ 0.1	

tissue from rabbits pregnant for 16 days (see Table 1) and 23 days (see Table 2). Although two experiments were done and the same general conclusions emerged from both, there was a rapid response to hormones in only one experiment and the results of this experiment are shown in Table 3.

Uncultured explants showed an overall rate of synthesis and a pattern of synthesized fatty acids (Table 3) which was similar to that obtained with freshly prepared mammary explants from rabbits pregnant for 16 days (see Table 1). The response to insulin or prolactin, or to both hormones, was also essentially similar. The effects of corticosterone were somewhat different. In mammary explants from pseudopregnant rabbits corticosterone appeared to prevent prolactin from increasing both the rate of fatty acid synthesis and the proportion of C<sub>8:0</sub> and C<sub>10:0</sub> acids synthesized (Table 3). In mammary explants from rabbits pregnant for 16 days, corticosterone prevented prolactin from stimulating the synthesis of medium-chain fatty acids but did not affect the overall rate of fatty acid synthesis (Table 1). With both preparations, the addition of corticosterone to cultures containing insulin and prolactin increased the overall rate of synthesis. However, the inhibition of medium-chain fatty acid synthesis by mammary explants from pseudopregnant rabbits (Table 3) was much less marked than with mammary explants from rabbits pregnant for 16 days (Table 1). The time-course of this inhibition by corticosterone was also different. With pseudopregnant rabbits, the proportion of C<sub>8:0</sub> and C<sub>10:0</sub> acids synthesized during culture with all three hormones increased with time in culture. After 6 days, the pattern of fatty acids synthesized was, in fact, similar to that after culture for 6 days with insulin and prolactin in the absence of corticosterone (Table 3); it was also similar to that synthesized by mammary explants from the lactating gland after culture for 4 days with all three hormones (Strong *et al.*, 1972). With mammary explants from rabbits pregnant for 16 days, the inhibition of medium-chain fatty acid synthesis in the presence of all three hormones persisted throughout the 6 days of culture (Table 1).

## Discussion

Explants of lobulo-alveolar mammary tissue obtained from pseudopregnant and pregnant rabbits contain a very high proportion of epithelial cells. Some connective-tissue stroma is present, but in serial sections adipose cells are rare or absent. It is therefore probable that the effects seen in the present experiments reflect overwhelmingly the biosynthetic activity of epithelial mammary cells and that all three hormones are acting on these.

Explants of mammary gland taken from rabbits on day 16 of pregnancy or day 11 of pseudo-pregnancy synthesized predominantly long-chain

Table 3. Effects of insulin, corticosterone and prolactin on fatty acid synthesis by mammary explants from a rabbit pseudopregnant for 11 days

Mammary explants prepared from a rabbit pseudopregnant for 11 days were cultured with the combinations of hormones shown (I = insulin; C = corticosterone; P = prolactin). Duplicate groups of five explants were then incubated for 1 h at 37°C in Krebs-Henseleit buffer containing 0.1 mM-sodium [ $^{14}$ C]acetate (2  $\mu$ Ci) plus 1.0 mM-glucose. With uncultured explants, the mean value  $\pm$ S.E.M. of the rate of fatty acid synthesis observed in four incubations is given; the combined lipid extract from these incubations was analysed by radio-g.l.c. With explants cultured for 2 days with insulin or for 6 days with corticosterone and insulin, the combined lipid extract from duplicate incubations was analysed by radio-g.l.c. For all other values, the mean of duplicate results  $\pm$  half the range between the two results is given.

Time in culture (days)	Hormones present	Rate of fatty acid synthesis (nmol of acetate incorporated/h per five explants)	Percentage distribution of radioactivity in fatty acids							
			C <sub>8:0</sub>	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub> +C <sub>16:1</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	
0	-	0.06 $\pm$ 0.06	0	0	10	15	42	21	12	
2	I	0.06 $\pm$ 0.00	0	0	0	22	47	26	5	
2	P	0.87 $\pm$ 0.03	24 $\pm$ 1	50 $\pm$ 1	15 $\pm$ 1	5 $\pm$ 1	4 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	
4	P	0.57 $\pm$ 0.10	37 $\pm$ 2	44 $\pm$ 2	11 $\pm$ 1	5 $\pm$ 2	3 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 0	
6	P	0.03 $\pm$ 0.02			Insufficient radioactivity for analysis					
2	I+P	0.39 $\pm$ 0.04	37 $\pm$ 1	42 $\pm$ 1	9 $\pm$ 1	5 $\pm$ 0	5 $\pm$ 0	2 $\pm$ 1	1 $\pm$ 0	
4	I+P	1.38 $\pm$ 0.01	32 $\pm$ 3	48 $\pm$ 1	11 $\pm$ 1	4 $\pm$ 2	4 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 0	
6	I+P	0.20 $\pm$ 0.01	40 $\pm$ 6	42 $\pm$ 3	8 $\pm$ 4	7 $\pm$ 3	5 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 0	
2	C+P	0.14 $\pm$ 0.01	2 $\pm$ 1	11 $\pm$ 1	17 $\pm$ 0	20 $\pm$ 1	30 $\pm$ 6	10 $\pm$ 2	8 $\pm$ 3	
4	C+P	0.20 $\pm$ 0.03	2 $\pm$ 1	6 $\pm$ 0	16 $\pm$ 2	24 $\pm$ 2	35 $\pm$ 3	9 $\pm$ 1	8 $\pm$ 1	
6	C+P	0.10 $\pm$ 0.01	7	11	11	19	30	15	7	
2	I+C+P	2.43 $\pm$ 0.07	9 $\pm$ 1	35 $\pm$ 1	23 $\pm$ 1	18 $\pm$ 1	14 $\pm$ 1	1 $\pm$ 1	0 $\pm$ 0	
4	I+C+P	2.28 $\pm$ 0.03	15 $\pm$ 1	39 $\pm$ 1	23 $\pm$ 1	14 $\pm$ 1	8 $\pm$ 1	1 $\pm$ 1	0 $\pm$ 0	
6	I+C+P	1.61 $\pm$ 0.08	35 $\pm$ 2	45 $\pm$ 1	12 $\pm$ 1	5 $\pm$ 2	3 $\pm$ 1	0 $\pm$ 0	0 $\pm$ 0	

fatty acids (Tables 1 and 3). After culture for 2 or 4 days with prolactin, they synthesized predominantly medium-chain fatty acids at an increased rate, which was similar to that observed with freshly prepared mammary explants from rabbits pregnant for 23 days (Table 2). The stimulation of lipogenesis and the initiation of predominant synthesis of medium-chain fatty acids, which occur between days 18 and 23 of pregnancy (Strong & Dils, 1972*b*), can therefore be reproduced in organ culture with prolactin alone. To obtain rates of lipogenesis similar to those seen in early lactation (Strong *et al.*, 1972) culture with the three hormones, insulin, corticosterone and prolactin was required (Tables 1 and 3). Delouis & Denamur (1972) have similarly shown that lactose synthesis by mammary explants from pseudopregnant rabbits can be initiated by culturing for 2 days with insulin and prolactin in the absence of corticosterone, although the inclusion of corticosterone in the medium increased lactose synthesis by approx. 2.5-fold.

These results contrast considerably with those reported for the mouse. In this species, synthesis of milk fatty acids was induced by culture with insulin + corticosterone + prolactin (Wang *et al.*, 1972). Prolactin alone was ineffective in stimulating lipogenesis, and although insulin was stimulatory (Moretti & Abraham, 1966), the pattern of fatty acids synthesized resembled that of tissue rather than milk lipid (Wang *et al.*, 1972).

The effects of these hormones on cell division and protein synthesis *in vitro* in the mid-pregnant mouse mammary gland are well documented (Topper, 1970; Mills & Topper, 1970). Culture with insulin stimulates epithelial cells to synthesize DNA and to divide. The daughter cells are then sensitive to cortisol, which stimulated the production of abundant rough endoplasmic reticulum. Only then can the epithelial cells respond to the synergistic actions of insulin and prolactin by synthesizing casein.

It is already clear that the rabbit mammary gland responds very differently *in vitro*. Prolactin alone will induce milk-fat synthesis, and preliminary ultrastructural studies suggest that dilated rough endoplasmic reticulum is also increased and that casein-like protein granules appear in the epithelial cells and alveolar lumina (B. E. Brooker & I. A. Forsyth, unpublished work). It is not yet known whether mitosis occurs *in vitro* in the presence of prolactin alone, or whether the hormone is acting on pre-existing cells to produce its effects.

Although the combination of the three hormones insulin, corticosterone and prolactin gave rates of lipogenesis similar to those in early lactation, the proportion of medium-chain fatty acids synthesized was decreased, with octanoic acid being most markedly affected. Insulin appears to increase synthetic rates in this system without affecting the pattern of fatty acids synthesized, but corticosterone

apparently antagonizes prolactin-stimulated medium-chain fatty acid synthesis by mammary gland from both pregnant and pseudopregnant rabbits, and this effect requires further investigation. The effect is not an absolute one since, at least in the pseudopregnant rabbit, if culture with all three hormones was continued for long enough, a pattern of predominantly medium-chain fatty acid synthesis ultimately emerged (Table 3). It is possible that the effect relates in some way to the division and differentiation of cells.

These results *in vitro* agree well in general with findings *in vivo*. Prolactin alone will restore milk yields and milk composition to normal in the hypophysectomized lactating rabbit (Cowie *et al.*, 1969) and will induce lactation in pseudopregnant rabbits after removal of the ovaries and adrenals (Cowie & Watson, 1966) or pituitary, ovaries and adrenals (Denamur, 1971). Nevertheless adrenal corticoids may have a role in normal lactogenesis, since milk secretion is induced by injections of adrenocorticotrophic hormone in intact pseudopregnant rabbits (Chadwick, 1971) and by cortisol acetate in the pregnant rabbit (Talwalker *et al.*, 1961).

The fatty acid composition of the milk after adrenocorticotrophic hormone or corticoid injections *in vivo* is, however, not known.

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