Effects of Progesterone on Protein Metabolism in Chicken Oviduct Tissue Pretreated with Oestrogen

By K. R. MULLER,* R. F. COX AND N. H. CAREY
Searle Research Laboratories, High Wycombe, Bucks., U.K.

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1. The effect of simultaneous injections of oestrogen benzoate and progesterone (0.5mg/day each) on immature chicken oviduct tissue pretreated with oestrogen benzoate (0.5mg/day) was studied. 2. After 3 days of treatment with both hormones, the weight of the tissue doubles as compared with tissue treated only with oestradiol benzoate. 3. The progesterone-induced weight increase had no effect on total DNA content of the tissue, but greatly increased the protein/DNA and RNA/DNA ratios. 4. Amino acid incorporation *in vivo* after progesterone treatment was elevated as measured by using 2h pulses of amino acids; this effect could be accounted for by observed alterations in the concentrations of free amino acids in the tissue. 5. With longer pulses of amino acid the specific radioactivity of total protein remained high in tissue treated with progesterone, at times when specific radioactivity of protein in oestrogen-treated animals was diminishing. 6. From a knowledge of the specific radioactivity of labelled amino acids in the free amino acid pool and in newly synthesized protein, the rate of protein synthesis was estimated in differently treated tissues. 7. The results suggest that progesterone treatment does not cause an increase in protein synthesis. It is concluded that the observed accumulation of oviduct protein is achieved via an effect of progesterone on protein catabolism.

The oviduct of the laying hen appears to be under the control of at least two hormones, namely an oestrogen and a progestagen. Our work has been concerned with the interaction of these two classes of hormones in the magnum region of this tissue.

The effects of oestrogen are well documented. The hormone causes marked growth of the oviduct (Brandt & Nalbandov, 1956) and is accompanied by cellular differentiation of the epithelial tissue (Kohler, Grimley & O'Malley, 1969). However, the precise action of progesterone on tissue pretreated with oestrogen is more obscure. Korenman & O'Malley (1968) showed that progesterone elicits the synthesis of a specific protein, avidin, in the absence of overall increases in weight and rate of protein synthesis, in tissues receiving massive doses of hormone. Oka & Schimke (1969) have confirmed the latter effect under similar experimental conditions. In contrast, our own work demonstrates that, if much lower doses are administered, progesterone can enhance oestrogen-induced growth, 3 days of simultaneous progesterone treatment being sufficient to double the weight of the magnum.

This paper describes experiments carried out to

define the mechanism of this alteration in terms of the effects on protein metabolism. Our results demonstrate that the observed effects of progesterone on protein turnover cannot be explained in terms of increases in protein synthesis, but are probably due to an action at the level of protein degradation.

MATERIALS AND METHODS

Materials. Oestradiol benzoate (Oestroform; BDH Chemicals Ltd., Poole, Dorset, U.K.) for injection was dissolved in arachis oil-ethyl oleate (4:1, v/v). Progesterone was prepared for injection by diluting Progestin (BDH Chemicals Ltd.) with sterile arachis oil. Radioactive amino acids obtained from The Radiochemical Centre, Amersham, Bucks., U.K., were used at the following specific radioactivities: L-[4,5-³H]lysine hydrochloride, 256 Ci/mol and 510 Ci/mol; L-[G-³H]phenylalanine, 750 Ci/mol; L-[G-³H]methionine, 107 Ci/mol; L-[4,5-³H]leucine, 1000 Ci/mol; L-[U-¹⁴C]lysine, 7.6 Ci/mol; L-[U-¹⁴C]leucine, 9.7 Ci/mol. Salmon testis DNA was obtained from BDH Chemicals Ltd., bovine serum albumin was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K., and yeast RNA type XI, ovalbumin grade V (crystalline) and conalbumin type I were from Sigma Chemical Co., St Louis, Mo., U.S.A.

Animals. White Leghorn chickens, 9½-10½ weeks old,

* Present address: CIBA Ltd., 4000 Basle, Switzerland.

were obtained from Messrs Withers, Appleby Farm, Egerton, Ashford, Kent, U.K. Intramuscular injections of 0.5mg of oestradiol benzoate were given daily into the breast muscle. Progesterone (0.5mg/day) was also injected intramuscularly, commencing on the seventh day of oestrogen treatment. Labelled amino acids were injected in 0.20–0.25ml of sterile 0.9% NaCl into a wing vein. Animals were killed by bleeding from the neck. The entire oviduct was removed into ice-cold 0.9% NaCl or 0.44M-sucrose and the magnum portion was used for all experiments. The word 'oviduct' is used in the text to describe this tissue.

Preparation of cell fractions. Particulate fractions I_v and P_3 were prepared by the method of Carey (1966) with the following modification. Tissues were homogenized in an all-glass Dounce homogenizer (Dounce, Witter, Monte, Pate & Cottone, 1955) by applying six strokes with a plunger of 0.002in clearance. After filtration through several layers of surgical gauze the homogenate was given two strokes with a plunger with 0.001in clearance. Particulate fractions were resuspended in 0.44M-sucrose in a 3ml Dounce homogenizer with a loosely fitting plunger.

Chicken liver cell sap was prepared from young male birds that had been starved for 16h before they were killed by bleeding from the neck. The liver was minced and homogenized in 3 vol. of 0.44M-sucrose in medium M (80mM-KCl–10mM-MgSO₄–50mM-NaCl–20mM-tris-HCl buffer, pH7.6) and centrifuged at 13000g_{av} for 10min and then at 140000g_{av} for 2h after the pellet had been discarded. The top two-thirds of the supernatant was removed and passed through a column of 200g of Sephadex G-25 equilibrated with 0.44M-sucrose in medium M. The effluent, stored in 5ml batches at –20°C, was stable for at least 2 months. One liver yielded about 85ml of cell sap containing 11.5mg of protein/ml.

Chemical fractionation. For fractionation of the whole tissue, 1–2g was minced and homogenized in 10ml of water. Samples corresponding to 200mg of tissue were treated with 5% (w/v) trichloroacetic acid at 4°C, allowed to stand for 1h and the precipitates washed twice. The supernatants were pooled and used for determination of acid-soluble radioactive amino acid. The precipitate was extracted with 5% trichloroacetic acid at 90°C once for 15min and twice for 10min. The combined supernatants were considered to contain hydrolysed RNA and DNA and the precipitate to contain the protein. Where protein only was required for determination of incorporated amino acid the procedure was abbreviated slightly in that samples corresponding to 400mg of tissue were treated with 5% trichloroacetic acid twice at 4°C and twice at 90°C for 10min.

Chemical determination. RNA was determined by the method of Webb & Levy (1958) after destruction of hexoses as described by Solomon (1957). Yeast RNA treated similarly was used as standard. DNA was determined by the diphenylamine method (Dische, 1930; Webb & Levy, 1958) with salmon testis DNA as standard.

Protein precipitates were dissolved in M-NaOH at 90°C and determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin (Armour Pharmaceutical Co. Ltd.) as standard.

Determination of radioactivity. Protein pellets or 0.5ml M-NaOH solutions of protein containing radioactive amino

acid were dissolved in 0.5ml of Hyamine 10X and 10ml of scintillator solution (Carey, 1964) was added to each. If the radioactivities of NaOH solution were counted 1ml of acetic acid was added to the counting vials to ensure neutralization. The radioactivities of samples were counted to 10000 counts in a Packard Tri-Carb liquid-scintillation counter.

Incubation conditions. Isolated cell fractions were incubated in 1ml of the following mixture: 4mM-ATP, 2mM-GTP, 20mM-phosphoenolpyruvate, 8μg of pyruvate kinase, 20mM-tris-HCl buffer, pH7.5, 50mM-NaCl, 80mM-KCl, 10mM-MgSO₄, 440mM-sucrose, 1μCi of [¹⁴C]lysine (11.7Ci/mol) or [¹⁴C]leucine (9.7Ci/mol) and 0.1ml of liver cell sap. The reaction was started by the addition of 0.1ml of particle suspension and after 20min at 37°C stopped by the addition of 1ml of 10% (w/v) trichloroacetic acid containing unlabelled amino acid.

Preparation of antisera and immunodiffusion methods. Ovalbumin antiserum was prepared from rabbits. It was found that the sample of ovalbumin contained conalbumin (cf. Herbert, 1967). Anticonalbumin was absorbed out of the antiserum with conalbumin after preliminary determination of the minimum quantity of conalbumin required. Immunodiffusion was carried out in 0.85% agar (Oxoid Ionagar no. 2) in borate-saline buffer (0.1M-boric acid–0.025M-borax–0.22M-NaCl, pH8.4) on microscope slides (1ml) or Petri dishes (20ml). Diffusion was allowed to proceed for 1–7 days: slides were then rinsed for 3 days in 0.9% NaCl. After the rinsing with 3% trichloroacetic acid the agar was stained in 0.2% Ponceau S and dried. Homogenates of tissue in water were centrifuged at 20000g_{av} for 30min and tested at various dilutions for the presence of ovalbumin.

Amino acid analysis. In pulse-labelling experiments animals were injected intravenously (wing vein) with 0.1mCi of [4,5-³H]lysine hydrochloride (510Ci/mol) in 0.1ml of 0.9% NaCl. At appropriate times 5ml of blood was removed by cardiac puncture. Blood samples were allowed to clot (30min) before centrifugation (2000g_{av} for 10min). Portions (2ml) of the supernatant were mixed with 4ml of 3% (w/v) sulphosalicylic acid containing 26μg of homoarginine/ml, centrifuged as above and duplicate supernatants pooled.

After the animal had been stunned electrically, the magnum was removed, weighed and frozen by immersion in liquid N₂ (Wollenberger, Ristau & Schoffa, 1960) and weighed. Frozen material (2g) was dropped into 8ml of 5% trichloroacetic acid and immediately homogenized in a Teflon-glass Potter-Elvehjem homogenizer. To portions of supernatant obtained after centrifugation of the homogenate (2000g_{av} for 10min), 2 vol. portions of sulphosalicylic acid-homoarginine solution were added.

Samples were stored frozen before analysis on a Technicon amino acid analyser. In some experiments samples were dissolved in 0.5ml of Hyamine and their radioactivities measured as described above. Samples of sulphosalicylic acid-precipitable material were treated with 5% trichloroacetic acid and their protein content and radioactivity determined as described above.

RESULTS

Effect of oestrogen and progesterone on oviduct growth and nucleic acid and protein contents. Pre-

Fig. 1. Effect of oestradiol benzoate and progesterone on growth of the oviduct. Immature hens received daily injections of oestradiol benzoate (0.5mg) alone or in combination with progesterone (0.5mg). ●, Treated with oestradiol benzoate; ○, treated with oestradiol benzoate and progesterone. Points and bars represent the means and standard deviations for a minimum of three animals per treatment.

liminary experiments indicated that 0.5mg of oestrogen/day caused an increase in oviduct wet weight that reached a maximum between the seventh and tenth day and remained constant until the fourteenth day. With doses of 1mg/day the maximum weight reached in our studies was about 50% of that obtained by using 0.5mg/day. Fig. 1 shows the growth of the tissue with 0.5mg of oestrogen/day and the effects of 0.5mg of progesterone/day treatment started on the seventh day of oestrogen treatment. Progesterone at this dose almost doubles the weight of the oestrogen-primed tissue. At 0.1mg/day progesterone was also found to increase the tissue weight, but at least 2 days were required before a difference was observed.

Under oestrogen treatment the DNA content of the oviduct increased in proportion with the wet weight (Fig. 2) so that the quantity of DNA/g of tissue does not change. On the contrary, with simultaneous oestrogen and progesterone treatment the DNA content/g of tissue falls sharply (Fig. 2b). This results from the fact that the rate of DNA synthesis is not changed by progesterone (Fig. 2a) whereas the tissue mass is greatly increased.

Fig. 2. Effect of oestradiol benzoate and progesterone on the DNA content of the oviduct. Conditions of hormonal administration and treatment of results were as described for Fig. 1. ●, Treated with oestradiol benzoate; ○, treated with oestradiol benzoate and progesterone.

The RNA content of the gland did not increase exactly in proportion with the weight of the tissue, showing a tendency to a somewhat lower ratio at later times in the treatment after an early rise (Fig. 3a). Progesterone had the effect of making the RNA content/g of tissue slightly lower although the total content of RNA increased with the growth produced by progesterone. The protein content of the tissue increased at a greater rate than the wet weight so that the tissue became richer in protein. Progesterone enhanced this effect (Fig. 4a).

The increase in wet weight caused by progesterone treatment was thus accompanied by an increase in dry weight that was largely accounted for by an increase in the total protein of the gland, which more than doubled within 3 days. Since the DNA content of the oestrogen-primed gland was unaltered by progesterone, both the protein/DNA and RNA/DNA ratios were greatly increased by this treatment (Figs. 3b and 4b).

Fig. 3. Effect of oestradiol benzoate and progesterone on the RNA content of the oviduct. Conditions of hormonal administration and treatment of results were as described for Fig. 1. ●, Treated with oestradiol benzoate; ○, treated with oestradiol benzoate and progesterone.

Fig. 4. Effect of oestradiol benzoate and progesterone on the protein content of oviduct. Conditions of hormonal administration and treatment of results were as described for Fig. 1. ●, Treated with oestradiol benzoate; ○, treated with oestradiol benzoate and progesterone.

Effect of hormones on amino acid incorporation in vivo. The observed increase in total protein content could be achieved either by an increased rate of protein synthesis, as has been observed in many other hormone-stimulated tissues, or by a decrease in breakdown. Experiments were carried out to choose between these possibilities.

In the first group of experiments each of four labelled amino acids was injected intravenously and allowed to incorporate for 2h. The radioactivity of the total protein of the gland was determined. The specific radioactivity of the protein increased in the first 2-3 days of progesterone treatment (Fig. 5) as compared with that in the control. This increase occurs at the time when growth due to progesterone treatment is maximal.

In the second group of experiments the radioactivity recovered in protein was measured at longer intervals after a single injection of amino acid. The protein radioactivity in the oviducts of oestrogen-treated birds reached a maximum within

10h, then fell during the next 4 days to less than 10% of the highest value (Fig. 6). In birds treated with both hormones the maximum incorporation was reached in about 20h and remained high for another 3 days at least.

Effect of progesterone on the distribution of free amino acids between oviduct and plasma in oestrogen-primed tissues. It was necessary to determine at this stage whether the observed increase in specific radioactivity of pulse-labelled protein and the apparent decrease in turnover of this protein after progesterone treatment could in any way be attributed to alterations in the concentrations of free amino acids in the tissue. Concentrations of four amino acids were therefore determined in the plasma and the oviduct after 1 and 5 days of treatment of oestrogen-primed tissue with progesterone. A measure of the ability of the oviduct to concentrate amino acids after progesterone treatment was obtained by calculating oviduct/plasma ratios for each amino acid. Results are shown in Table 1.

Progesterone treatment for 1 day tends to lower

Fig. 5. Effect of progesterone on the amino acid incorporation *in vivo* into oviduct proteins. Immature hens at various stages of the usual hormone treatment each received 20–25 μ Ci of one labelled amino acid by intravenous injection, were killed 2 h later and the protein-bound radioactivities were determined. Four amino acids were used: ●, lysine; ○, leucine; ▼, methionine; Δ, phenylalanine. One animal was used for each experimental point.

overall the amino acid concentrations in the oviduct, a result that is in accord with the observed increase in specific radioactivity of amino acid incorporated into protein (Fig. 5). This situation is reversed after 5 days of treatment, when the concentration of the free amino acids was increased.

Effect of progesterone on the kinetics of uptake of lysine and its incorporation into protein in oestrogen-primed tissue. In an attempt to ascertain the approximate rate of synthesis of proteins in differently treated tissues the incorporation of labelled lysine into oviduct protein was measured after a single injection of the amino acid. The specific radioactivity of free lysine present in the intracellular pool and the specific radioactivity of the total protein were determined at 30 and 60 min after the initial lysine injection. Animals were treated with the usual combinations of oestrogen and progesterone. The results of these experiments are shown in Table 2. Progesterone treatment for 1 day clearly elevates the specific radioactivity of free lysine, whereas 5 days of such treatment lowers this value as compared with that in tissues treated with oestrogen daily. Changes in specific radio-

Fig. 6. Effect of oestradiol benzoate and progesterone on the turnover of oviduct protein. All animals received 0.5 mg of oestradiol benzoate daily. Progesterone treatment (0.5 mg/day) was started on the seventh day and continued until the end of the experiment. At 20 h after the first dose of progesterone, all animals received 25 μ Ci of [4,5- 3 H]lysine intravenously. Protein-bound radioactivity was determined at different time-intervals. One animal was used for each experimental point. ●, Treated with oestradiol benzoate; ○, treated with oestradiol benzoate and progesterone.

activity of protein between 30 and 60 min in each tissue parallel effects seen in the lysine pool, 1 day of progesterone treatment producing a large change in specific radioactivity of protein (54 000 to 113 000 c.p.m./g of oviduct) and 5 days of treatment producing little change (33 000 to 57 000 c.p.m./g of oviduct).

Effect of hormones on amino acid incorporation in vitro. The incorporation of amino acid by separated cell fractions was measured as described in the Materials and Methods section by using lysine or leucine. Results are shown in Table 3. In these experiments the variation in activity of the isolated particles was so great that the conclusions that can be drawn are very limited. The conditions used were standardized as far as possible, but it must be presumed that other uncontrollable factors interfere. The results of this study suggest that progesterone does not affect the activity of isolated particulate fraction Iv, which contains the main amino acid-incorporating particles of the adult oviduct (Carey, 1966). There is a slight indication that during oestrogen treatment there is a gradual decline in radioactivity of the isolated P₃ fraction (105 000 g_{av}. pellet) that is accelerated by progesterone. In the adult this fraction is completely inactive.

Detection of ovalbumin in the oviduct. In view of the large increase in protein content with progesterone treatment it was decided to determine at what stage ovalbumin appeared in the tissue. While these experiments were in progress Kohler *et al.* (1969) published results indicating that ovalbumin appeared in the tissue after 6 days with their regime of treatment. We have confirmed these

Table 1. *Concentration of free amino acids in the oviduct of hormone-treated immature hens*

Animals were injected with oestradiol benzoate and progesterone in the usual way. After appropriate treatment, samples of blood and oviduct tissue were removed and analysed for their free basic amino acid content as described in the Materials and Methods section.

Oestrogen treatment (days)	Pro-gestosterone treatment (days)*	Free amino acid ($\mu\text{mol/g}$ of oviduct)				Free amino acid in oviduct ($\mu\text{mol/g}$)/ free amino acid in plasma ($\mu\text{mol/ml}$)			
		Arg	His	Lys	Phe	Arg	His	Lys	Phe
8	—	0.240	1.774	0.481	0.158	1.18	5.52	1.41	1.50
8	1	0.248	1.265	0.309	0.130	1.12	6.16	1.36	1.37
12	—	0.162	1.346	0.757	0.193	1.53	6.90	1.54	2.01
12	5	0.290	1.698	0.637	0.254	1.44	6.76	1.95	2.32

* Commencing on the seventh day of oestrogen treatment.

Table 2. *Incorporation of [^3H]lysine in vivo into the acid-soluble pool and protein-fractions of oviducts from hormone-treated immature hens*

After appropriate hormone treatment, animals were injected intravenously with 0.1 ml of [$4,5\text{-}^3\text{H}$]lysine. After 30 or 60 min samples of tissue were analysed for acid-soluble and acid-precipitable radioactivity and lysine content as described in the Materials and Methods section.

Oestrogen treatment (days)	Progesterone treatment (days)*	Duration of [^3H]lysine incorporation (min)	Free lysine ($\mu\text{mol/g}$ of oviduct)	Radioactivity in free lysine (c.p.m./g of oviduct)	Sp. radio-activity of free [^3H]lysine (c.p.m./ μmol)	Radioactivity in protein (c.p.m./g of oviduct)
8	—	30	0.482	9930	20600	73600
8	—	60	0.481	12620	26300	105100
8	1	30	0.331	14120	42700	54700
8	1	60	0.287	10390	36200	113200
12	5	30	0.517	8800	17000	33600
12	5	60	0.758	11620	15400	57200

* Commencing on the seventh day of oestrogen treatment.

Table 3. *Incorporation of amino acids in vitro by cell fractions from hormone-treated immature hens*

Particulate fractions Iv and P₃ (Carey, 1966) were isolated from oviducts of appropriately treated animals, and incubated as described in the Materials and Methods section (particle suspensions were adjusted such that 93 μg of particle protein was contained in each incubation flask). Trichloroacetic acid-precipitable radioactivity was then measured.

Amino acid	Oestrogen treatment (days)	Progesterone treatment (days)*	Incorporation (c.p.m.)/mg of particle protein					
			Fraction Iv			Fraction P ₃		
			Oestrogen only	Oestrogen+ 0.1 mg of progesterone	Oestrogen+ 0.5 mg of progesterone	Oestrogen only	Oestrogen+ 0.1 mg of progesterone	Oestrogen+ 0.5 mg of progesterone
Lys	7	—	837	—	—	1100	—	—
	8	1	1510	1240	1470	1070	1170	124
	9	2	915	1390	1090	132	334	0
	10	3	1060	681	—	175	0	—
Leu	7	—	1185	—	—	811	—	—
	8	1	1660	1430	743	670	723	147
	9	2	1580	1125	966	236	0	0
	10	3	834	391	—	26	0	—

* Commencing on the seventh day of oestrogen treatment.

results by using immunodiffusion. Ovalbumin was detected at the earliest time observed (48h) whereas it was not observed in the unstimulated

gland by this method. Conalbumin was easily detected in the unstimulated gland as well as in the stimulated and the adult tissue.

DISCUSSION

Oestrogen has been known for some time to cause a hyperplasia in target organs, notably the uterus and mammary gland in mammals and the oviduct in birds. It is probable that the response in immature birds resembles that which occurs on maturation of the animal. One condition required to maximize this similarity is that the dose of hormone given to immature animals should be similar to the amount produced by the adult. In this study the dose of oestrogen was chosen on the basis of data given by Brandt & Nalbandov (1956) and our own experience. Daily doses lower than 0.5mg produce slow growth of the oviduct; 1.0mg also gives a lower growth rate in tissue than that produced by 0.5mg of oestrogen daily. A dose of 0.5mg therefore appears to be the optimum for this size of bird (about 800g), higher doses giving visible signs of toxicity. Compared with doses required to stimulate uterine growth rates this dose is very high, but more recent data (R. F. Cox & N. H. Carey, unpublished work) suggest that the blood concentration achieved is comparable with that of the laying adult.

The growth-promoting effects of the two hormones used in this study are clearly of two types. Treatment with oestrogen causes a rapid growth for the first week. During this growth the chemical composition of the tissue is not greatly changed. The increase in DNA content and microscopic observations (Kohler *et al.* 1969; Oka & Schimke, 1969) demonstrate that this growth is due to cell division leading to an increase in cell number. On the other hand, treatment of the oestrogen-primed tissue with progesterone causes growth in which the DNA content of the whole tissue remains constant, causing a rise in the RNA/DNA and protein/DNA ratios. Most of the increase in dry weight is due to the increase in protein and the cells increase in size without division.

The results in this paper and the work of other authors show that progesterone can have two effects on the chicken oviduct: it may either potentiate or inhibit the oestrogen-induced growth. The differences in effects observed will almost certainly be found to be due to the doses chosen in different studies. Oka & Schimke (1969) find an inhibitory effect of progesterone when the daily oestrogen dose is 20mg/kg, and in the studies by Kohler *et al.* (1969) the daily dose was approx. 100mg of stilboestrol/kg for several weeks. The daily dose of oestrogen used in the present studies was about 0.6mg/kg for 7 days. It has been shown (Steel & Hinde, 1963) that with higher doses of oestrogen the effect of progesterone in potentiating oviduct growth diminishes. No results indicating oestrogen antagonism were given. However, it is clear that

progesterone can have a variety of effects on the tissue, depending to a large degree on the dose of oestrogen, and that high oestrogen doses may obliterate many of the progesterone effects. It is not possible at this stage to decide which of the experimentally observed effects of progesterone are involved in the function of the hormone in the normal development of the animal.

It has been claimed by O'Malley (1967) that one effect of progesterone on the oviduct is to elicit the synthesis of one specific protein, avidin, in the absence of tissue growth and that this effect is on one type of cell only in the tissue (Kohler, Grimley & O'Malley, 1968). The results we obtain clearly indicate that progesterone is capable of causing tissue growth. Whether the effects observed by O'Malley (1967) and by ourselves are independent or related remains to be defined.

Our results do not confirm the observations by Brandt & Nalbandov (1956) that progesterone is responsible for eliciting ovalbumin production, but confirms those of Kohler *et al.* (1969) that ovalbumin is produced by oestrogen treatment alone. Our results are also in agreement with Oades & Brown (1965) in showing that conalbumin production is not under hormonal control, since it is easily detected in the unstimulated oviduct.

In this study the most clear-cut effect of progesterone, an increase in weight of the oviduct in the absence of cell division, can clearly be ascribed to a cessation of protein breakdown during turnover. It has not been possible to demonstrate any effect on protein synthesis either *in vitro* or *in vivo*. In cell-free systems there was no difference between the amino acid-incorporating activity of particles from animals treated with oestrogen or oestrogen plus progesterone. With other growth-stimulating hormones (Korner, 1965; Tata & Williams-Ashman, 1967) an increase in amino acid incorporation has been easily demonstrated.

When amino acid was injected and allowed to be incorporated *in vivo* it appeared that there was a period of 1–2 days in which synthesis might have been stimulated. As shown below, however, this is probably due to alteration in the amino acid pool. Either a smaller pool size (Table 1) or a decreased dilution of the pool by non-radioactive amino acid resulting from decreased protein breakdown may occur. Both would result in a higher specific radioactivity, higher incorporation and an apparent increase in synthesis.

The lack of effect of progesterone on the rate of protein synthesis can be shown by calculation from the results in Table 2. The molar rate of incorporation of lysine into protein, which is proportional to the rate of protein synthesis assuming that the hormone treatment does not cause any major change in the proportion of lysine in the total

Table 4. *Rate of incorporation of lysine in vivo into protein by oviducts from hormone-treated immature hens*

Oestrogen treatment (days)	Progesterone treatment (days)*	Lysine incorporated into protein ($\mu\text{mol/h}$)
8	—	2.70
8	1	2.96
12	5	2.92

* Commencing on the seventh day of oestrogen treatment.

protein, was calculated. The amount of [^3H]lysine label incorporated into protein between 30 and 60 min after injection was presumed to be derived from the free lysine in the intracellular pool; the specific radioactivity of the latter was calculated as the average of the values obtained at 30 and 60 min after the lysine pulse. Hence the lysine (in μmol) incorporated into protein/h equals $2 \times L_p/L_r$, where L_p is the [^3H]lysine radioactivity incorporated between 30 and 60 min and L_r is the average specific radioactivity of free lysine of the pool between 30 and 60 min. The rates of lysine incorporation for three hormone treatments are shown in Table 4. The results show that the rates of protein synthesis are remarkably similar in the tissues from all three treatments.

It is clear from the present results that the growth occurring after progesterone treatment results from a cessation in protein breakdown. Very little is known of the mechanisms of catabolism, but they are usually assumed to be hydrolytic and to involve lysosome function. A few preliminary experiments that we have carried out did not suggest any change in the fragility of lysosomal membranes in the oviduct under various hormone treatments. Regardless of the precise mechanism, this represents one of the few examples of control acting via a degradative pathway rather than a synthetic one. Schimke (1965) reported that increases in arginase content in rat liver were brought about by diminished breakdown, and Sjöqvist & Hultin (1967) showed that the regression of Müllerian ducts during chick embryo development occurred under conditions of unaltered protein synthesis, implicating control at the level of breakdown.

It is not possible to decide from our results whether progesterone has a similar action to this

during normal development. It should be noted that, after an initial period with no breakdown, turnover is re-established with the tissue at a new weight. In view of the fact that progesterone can act as an antagonist or stimulator of oestrogen action in its effect on tissue growth, depending on the doses, it is possible that one of the functions of progesterone is to modulate the effect of oestrogen on growth or secretory protein production and that this effect is achieved via an effect on protein catabolism.

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