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Further Studies on the Stimulation of Protein Synthesis in Androgen-Dependent Tissues by Testosterone

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1. By using centrifugation through a discontinuous sucrose gradient, four microsomal fractions are obtained from the prostate gland. 2. Administration of androgens to castrated rats stimulates protein synthesis in all fractions, particularly in the heavy rough fraction. 3. Androgens also increase the content of protein, RNA and phospholipid in the heavy rough fraction. 4. Time-course experiments *in vivo* show that androgens induce a rapid increase in the synthesis of ribosomal precursor RNA preceding the synthesis of new microsomal fraction and the increase in protein synthesis.

Protein synthesis in many tissues is stimulated by both steroid and polypeptide hormones. In androgen-dependent tissues, such as the ventral prostate gland of the rat, the administration of testosterone to castrated animals stimulates the incorporation of radioactive amino acids into protein (see reviews by Williams-Ashman *et al.*, 1964; Frieden, 1964). Previous studies have indicated that androgens may influence the ability of prostate ribosomes to promote protein synthesis. Liao & Williams-Ashman (1962) and Mangan *et al.* (1967) have demonstrated a decreased incorporation of amino acids into protein in cell-free systems *in vitro*, with ribosomes prepared from castrated animals by treatment with detergents. On the basis of these findings it was suggested that the amount of mRNA in prostate ribosomes was controlled *in vivo* by androgens. Similar investigations with other hormones, including growth hormone (Korner, 1965), tri-iodothyronine (Tata, 1966), oestradiol-17β (Teng & Hamilton, 1967) and insulin (Wool *et al.*, 1968), have confirmed that the synthetic ability of membrane-free ribosomes prepared by treatment with detergents is greatly influenced by a diversity of hormones.

In the majority of these cases the ribosomes were freed of associated membranes of the endoplasmic reticulum by treatment with surface-active agents such as sodium deoxycholate and various non-ionic detergents. More recent studies, however, have indicated that the endoplasmic reticulum plays an equally important part in controlling protein synthesis in highly differentiated cells. Accordingly, in previous studies on the effects of androgens on protein synthesis with membrane-free ribosomal particles, additional means of hormonal control may have been overlooked. J. R. Tata and his colleagues (Tata & Widnell, 1966; Tata, 1967*a*,*b*,*c*; Kerkof & Tata, 1969; Tata, 1970) have demonstrated that an increase in the synthesis of ribosomes accompanies enhancement of protein synthesis and that hormones may influence the attachment of the newly formed membrane-free ribosomal particles to the endoplasmic reticulum. In addition, Tata (1967*a*) showed that a proliferation of cytoplasmic membrane accompanied the hormone-induced synthesis of membrane-free ribosomal particles. An explanation of the involvement of the membranes of the endoplasmic reticulum in controlling protein synthesis has been offered by P. N. Campbell and his colleagues (complete references are given in Campbell & Lawford, 1967). From a series of elegant studies on the synthesis of specific proteins in liver they concluded that the release of 'exported' proteins for use outside the liver itself, such as serum albumin, was mediated by the membranes of the endoplasmic reticulum. The hormone-induced synthesis of membranes could be particularly important in accessory sexual tissues since these all synthesize and release complex proteinaceous secretions into the male reproductive tract. In the light of these findings the principal objectives of the present work were twofold. First, to ascertain if androgenic stimulation of protein synthesis in the prostate gland was due to the provision of newly synthesized ribosomes and, second, to ascertain whether the subsequent increase in protein synthesis was paralleled by a proliferation of membranes in the endoplasmic reticulum.

Materials and Methods

Chemicals

[5-^{3}H]Uridine (sp. radioactivity 2.1 Ci/mmol), [*methyl*-^{14}C]choline chloride (sp. radioactivity 41 mCi/mmol) and L-[U-^{14}C]phenylalanine (sp. radioactivity $\rightleftharpoons$ 405 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. U-^{14}C-labelled protein was obtained from *Chlorella vulgaris* grown in a medium containing [^{14}C]$NaHCO_3$

as a sole carbon source (Catch, 1954) and hydrolysed overnight in 6M-HCl under vacuum at 120°C. Specific radioactivity of the labelled amino acid mixtures was 45mCi/mg-atom of carbon. Tris base (99.5% pure), ATP, GTP, phosphoenolpyruvate (sodium salt), dithiothreitol and pyruvate kinase (suspension in ammonium sulphate) were obtained from the Sigma (London) Chemical Co. Ltd., London, S.W.6, U.K.

Animals

All experiments were performed on adult male Sprague–Dawley rats weighing 250–300g. Bilateral orchidectomy was by the scrotal route under fluothane anaesthesia. Androgen treatment consisted of a subcutaneous injection of 0.25ml of testosterone phenyl propionate (10mg/ml) in arachis oil. Control animals received a sample volume of oil only. In time-course experiments animals were given testosterone at various times before they were killed, 72h after orchidectomy.

Preparation of subcellular fractions

All preparations were conducted at temperatures as near 0°C as possible.

Microsomal fraction. A total microsomal fraction was prepared by a method based on that of Campbell *et al.* (1963). Prostate glands were cooled in ice-cold medium A (0.35M-sucrose, 25mM-KCl, 10mM-$MgCl_2$ and 5mM-β-mercaptoethanol in 35mM-tris–HCl buffer, pH7.8), rinsed three times with medium A, finely minced with scissors and homogenized in a Potter–Elvehjem homogenizer with sufficient medium B (medium A with the omission of the β-mercaptoethanol) to give a 30% (w/v) tissue suspension. The homogenate was filtered through a stainless-steel grid (100 gauge) and centrifuged at 16000*g* for 10min. The supernatant was centrifuged at 105000*g* for 50min in the A60 rotor of a Christ ultracentrifuge. The sediment of microsomal fraction was resuspended by gentle hand homogenization in 10ml of medium B and centrifuged at 105000*g* for 35min. The final preparations of washed microsomal fraction were resuspended in sufficient medium B to give a suspension of approx. 0.3mg of microsomal RNA/ml.

Microsomal subfractions. Microsomal fractions were separated into four subfractions by the method of Tata & Williams-Ashman (1967), with modifications in volume to suit the rotors of the Christ ultracentrifuge. Eight prostate glands were homogenized in 15ml of medium B and a 16000*g* supernatant fraction was prepared as described above. Samples (5ml) were layered over 4ml of 1.3M-sucrose containing 10mM-$MgCl_2$ and centrifuged at 105000*g* in the A60 rotor for 90min. The material collected at the interface (smooth membranes) was recovered with a Pasteur pipette, diluted to 11ml with medium B and centrifuged at 105000*g* for 60min. The sediment of smooth membranes was resuspended in 2.0ml of medium B and stored frozen at −20°C. The material sedimenting through the discontinuous 1.3M-sucrose gradient was evenly resuspended in 2ml of medium B and layered over 1.5ml of 2.0M-sucrose and 1.5ml of 1.5M-sucrose containing 10mM-$MgCl_2$ in 5ml tubes. Centrifugation was carried out at 64000*g* for 14–16h in a swing-out S40 rotor. Material sedimenting at the interface of the 0.32M-sucrose and the 1.5M-sucrose was termed 'light rough membrane' and that above the 2.0M-sucrose was termed 'heavy rough membrane'. Free polyribosomes were collected from the bottom of the tubes. All fractions were suspended in 2.0ml of medium B and stored frozen at −20°C. All the preparations could be stored for several days without deleterious change.

pH5 enzyme. Liver was homogenized in a Potter–Elvehjem homogenizer with sufficient medium A to give a 10% (w/v) homogenate. The homogenate was filtered through a stainless-steel grid (100 gauge) and centrifuged at 105000*g* for 2h. The clear supernatant below the small floating layer of fat was carefully aspirated to within 1cm of the heavy sediment and brought to pH5.2 by the dropwise addition of 1.0M-acetic acid. The precipitate was collected by centrifugation at 10000*g* for 10min and resuspended in a volume of storage medium (50% glycerol, 25mM-KCl, 10mM-$MgCl_2$ and 2mM-dithiothreitol in 20mM-tris–HCl buffer, pH7.8) equal to the initial homogenizing volume. The enzyme was stored in 1ml portions at −20°C. Under these storage conditions the enzymic activity was preserved for over 3 months.

Measurement of incorporation of ^{14}C-labelled amino acids into protein

Incorporation in vitro. Each assay (total volume 1.0ml) contained 0.1ml of membrane-free ribosomal particle suspension, 0.2ml of pH5 enzyme, 2μmol of ATP, 0.5μmol of GTP, 7.5μmol of phosphoenolpyruvate, 10μg of pyruvate kinase, 0.1μCi of [^{14}C]-phenylalanine and sufficient 50mM-$MgCl_2$ and 10mM-tris–HCl buffer, pH7.8, to give a final concentration of 10mM with respect to magnesium in a total volume of 1ml. Poly(U) (100μg) was added to some assays. Samples were incubated for 40min at 37°C, when 0.3ml of 1M-KOH was added and the incubation continued for a further 10min to release radioactivity bound to tRNA. Protein was precipitated by the addition of 2ml of ice-cold 0.5M-$HClO_4$ and, after standing in ice for 30min, the precipitate was collected on glass-fibre filters (Whatman GF/A), washed three times with 5ml of ice-cold 0.5M-$HClO_4$ containing 1mM-[^{12}C]phenylalanine and finally washed twice with 5ml of cold ethanol. Filters were allowed to dry thoroughly in air and radioactivity was counted in toluene-based counting fluid.

Incorporation in vivo. Owing to the low incorporation of labelled amino acids into prostate proteins when precursors were injected systemically or intraperitoneally, proteins were labelled by injection of radioactive precursors directly into the gland. Animals were anaesthetized with an intraperitoneal injection of 0.25ml of Nembutal (sodium pentobarbitone). The skin was cleansed with 4% Cetavlon soln. and the gland exposed by a ventral incision adjacent to the base of the bladder under aseptic conditions. Radioactive amino acids were injected in 0.1ml of 0.9% NaCl and the incision was sealed with suture clips. In short-term experiments, of 1h duration, animals remained under anaesthesia throughout.

Measurement of incorporation of [^{14}C]choline into cytoplasmic membranes in vivo

Tata (1967*a*) has pointed out the importance of the choice of a phospholipid precursor for measurement of the rate of synthesis of membranes, and [^{14}C]-choline chloride was selected for this purpose. The radioactive precursor was injected directly into the prostate gland as described above.

Measurement of radioactivity

Acid-insoluble materials, retained on glass-fibre discs, were counted in a toluene-based scintillation fluid (5g of 2,5-diphenyloxazole/litre) in a Nuclear–Chicago mark II scintillation spectrometer. Counting

Table 1. *Effect of androgens in vivo on the incorporation of [^{14}C]phenylalanine into protein in a cell-free system containing microsomal fraction and pH5 enzymes from prostate gland or liver*

In all cases microsomal fraction (50μg of RNA) was incubated with pH5 enzyme (400μg of protein) from the same tissue. Complete details of the assay system are given in the Materials and Methods section. Animals were used 48h after bilateral orchidectomy. Where indicated, testosterone (2.5mg) was given subcutaneously at the time of operation and again 24h later. Tissue was pooled from 12 animals in each experimental group. The activities obtained after 2 days' treatment of castrated animals with testosterone were almost identical with those found in normal (untreated) animals.

	Incorporation of [^{14}C]phenylalanine (c.p.m./100μg of microsomal RNA)	
Source of pH5 enzyme ...	Castrated animals	Castrated and androgen-treated animals
Source of microsomal fraction		
Liver		
Castrated animals	1802	1922
Castrated and androgen-treated animals	1896	2064
Prostate gland		
Castrated animals	275	406
Castrated and androgen-treated animals	424	825

Table 2. *Incorporation of [^{14}C]phenylalanine into protein in a system containing prostate microsomal fraction and liver pH5 enzyme from normal animals*

Complete details of the assay system are given in the Materials and Methods section. Results are the means of determinations in triplicate.

	Incorporation of [^{14}C]phenylalanine (c.p.m./100μg of microsomal RNA)
Complete system	996
Minus ATP	542
Minus GTP	530
Minus phosphoenolpyruvate+ pyruvate kinase	730
Minus pH5 enzyme	130
Minus microsomal fraction	101
Plus 50μg of puromycin	121
Plus 50μg of cycloheximide	182

efficiency for ^{14}C was 75%. Acid-soluble samples were counted in a water-miscible scintillation fluid (7g of 2,5-diphenyloxazole and 200g of naphthalene dissolved in 440ml of ethanol, 770ml of xylene and 770ml of dioxan). Counting efficiency was 60% for ^{14}C.

Chemical determinations

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as reference. RNA was estimated by the spectrophotometric procedure of Munro & Fleck (1966), with yeast RNA as standard. Phospholipids were extracted with chloroform–methanol (2:1, v/v) as suggested by Folch (1957) and estimated by analysis for inorganic phosphate (Chen, 1956).

Results

Development of an assay system in vitro for protein synthesis

In preliminary experiments a 'cross-over' was made of pH 5 enzyme fractions and microsomal fractions derived from tissues of castrated animals, some of which were given testosterone *in vivo*. Castration had little effect on the ability of subcellular liver fractions to promote the incorporation of [^{14}C]phenylalanine into protein (Table 1). In marked contrast, the synthetic ability of similar preparations from the prostate gland was severely impaired by deprivation of androgenic steroids. The activities of both the microsomal fraction and the pH 5 enzyme fraction from the prostate were significantly enhanced by the administration of testosterone *in vivo*. Since the major objective of the present study was to investigate the effects of androgenic steroids on the microsomal fraction of the prostate, the use of a more readily available source of pH 5 enzyme was permissible. That from rat liver was an ideal choice, since it is readily available in large quantities and the additional presence of a potent ribonuclease inhibitor (Roth, 1956) is clearly advantageous for assay systems *in vitro*. From analysis of the time-course of the incorporation of labelled amino acids into protein with microsomal fraction from castrated and androgen-treated–castrated animals, the differences in activity of the microsomal fraction could not be attributed to the selection of a 40min period of incubation. The differences persisted at all times of incubation.

The characteristics of the prostate microsomal fraction–liver pH 5 enzyme assay system are summarized in Table 2. The incorporation of [^{14}C]-phenylalanine into polypeptide linkage was strongly dependent on the presence of ATP and GTP, whereas the requirement for an energy-generating system was less important. The system was inhibited by puromycin and cycloheximide. Additional experiments

Table 3. *Effect of androgens on the chemical composition of the microsomal subfractions from the prostate gland*

Full details of the preparation of the microsomal subfractions are given in the Materials and Methods section. The results are the means of estimations from three experiments, with preparations derived from six animals (approx. 1–1.5g wet wt. of tissue) per experimental group. Values from separate experiments were within ±8%. All animals were castrated and used 48h after bilateral orchidectomy. One group received testosterone (2.5mg/animal) at the time of the operation only and the other was given testosterone additionally 24h after castration. All values are quoted in mg/g wet wt. of tissue. The recovery of total RNA was within the range 60–65%.

Microsomal fraction ...	Smooth			Light rough			Heavy rough			Free polyribosomes		
	RNA	Protein	RNA/protein ratio	RNA	Protein	RNA/protein ratio	RNA	Protein	RNA/protein ratio	RNA	Protein	RNA/protein ratio
Castrated animals	0.46	6.22	0.07	0.18	1.62	0.11	0.82	4.04	0.20	0.18	0.26	0.69
Castrated animals: 1 day of testosterone treatment	0.40	8.02	0.05	0.18	2.02	0.09	1.66	5.48	0.31	0.20	0.24	0.83
Castrated animals: 2 days of testosterone treatment	0.46	8.48	0.05	0.22	2.18	0.09	2.39	6.76	0.35	0.24	0.24	1.00
Normal animals	0.5	9.24	0.052	0.24	2.18	0.11	2.6	7.4	0.35	0.40	0.40	1.00

showed that the optimum $MgCl_2$ concentration for the assay system was 10mM and that the reaction was essentially complete after 30min incubation. Incorporation of [^{14}C]phenylalanine was strictly proportional to the concentration of added microsomal fraction in the range 25–100μg of microsomal RNA, and to the amount of pH5 enzyme present up to a maximum of 400μg of protein. Comparative studies on the activity of microsomal preparations were conducted at saturating amounts of pH5 enzyme, namely 400μg of protein/assay. Other experiments showed that the qualitative nature of protein synthesis was similar with homologous (prostate) pH5 enzyme but less extensive than with heterologous (liver) pH5 enzyme.

Effect of androgens on the chemical composition of prostate microsomal fraction

The administration of testosterone to castrated animals *in vivo* had a significant effect on the chemical composition of prostate microsomal fraction. The results of these experiments are summarized in Table 3. There was a small increase in RNA content of the free polyribosome fraction after testosterone treatment and an increase in the amount of smooth membrane. By far the most important response to hormonal stimulation, however, was the increase in the overall amount of heavy rough membrane fraction and a pronounced rise in the amounts of RNA associated with these membranes. The low RNA:protein ratio in the heavy rough membrane fraction in castrated animals is indicative of progressive degranulation of the endoplasmic reticulum in the absence of androgenic steroids. A similar effect has been found in the seminal vesicles of castrated rats (Mills & Topper, 1969) and in the liver of adrenalectomized animals (Rancourt & Litwack, 1969). The overall recovery of microsomal RNA in the total purified membrane fractions was, at best, within the range 60–65%. This relatively low recovery was constant throughout the experiments and is attributable to the necessity of including the bivalent cation Mg^{2+} in the homogenizing medium and all preparative media. Earlier work by Harding & Samuels (1961) demonstrated that the complete recovery of prostate microsomal fraction from tissue homogenates is possible only in the presence of EDTA. In the absence of EDTA, and certainly in the presence of bivalent cations, a considerable proportion of the microsomal fraction associates with other subcellular particles, particularly nuclei, and is prematurely sedimented at low gravitational forces. The chemical composition of the microsomal subfractions is in agreement with the values given by Campbell *et al.* (1963) and Tata (1967*b*) for similar preparations from rat liver.

Stimulation of protein synthesis in prostate microsomal fraction by testosterone

The effects of the administration of testosterone *in vivo* on the incorporation of phenylalanine into

Table 4. *Stimulation of protein synthesis in prostate microsomal fraction by the administration of testosterone in vivo*

Microsomal subfractions were prepared from pooled prostate glands from 12 animals in each experimental group and the incorporation of [^{14}C]phenylalanine into protein was assayed, in the system described in the Materials and Methods section, with liver pH5 enzyme. The results are the means of triplicate determinations. The schedule of injections of testosterone *in vivo* is given in the legend to Table 3. Where indicated, 100μg of poly(U) was added to the standard assay system.

	Incorporation of [^{14}C]phenylalanine into protein (c.p.m./100μg of microsomal RNA)							
	Smooth		Light rough		Heavy rough		Free polyribosomes	
		With poly(U)		With poly(U)		With poly(U)		With poly(U)
Castrated animals (controls)	364	576	1810	6700	1090	7300	3630	6350
Castrated animals: 1 day of testosterone treatment	380	660	2340	8010	1762	11580	5720	16800
Castrated animals: 2 days of testosterone treatment	441	751	2440	8100	2691	10800	11050	22400
Normal animals	424	980	2400	7700	3090	13672	11010	23600

protein by prostate microsomal fractions are presented in Table 4. In the absence of the synthetic mRNA, poly(U), testosterone induces a very large stimulation of protein synthesis in the heavy rough membrane fraction and free polyribosome fraction. The latter was particularly active in protein synthesis in all experimental groups. Little effect of androgenic stimulation was shown in either the smooth membrane or light rough membrane fractions. To gain some insight into the endogenous mRNA content of the various fractions, a series of incubations were performed in the presence of poly(U). Even in the presence of the synthetic mRNA, the smooth membrane fraction was relatively inactive in promoting protein synthesis. A modest stimulation in the incorporation of [^{14}C]phenylalanine was observed with the free polyribosome fraction and this remained at approximately a twofold stimulation in all the experimental groups. In the remaining membrane fractions there was a significant stimulation of synthetic ability in the presence of poly(U) but this was less pronounced after hormonal stimulation. This finding could suggest an increase in the concentration of endogenous mRNA as a result of androgen stimulation. Since about 75% of the total RNA present in the androgen-stimulated prostate gland is associated with the heavy rough membrane fraction (see Table 3), the overall stimulation of protein synthesis by androgens *in vivo* is very large indeed.

It was clearly desirable to confirm the site of androgen stimulation of protein synthesis by similar studies conducted entirely *in vivo*. The kinetics of the incorporation of ^{14}C-labelled amino acid mixture into microsomal protein after direct injection of ^{14}C into the prostate gland are given in Fig. 1. Maximum

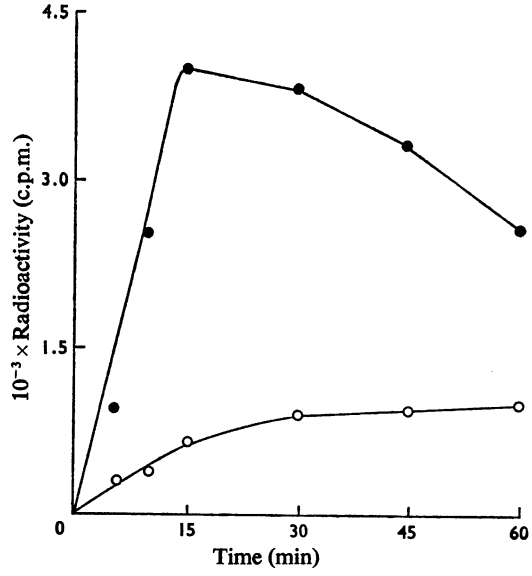

Fig. 1. *Incorporation of radioactivity into prostate microsomal fraction after the injection of* 1 μCi *of a* ^{14}*C-labelled amino acid mixture directly into the gland*

Details of the injection procedure are given in the Materials and Methods section. Each point represents the radioactivity associated with the preparations derived from four normal untreated animals. Each preparation was treated with 1.0% (v/v) Triton X-100 and centrifuged at 105000*g* for 2h. The acid-insoluble radioactivity recovered in the sediments and supernatant fractions was measured. ●, Radioactivity in ribonucleoprotein particle; ○, radioactivity in soluble supernatants.

Table 5. *Comparison of the incorporation of* ^{14}*C-labelled amino acids into prostate microsomal subfractions after labelling in vivo*

The results are the means of determinations of microsomal fraction-bound radioactivity after 15min labelling *in vivo* by direct injection of 5 μCi of ^{14}C-labelled amino acids into the prostate gland. The preparations were derived from eight animals per experimental group. The chemical composition of the fractions and the schedule of injections of testosterone are given in the legend to Table 3. Samples of labelled microsomal fraction were repeatedly washed with cold 0.5M-$HClO_4$ and radioactivity was counted.

	Incorporation of [^{14}C]phenylalanine into protein (c.p.m./100 μg of microsomal RNA)			
	Smooth	Light rough	Heavy rough	Free polyribosomes
Castrated animals (controls)	681	2760	1690	567
Castrated animals: 1 day of testosterone treatment	1224	5520	5850	1530
Castrated animals: 2 days of testosterone treatment	1103	5670	8760	2490
Normal animals	1340	5490	9870	2960

incorporation of radioactivity into the microsomal fraction occurred 15min after the administration of the ^{14}C-labelled amino acid mixture. From measurement of the radioactivity rendered soluble by treatment with Triton X-100, presumably newly synthesized membrane proteins, over 80% of the radioactivity incorporated is associated with the synthesis of nascent polypeptide chains, which are not components of the endoplasmic reticulum. The maximum incorporation of radioactivity into protein occurred some 15min after administration of ^{14}C-labelled amino acids into the gland. A summary of the results of the labelling of the microsomal subfractions *in vivo* is presented in Table 5. The results *in vivo* are very similar to those obtained from incubations *in vitro*. The effect of testosterone administration is an increased activity in the rough fractions. One major difference between the results of the experiments *in vivo* and *in vitro*, however, was the low synthetic activity of the free polyribosome fraction *in vivo*. It is difficult to readily explain this anomaly but it remains a distinct possibility that the liver pH5 enzyme fraction, employed in the cell-free assay system *in vitro*, may contain factors that are not present in the prostate gland. Differences in ribonuclease activity, for example, could provide a possible explanation and this activity would certainly be lower in the liver enzyme fraction owing to the presence of the ribonuclease inhibitor (Roth, 1956).

Control of membrane synthesis in the prostate by androgens

On the basis of the experiments summarized in Table 3, it seemed most probable that the synthesis of the membranes of the endoplasmic reticulum of the prostate gland was controlled by testosterone *in vivo*. To confirm this important point the rate and extent of the incorporation of radioactive precursors of the membrane-associated phospholipid components after hormonal treatment were investigated. In preliminary experiments, the time-course of the incorporation of [^{14}C]choline into microsomal fraction was established, after direct injection of precursor into the prostate gland *in vivo*. Maximum incorporation occurred 15min after the administration of the radioactive precursor. Unlike the time-course shown previously for the incorporation of ^{14}C-labelled amino acids (see Fig. 1), however, the loss of radioactivity from the gland was far more rapid. This has been reported by other workers from studies of liver cell suspensions (Jungalwala & Dawson, 1970) and attributed to the very rapid turnover of the pool of phospholipid precursors. As shown in Table 6, the administration of testosterone *in vivo* to castrated rats stimulates the incorporation of [^{14}C]choline into microsomal fraction-associated phospholipids to a very considerable extent. The stimulation of phospholipid synthesis

Table 6. *Stimulation of phospholipid synthesis in the prostate gland after the administration of testosterone in vivo*

The eight animals in each experimental group were each given 5μCi of [^{14}C]choline by direct injection into the prostate gland under Nembutal anaesthesia. The prostates were removed 15min later and the microsomal subfractions prepared. Samples were extracted with chloroform–methanol to isolate the phospholipids. Other samples were precipitated with cold 0.5M-$HClO_4$ and the acid-insoluble radioactivity was counted. All animals were castrated and used 48h after bilateral orchidectomy. Details of the administration of testosterone are given in Table 3. All values are quoted in mg/g wet wt. of tissue.

Microsomal subfraction ...	Smooth		Light rough		Heavy rough	
	Total phospholipid (mg)	Incorporation (c.p.m./mg of phospholipid)	Total phospholipid (mg)	Incorporation (c.p.m./mg of phospholipid)	Total phospholipid (mg)	Incorporation (c.p.m./mg of phospholipid)
Castrated animals (controls)	1.26	7232	0.48	1772	0.70	3570
Castrated animals: 1 day of testosterone treatment	1.64	7450	0.46	2520	0.176	6182
Castrated animals: 2 days of testosterone treatment	2.38	8122	0.68	4140	2.62	8472
Normal animals	2.2	8410	0.70	4100	3.04	8741

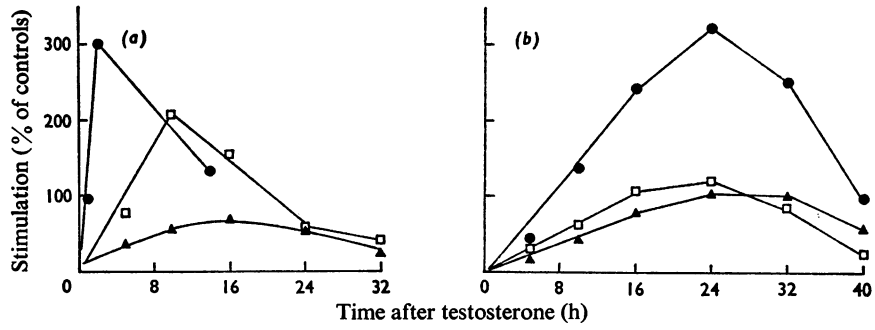

Fig. 2. *Stimulation of activities in the prostate gland after the administration of testosterone in vivo*

In each experiment 24 rats were used 48h after bilateral orchidectomy. Testosterone (2.5mg) was given subcutaneously and four animals were left untreated as controls. At timed intervals four animals were killed and various biochemical activities were measured. Full details of each assay system are given in the Materials and Methods section. (*a*) Assays employing the incorporation of radioactive precursors: ●, formation of 45S ribosomal RNA precursor by labelling *in vivo* (P. A. Wilce and W. I. P. Mainwaring, unpublished observations); □, uptake of [^{14}C]choline into microsomal fraction *in vivo*; ▲, incorporation of [^{14}C]phenylalanine into protein with microsomal fraction *in vitro*. (*b*) Chemical composition of total microsomal fraction: ●, phospholipid; ▲, protein; □, RNA. The alterations in these components were measured per unit amount of DNA.

is particularly marked in the heavy rough membrane fraction. The phospholipid content of the free polyribosome fractions was below the sensitivity of the analytical methods and is consequently omitted from Table 6. Further experiments with $H_3{}^{32}PO_4$ *in vivo* have confirmed the pattern of labelling achieved with [^{14}C]choline. It is therefore unlikely that the results in Table 6 can be simply explained by changes in pool sizes or in the uptake of choline. A similar hormonally induced stimulation of phospholipid synthesis was found by Tata (1967*a*) in the livers of thyroidectomized rats after treatment with triiodothyronine *in vivo*.

Despite the increase in membrane synthesis after testosterone treatment, no selective qualitative difference either in the membrane-associated proteins or phospholipids could be detected. In keeping with the previous findings of Tata (1967*a*), hormones stimulate the synthesis of components of the microsomal membranes, particularly in the heavy rough fraction, in a general rather than a selective manner.

Time-course of the hormone-induced changes in the rat prostate gland

In a concluding series of experiments an investigation was made into the rate of stimulation of many metabolic processes in the prostate gland after the administration of a single injection of testosterone *in vivo*. From the results presented in Figs. 2(*a*) and 2(*b*) it may be seen that the earliest detectable change was in the synthesis of 45S RNA. Later, the increase in the incorporation of radioactive precursors into the phospholipid and RNA components of the microsomal fraction was found and this preceded the gross proliferation of the microsomal fraction measured by standard chemical methods rather than by radioactive isotope techniques. It was significant that the increases in the various components of the microsomal fraction, in terms of phospholipid, protein and RNA, were in strict parallel. Some 24h after the single injection of testosterone *in vivo* there was a decline in the amount and synthetic activity of prostate microsomal fraction. This observation is in accordance with the known retention time of androgenic steroids in the rat prostate gland (Tveter & Attramadal, 1968; Mainwaring, 1971) of between 16 and 24h. Accordingly, 24h after the administration of androgenic hormones to castrated animals, the effects of hormonal deprivation should again be manifest.

Discussion

On the basis of the results presented here it is concluded that the androgens maintain the structure and function of the cytoplasmic membranes in the rat prostate. When the amount of circulating androgens is decreased by castration, the synthetic ability and chemical composition of the microsomal fractions is dramatically altered. With androgen treatment *in vivo* there is a pronounced stimulation of the synthesis of the phospholipid, RNA and protein components of the microsomal fraction, resulting in a

marked rise in the amount of heavy rough membrane fraction, rich in membrane-free ribosomal particles. Recent work involving electron microscopy (P. A. Wilce and W. I. P. Mainwaring, unpublished observations) has confirmed this viewpoint. These studies confirm the findings of Dallner *et al.* (1966*a*,*b*) and Arias *et al.* (1969) that the endoplasmic reticulum is a dynamic system with a rapid rate of turnover and renewal. In the prostate, the maintenance of the endoplasmic reticulum is acutely dependent on the presence of androgenic steroid hormones. Despite the rapidity of the binding of androgens (Mainwaring, 1971) there is a considerable delay before a gross stimulation of protein synthesis is detectable. Certainly there is no exceedingly rapid response in protein synthesis to match that reported in muscle after insulin treatment (Wool *et al.*, 1968). Nevertheless, there could be a limited but rapid synthesis of selected proteins that is below the level of sensitivity of the methods employed in this study.

The rapid stimulation in the synthesis of 45S RNA in the prostate after hormonal stimulation is in keeping with the results with other hormone-sensitive systems (Knowler & Smellie, 1971) and with the increased activity of solubilized prostate RNA polymerase (Mainwaring *et al.*, 1971). The relatively late stimulation of protein synthesis in the prostate microsomal fraction may depend on the provision of newly formed ribosomes themselves or more indirectly on the increased supply in mRNA. It has been suggested that much of the RNA synthesized in the nuclei of higher organisms is retained at the site of synthesis and is not released into the cytoplasm (Church & McCarthy, 1970). Transport of mRNA into the cytoplasm, however, could be effected as a complex with the newly formed RNA (Girard *et al.*, 1965; Henshaw *et al.*, 1965; Samarina *et al.*, 1968). The decreased effect of poly(U) on the incorporation of [^{14}C]phenylalanine into protein by the heavy rough membrane fraction could be explained by a hormonally induced increase in endogenous mRNA, as has been suggested by others (Liao & Williams-Ashman, 1962).

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