Specific Changes in the Messenger Ribonucleic Acid Content of the Rat Ventral Prostate Gland after Androgenic Stimulation

EVIDENCE FROM THE SYNTHESIS OF ALDOLASE MESSENGER RIBONUCLEIC ACID

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1. Aldolase was selected as a suitable marker for following the androgenic regulation of mRNA synthesis in the prostate gland. 2. Antibodies raised in rabbits against crystalline prostate aldolase were used to monitor the synthesis of this androgen-induced enzyme after hormonal stimulation of castrated animals, by using procedures *in vivo* and *in vitro* for the translation of prostate poly(A)-rich mRNA. 3. After androgenic stimulation *in vivo* the poly(A)-rich mRNA was isolated from the prostate gland and other tissues of castrated rats, and added to a protein-synthesizing system *in vitro* derived from Krebs II ascites-tumour cells. By using this approach it was found that androgens regulate the synthesis of aldolase mRNA in a highly tissue-specific manner. Stimulation of aldolase mRNA synthesis reached a maximum after 8 h of androgenic treatment and then declined. 4. The androgenic control of aldolase mRNA synthesis was also investigated *in vivo*. After treatment of castrated animals with various steroids *in vivo* [³⁵S]methionine was injected directly into the prostate gland, and labelled aldolase was selectively precipitated from isolated polyribosomes with anti-aldolase serum. The regulation of aldolase mRNA synthesis in the prostate gland was stringently steroid-specific and could only be evoked by androgens. After a single injection of testosterone, aldolase synthesis reached a maximum after 16 h of hormonal stimulation and then declined. 5. Although androgens exert significant control over transcriptional processes in the prostate gland, and appear to regulate the synthesis of aldolase mRNA *de novo*, the possibility exists for additional means of control at the translational level of aldolase synthesis. The results are discussed in the context of the overall mechanism of action of androgens.

There is now an impressive body of evidence supporting the unique presence of poly(A)-rich RNA sequences at the 3'-terminus of eukaryotic mRNA (Darnell *et al.*, 1971; Edmonds *et al.*, 1971; Lee *et al.*, 1971) with the exception of that coding specifically for the synthesis of histones (Adesnik & Darnell, 1972; Schochetman & Perry, 1972). Mainwaring *et al.* (1974) presented evidence in support of the view that synthesis of mRNA *de novo* occurred during the androgenic response in the rat ventral prostate gland. A severe limitation to this work (Mainwaring *et al.*, 1974), however, was that the prostate proteins induced by androgenic stimulation were not extensively characterized and were solely identified on the basis of changes in the pattern of protein synthesis revealed by means of a double-labelling technique *in vitro*. One obvious problem accompanying such a relatively unsophisticated approach is that damage to the poly(A)-rich mRNA fraction during its isolation could ultimately be reflected in an artifactual change in protein synthesis measured in cell-free systems. Although it remains true that accurate translation of

eukaryotic mRNA can occur even after removal of the poly(A)-rich sequences (Humphries *et al.*, 1974; Williamson *et al.*, 1974), damage to other regions of the polyribonucleotide sequence would be exceedingly difficult to detect. Perhaps the only means of circumventing such criticisms is to study the androgenic regulation of the synthesis of a distinctive species of mRNA coding for a specific protein. The selection of an appropriate marker of the androgenic response is by no means easy since androgens are responsible for the maintenance of the morphology and secretory activity of the prostate gland. Accordingly, the androgenic response is likely to involve a broad spectrum of mRNA molecules. Potential protein markers could include various glycosidases (Conchie & Findlay, 1959), enzymes engaged in the synthesis of citric acid (Williams-Ashman & Banks, 1954), aldose reductases (Samuels *et al.*, 1962), cytochrome *c* oxidase (Davis *et al.*, 1949), malate dehydrogenase (Williams-Ashman, 1954), fructose disulphate aldolase (Butler & Schade, 1958), adenosine triphosphatase (Ahmed & Williams-Ashman, 1969) and nuclear non-histone

proteins (Chung & Coffey, 1971). The final choice of marker rested upon the protein that could be unequivocally identified as a minor component within a complex mixture of proteins. Two factors favoured fructose diphosphate aldolase (fructose 1,6-diphosphate D-glyceraldehyde 3-phosphate lyase; EC 4.1.2.13) as the best indicator of the androgenic response. First, this enzyme may be extensively and readily purified by means of affinity chromatography on a matrix of phosphocellulose, and secondly, it possesses distinctive characteristics in terms of isoelectric point and sedimentation coefficient, which may be profitably exploited for purposes of identification. To facilitate the identification of the aldolase formed *in vitro* and *in vivo*, extensive use was made throughout the present study of rabbit antibodies against crystalline rat prostate aldolase.

Materials and Methods

Animals

Castration of male Sprague-Dawley rats (230–260 g body wt.) was performed by the scrotal route under fluothane anaesthesia. Where indicated, steroids were injected subcutaneously into the flank region in oil (0.25 ml) or 1,2-propanediol-ethanol (3:1, v/v; 0.25 ml). Doses of steroids and the duration of individual experiments are given in the legends to Tables. Male rabbits (1.5–2 kg body wt.) were of the New Zealand White strain.

Chemicals

L-Phenyl[2,3-³H]alanine (9.1 Ci/mmol), L-[G-³H]-phenylalanine (0.66 Ci/mmol), L-[Me-³H]methionine (9.0 Ci/mmol) and L-[³⁵S]methionine (165 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Tris base (99.5% pure), L-amino acids, creatine phosphate, creatine kinase, 2-mercaptoethanol, dithiothreitol, fructose 1,6-diphosphate (tetrasodium salt) and crystalline bovine serum albumin were supplied by Sigma (London) Chemical Co., London S.W.6, U.K. Crystalline rabbit muscle aldolase was obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Poly(U) and poly(A-U-G) were purchased from Miles-Seravac Ltd., Maidenhead, Berks., U.K. Phosphocellulose (Whatman P11) was recycled before use according to the manufacturer's recommendations. Ribonuclease-free deoxyribonuclease was purchased from Worthington Chemical Corp., Freehold, N.J., U.S.A. Steroids were obtained from Steraloids Ltd., Croydon, Surrey, U.K.; testosterone phenylpropionate (10 mg/ml; in oil) was supplied by Organon Ltd., Morden, Surrey, U.K. Other chemicals were of the highest available purity and glass-distilled water was

used in the preparation of all solutions. A mandatory requirement of the work was that all solutions and glassware were autoclaved before use to inactivate ribonuclease.

Isolation of RNA fractions

(a) *Messenger RNA*. Preparations of total cellular RNA were isolated as described in detail by Mainwaring *et al.* (1974) by extraction with phenol, first at ambient temperature and then at 55°C. After incubation with ribonuclease-free deoxyribonuclease, up to 20 E_{254} units of RNA were slowly filtered through nitrocellulose discs (Millipore HAWP 02500) under the conditions prescribed by Mainwaring *et al.* (1974). Discs were then placed in a tightly capped vial with 1.0 ml of 0.1 M-Tris-HCl buffer, pH 9.0, containing 0.15 M-NaCl and 1% (w/v) sodium dodecyl sulphate, and shaken vigorously at room temperature (25°C) for 30 min. The fluid was drained into pointed centrifugation tubes and thoroughly cooled by standing in an ice bath for 30 min. The precipitate of the anionic detergent was sedimented by centrifugation at 5000 g for 5 min and the poly(A)-rich mRNA was precipitated from the clear supernatant fraction at -20°C after the addition of 2 vol. of ethanol and one-tenth vol. of 2 M-NaCl. The fine precipitate was collected by centrifugation at 100 000 g for 15 min, dissolved in 0.2–0.3 ml of water and stored in 50 μ l portions in a liquid N₂ refrigerator.

(b) *Rabbit globin mRNA*. Rabbits were given daily subcutaneous injections of 2.5% (w/v) phenylhydrazine hydrochloride in 0.9% (w/v) NaCl (0.30 ml/kg body wt.) for 5 days and blood was removed 2 days later by cardiac puncture under conditions of nembutal anaesthesia into heparin-coated syringes. Reticulocytes were collected by centrifugation at 1000 g and polyribosomal RNA was prepared by the method of Aviv & Leder (1972). The poly(A)-rich RNA, essentially globin mRNA, was isolated as described above.

(c) *Polyribosomes labelled in vivo*. Rats were subjected to complete surgical anaesthesia by an intraperitoneal injection of nembutal (60 mg/ml; 0.15 ml/100 g body wt.) and 5.0 μ Ci of [³⁵S]methionine was injected directly into the prostate gland (Mainwaring & Wilce, 1972). When the incorporation of radioactive precursor into nascent polypeptide chains was at a maximum approx. 15 min later, prostate polyribosomes were prepared by the procedure of Mainwaring & Wilce (1973).

(d) *rRNA and tRNA*. Suspensions of ascites-tumour cells or homogenates of prostate gland were extracted with phenol to prepare tRNA (von Ehrenstein & Lipmann, 1961). Prostate rRNA was isolated from ribonuclease-treated polyribosomes, as described by Mainwaring *et al.* (1974).

Ascites protein-synthesizing system

The 30000g supernatant (S-30) preparations were isolated from Krebs II ascites-tumour cells as described by Mainwaring *et al.* (1974) and stored frozen at -196°C in batches of approx. 1 ml. The constituents for the assay of protein synthesis (total volume 50 μl or 100 μl) were as described by Mainwaring *et al.* (1974) and either [^3H]phenylalanine, [^{35}S]- or [^3H]-methionine served as radioactive tracer (0.25–2.5 μCi /assay).

Isolation of crystalline prostate aldolase

In view of their small size, prostate glands were gradually accumulated before worthwhile preparations of aldolase could be undertaken. With the generous co-operation of colleagues, glands were pooled and frozen at -70°C over several weeks and preparations of aldolase were carried out with 100–140 prostate glands as starting material. The enzyme was isolated by the method developed by Gracy *et al.* (1970) for rat liver and hepatoma aldolases. The sequential purification steps included the fractionation of the 100000g supernatant fraction with $(\text{NH}_4)_2\text{SO}_4$ and two cycles of affinity chromatography on columns (2.5 cm $\times$ 20 cm) of phosphocellulose, with a salt gradient as eluent for the first column and elution with substrate (2.5 mM-fructose 1,6-diphosphate) for the second column. Crystallization of aldolase was accomplished by cautious dialysis against gradually increasing concentrations of $(\text{NH}_4)_2\text{SO}_4$ (enzyme grade, low in heavy metals; Fisons and Co., Loughborough, Leics., U.K.) as described by Gracy *et al.* (1969). On the basis of the units of enzyme activity defined by Gracy *et al.* (1970), our final preparations were of similar specific activity to rat liver aldolase (Gracy *et al.*, 1970). The yield of aldolase in terms of enzyme protein was within the range 600–850 μg /100 prostate glands. Three preparations were carried out, one to provide material for eliciting the formation of anti-aldolase antibodies in rabbits, the remaining preparations serving as carrier protein in the immunological precipitation of the labelled aldolase formed *in vivo* and in cell-free systems *in vitro*.

Electrophoretic analysis of aldolase

Two procedures were adopted. For the analysis of cell 100000g supernatant fractions, samples were analysed in polyacrylamide gels with protein migration in the direction of the anode, as described by Takeo (1970). Purified enzymes were also investigated by electrophoresis on cellular acetate membranes as described by Penhoet *et al.* (1966). The membranes were finally scanned in a Joyce-Loebl microdensitometer. In both procedures, enzyme activity was located by the formation of Nitro Blue BT formazan in a coupled reaction containing

glyceraldehyde 3-phosphate dehydrogenase and phenazine methosulphate.

Rabbit antibodies against crystalline prostate aldolase

Rabbits were injected every 2 weeks with 200 μg of aldolase (800 μg in all) in Freund's complete adjuvant into the foot-pad. Control animals received the course of adjuvant injections only. Blood was removed by cardiac puncture and samples of control and anti-aldolase serum were stored frozen at -20°C in 1 ml portions. Precipitation lines were observed in the standard Ouchterlony analysis only in the presence of crystalline enzyme and the anti-aldolase serum. A more rigorous examination of the specificity of the anti-aldolase serum is presented in the Results section.

Selective precipitation of aldolase synthesized in vitro and in vivo

The same immunological method was used for the precipitation of prostate aldolase from the proteins synthesized by the ascites-tumour S-30 system *in vitro* or in the protein complement synthesized on prostate polyribosomes *in vivo*. The immunoprecipitation reactions were conducted in small round-bottomed Pyrex tubes, capacity 1 ml, on 50 or 100 μl samples. The standard procedure for the 50 μl samples was a development of the method of Means *et al.* (1972) for the selective precipitation of oviduct ovalbumin. Components of the assay system were added in the following order, without deliberate mixing; 50 μl of serum (52 mg of protein/ml), 50 μl of freshly prepared sodium deoxycholate, 25 μl of 4% (v/v) Triton X-100 and 10 μl (10 μg) of crystalline prostate aldolase. After gentle mixing by tapping with the finger, the samples were left undisturbed for 30 min. Then 250 μl of medium A [10 mM- Na_2HPO_4 – NaH_2PO_4 buffer, pH 7.4, containing 0.15 M- NaCl , 3% (v/v) Triton X-100 and nonradioactive equivalent of the amino acid used as tracer (1 mM)] were added, and after mixing the tubes were centrifuged at 20000g for 15 min in a micro-centrifuge (Microchemical Specialities Inc., Berkeley, Calif., U.S.A.). The precipitates were carefully resuspended in 0.5 ml of medium A with the aid of a Pasteur pipette and transferred, with washings, on to pre-soaked Millipore filters (HAWP 02500). After washing four times with 3 ml of medium A, the filters were dried under an i.r. lamp and counted for radioactivity. Throughout this study, replicate assays were done in the presence of control and anti-aldolase serum.

Analytical procedures

Aldolase was assayed by the spectrophotometric method of Gracy *et al.* (1969). Alkaline phosphatase

activity was monitored by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (Garen & Levinthal, 1960). Glucose 6-phosphate dehydrogenase was measured by the method of Yue *et al.* (1969). Full details of the procedures for isoelectric focusing and sucrose-density-gradient centrifugation have been published elsewhere (Mainwaring & Irving, 1973)

Chemical determinations

Protein was determined by the Folin procedure (Lowry *et al.*, 1951), with bovine serum albumin serving as the standard. Concentrations of polyribosomes were calculated from their extinction at 254 nm, 1 unit of particles being equivalent to an extinction of 1.0. Solutions of total cellular RNA and mRNA were measured by the more precise spectrophotometric procedure of Munro & Fleck (1966), with yeast tRNA as reference.

Measurement of radioactivity

Acid-insoluble precipitates of labelled protein, retained and washed on glass-fibre discs (Whatman GF/A) as described by Mainwaring & Wilce (1972), were counted for radioactivity in a toluene-based phosphor (5 g of 2,5-diphenyloxazole/litre) in a Nuclear-Chicago mark II liquid-scintillation spectrometer. The counting efficiency for ^{35}S was 76%, and 46% for ^3H . Radioactive protein retained on Millipore filters at the terminal step of the immunoprecipitation assay for prostate aldolase was counted in water-soluble phosphor (Mainwaring & Wilce, 1973). In view of the low levels of radioactivity en-

countered in this assay, it was essential that the filters were totally dissolved by gentle shaking overnight in a cold room at 4°C. This prevented serious counting errors attributable to chemiluminescence in the course of the solubilization of the membranes. In this aqueous phosphor, radioactive counting efficiency for ^{35}S was 67%, and 38% for ^3H . In all radioactive counting procedures, the degree of quenching was determined by the channels-ratio method.

Results

Translation of prostate mRNA in ascites-tumour S-30 systems

Before embarking on a detailed investigation on the androgenic regulation of prostate mRNA synthesis, it was necessary to establish the optimum conditions for the translation of this RNA in the mRNA-depleted protein-synthesizing system derived from ascites-tumour cells. Of outstanding importance was the determination of the concentration range of prostate mRNA within which protein synthesis was proportional to the amount of exogenous mRNA. Fig. 1(a) shows that the incorporation of [^3H]phenylalanine into peptide linkage was essentially linear in the presence of up to 6 μg of prostate mRNA but declined sharply at higher concentrations. This finding contrasted markedly with our findings on the response of the ascites-tumour S-30 system to the synthetic mRNA, poly(U). Synthesis of polyphenylalanine continued linearly even at extremely high concentrations of poly(U) without any indication of saturation of the protein-synthesizing system. Inhibition of the

Fig. 1. Optimum conditions for the incorporation of phenylalanine into peptide linkage by ascites-tumour S-30 preparations and prostate mRNA

Incorporation of [^3H]phenylalanine (12.5 Ci/mmol) was measured in the ascites-tumour S-30 system (total assay volume, 50 μl ; 0.25 μCi of labelled amino acid) in the presence of poly(U) or prostate mRNA. (a) Dependence of incorporation on the amount of added mRNA at a constant MgCl_2 concentration of 5 mM. The results are corrected for controls not supplemented with mRNA. (b) Effect of MgCl_2 concentration on incorporation. The results represent the difference in incorporation between assays in the presence of 5 μg of poly(U) with no mRNA and assays in the presence of 1 μg of prostate mRNA at each MgCl_2 concentration. ●, Prostate mRNA; ○, poly(U).

ascites-tumour S-30 system by relatively low concentrations of naturally occurring mRNA has been reported by other investigators. During their innovative study of this cell-free system, Mathews & Korner (1970) observed a similar inhibition of protein synthesis at certain concentrations of encephalomyocarditis virus RNA. A similar optimum concentration of 5 mM-MgCl₂ was established for the translation of both prostate mRNA and poly(U) (Fig. 1*b*). Such an optimum with respect to MgCl₂ concentration is higher than that reported for the translation of ovalbumin mRNA in cell-free systems derived from rabbit reticulocytes (Comstock *et al.*, 1972; Palmiter, 1973) but is identical with the Mg²⁺ requirement for the translation of globin mRNA (Aviv & Leder, 1972; Metafora *et al.*, 1972) and encephalomyocarditis virus RNA (Mathews & Korner, 1970; Metafora *et al.*, 1972) in cell-free systems isolated from Krebs II cells. Mathews & Korner (1970) reported a much higher optimum MgCl₂ concentration for effecting the maximum response of the system to poly(U). One outstanding anomaly, however, was the marked response of the ascites-tumour S-30 system to exogenous prostate mRNA at very low concentrations of MgCl₂ (Fig. 1*b*). This effect may be due to a complement of membrane-bound or otherwise structurally distinct ribosomes in the ascites-tumour S-30 preparation that are characterized by a uniquely low requirement for Mg²⁺ ions. Notwithstanding this effect, incubations done at a concentration of 5 mM-MgCl₂ provided a sensitive and reproducible means for the assay of prostate mRNA.

Stimulation of the ascites-tumour S-30 system also demonstrated an absolute specificity with respect to poly(A)-rich prostate mRNA (Table 1). Prostate rRNA or tRNA, even at high concentrations, could not simulate this stimulation of protein synthesis. Further, supplementation of the ascites-tumour S-30

system with an excess of tRNA in the presence of prostate mRNA did not enhance the incorporation of amino acids into peptide linkage, as has been previously reported by other investigators (Aviv *et al.*, 1971; Metafora *et al.*, 1972). Disparities in the response to addition of tRNA may be explained by minor differences in the manner in which the pre-incubation of the S-30 preparations is done in an effort to deplete the endogenous mRNA. Under certain conditions, this may lead additionally to some degradation of tRNA and hence promote an apparent requirement for added tRNA. Alternatively, minor differences in the handling of intact cells can promote changes in ribonuclease activity (Kerr *et al.*, 1966; Mathews & Korner, 1970) and hence differences in the properties of the final S-30 preparations.

Analysis of prostate aldolase and its precipitation by anti-aldolase serum

Aldolase activity in eukaryotes is represented by a spectrum of isoenzymes, which are generally classified in relation to three basic aldolase forms A, B and C, as typified by the principal enzyme in muscle, liver and brain respectively (Rutter, 1964). Plate 1(*a*) shows that an aldolase activity could be detected in 100000g supernatant fractions of prostate and muscle but not liver, which had a limited migration towards the anode during electrophoresis at pH 8.6. Many additional bands were located by the coupled Nitro Blue BT tetrazolium reaction in all extracts, but since they were additionally located in the absence of fructose 1,6-diphosphate their nature remains obscure. They may be the 'nothing' dehydrogenase reported by Dietz & Lubrano (1967) and Prochazka & Wachsmuth (1972). This slowly migrating anodic form of aldolase was similarly demonstrated in the

Table 1. *Specificity of poly(A)-rich mRNA in the stimulation of ascites-tumour S-30 preparations*

Prostate RNA fractions were isolated from normal (non-castrated) animals. Details for the preparation of the various RNA fractions are in the Materials and Methods section. Samples of ascites-tumour S-30 system (total assay volume, 50 μ l) were incubated in triplicate with 1 μ g of mRNA (5 μ g in the case of synthetic mRNA species) or other RNA, as indicated, in the presence of either 0.25 μ Ci of [³H]phenylalanine (0.66 Ci/mmol) or [³H]methionine (9.1 Ci/mmol). Incorporation of labelled amino acid into protein was determined. Results are given as means $\pm$ S.E.M.

RNA added	Incorporation (c.p.m.)	
	[³ H]Phenylalanine	[³ H]Methionine
None (controls)	1710 $\pm$ 110	6700 $\pm$ 140
Poly(U)	3420 $\pm$ 70	6310 $\pm$ 100
Poly(A-U-G)	1590 $\pm$ 40	7990 $\pm$ 100
Prostate mRNA	2420 $\pm$ 100	7340 $\pm$ 200
Prostate rRNA (5 μ g)	1640 $\pm$ 170	6400 $\pm$ 70
Prostate rRNA (20 μ g)	1440 $\pm$ 200	6660 $\pm$ 210
Prostate tRNA (5 μ g)	1650 $\pm$ 100	6740 $\pm$ 30
Prostate mRNA + ascites-tumour tRNA (5 μ g)	2540 $\pm$ 190	7490 $\pm$ 70

Fig. 2. Electrophoretic analysis of purified aldolase on cellular acetate membranes

Electrophoresis was conducted in 50mM-sodium barbital buffer, pH 8.6, containing 10mM-2-mercaptoethanol, for 3 h at 200 V. After staining for enzyme activity the membranes were scanned in a microdensitometer. P marks the point of application; the cathode is to the right. (a) Muscle; (b) prostate; (c) liver.

purified enzyme preparations from muscle and prostate but again not that purified from liver (Plate 1b). The latter enzyme, in our hands at least, cannot be analysed by this electrophoretic procedure. Neither control nor anti-aldolase serum contained detectable aldolase activity, but the latter serum was able to precipitate totally purified prostate aldolase and, to some extent, purified muscle enzyme. In view of the electrophoretic similarity of the two enzymes and hence their probable structural homology, the cross reaction between the anti-prostate aldolase serum with muscle aldolase is to be expected.

There is evidence that purified liver aldolase has a much higher isoelectric point than the enzyme in muscle (Ikehara *et al.*, 1970) and therefore migrates to the cathode rather than anode during conventional polyacrylamide electrophoresis (see Plate 1). To ensure that prostate aldolase was related to the muscle rather than liver enzyme, purified preparations from all three tissues were analysed by electrophoresis on cellulose acetate membranes (Fig. 2). As reported by others (Penhoet *et al.*, 1966; Gracy *et al.*, 1970; Ikehara *et al.*, 1970), the liver enzyme migrated rapidly towards the cathode and is more negatively charged than either muscle or prostate enzyme. The latter two enzymes again demonstrated identical electrophoretic properties.

Fig. 3. Specificity of anti-aldolase serum to precipitate prostate aldolase

Samples (100 μl) of cell 100000g supernatant were treated with increasing amounts of either control or anti-aldolase serum. After sedimentation of the precipitate at 20000 g enzyme activities were determined in the clear supernatants. The results were corrected for enzyme activities present in the serum. (a) Tissue specificity in the precipitation of aldolase. The results are given in terms of source of supernatant fraction and serum used. ○, Prostate supernatant, control serum; ●, prostate supernatant, anti-aldolase serum; Δ, muscle supernatant, anti-aldolase serum; ▲, liver supernatant, anti-aldolase serum. (b) Selectivity in the precipitation of enzymes in prostate 100000g supernatant fractions by anti-aldolase serum. ○, Alkaline phosphatase; ●, aldolase; ▲, glucose 6-phosphate dehydrogenase.

EXPLANATION OF PLATE I

Analysis of aldolases by polyacrylamide electrophoresis

Analyses were done by the method of Takeo (1970) with the anode to the base of the gels. Enzyme activity was located by the coupled Nitro Blue BT tetrazolium reaction in the presence of 10mM-fructose 1,6-diphosphate; in certain cases, gels were incubated in the absence of substrate to locate aldolase-like artifacts. These were not present in purified enzyme preparations. (a) Cell 100000g supernatant fractions. 1 and 2, Muscle; 3 and 4, prostate; 5 and 6, liver. The even numbers represent controls where the gels were stained in the absence of added substrate. (b) Purified enzymes, including samples run after treatment with either control or anti-aldolase serum and concentration by ultrafiltration using Diaflo membranes. 7, Muscle; 8, muscle after treatment with control serum; 9, muscle after treatment with anti-aldolase serum; 10, prostate; 11, prostate after treatment with control serum; 12, prostate after treatment with anti-aldolase serum; 13, liver; 14, serum (control or anti-aldolase).

To establish further the specificity of the anti-aldolase serum, cell 100000g supernatant fractions were prepared from prostate, liver and muscle and treated with either control or anti-prostate aldolase serum (Fig. 3) as described in the Materials and Methods section. Enzyme activities were determined in the soluble supernatant fraction, and corrections made for dilution and the contribution of the serum to the reported enzyme activities. Fig. 3(a) shows that anti-aldolase serum precipitated aldolase activities in prostate and muscle extracts, but scarcely affected aldolase activity in liver. This result suggests that the serum was essentially specific for muscle-type aldolase. Further, as shown in Fig. 3(b), the anti-aldolase serum did not precipitate either alkaline phosphatase or glucose 6-phosphate dehydrogenase activities in prostate extracts. These results demonstrate the acute specificity of the anti-aldolase serum.

Identification of prostate aldolase synthesized in cell-free systems in vitro

(a) *On the basis of characteristic physicochemical properties of aldolase.* Aldolase was selected as a convenient marker of the androgenic response in the prostate gland on the basis of distinctive properties that could be exploited in its identification. Particularly useful physicochemical characteristics, reported for structurally related aldolase from other sources, include the selective elution from columns of phosphocellulose by its substrate, fructose 1,6-diphosphate (Gracy *et al.*, 1969; Penhoet *et al.*, 1970), a basic isoelectric point within the broad pH range 8.4–10.4 (Ikehara *et al.*, 1970) and a sedimentation coefficient of approx. 8.1–8.5S (Gracy *et al.*, 1969, 1970).

Ascites-tumour S-30 preparations were incubated with [³⁵S]methionine in the presence of exogenous mRNA from several sources and the labelled proteins were sequentially processed in a manner that provided satisfactory evidence for the identification of aldolase (Fig. 4). A labelled protein that was specific to syntheses in the presence of prostate mRNA was identified, which showed precisely the analytical behaviour of added crystalline prostate aldolase in selective elution from phosphocellulose columns (Fig. 4a), isoelectric focusing (Fig. 4b) and sucrose-gradient ultracentrifugation (Fig. 4c). The properties of crystalline prostate aldolase showed an excellent correlation with highly purified preparations of enzymes from other sources (Gracy *et al.*, 1969, 1970; Penhoet *et al.*, 1970; Ikehara *et al.*, 1970) with one minor exception. Our determination of the sedimentation coefficient of prostate aldolase of approx. 7.8–8.0S is marginally lower than the value of 8.1–8.5S quoted in the literature for aldolase from other tissues (Gracy *et al.*, 1969, 1970). However, our estimate is based on sedimentation behaviour in

sucrose gradients rather than in the Model E analytical ultracentrifuge (Gracy *et al.*, 1969, 1970) and small differences could be expected on methodological grounds. In a particularly important experiment (Fig. 4d), a radioactive protein (approx. 8S) was selectively precipitated with rabbit antibodies raised against prostate aldolase, from a class of proteins of distinctive chromatographic behaviour on phosphocellulose. Overall, the results provided conclusive evidence that prostate aldolase had been successfully synthesized in the ascites-tumour S-30 system *in vitro*.

(b) *By direct immunological precipitation.* Despite the success in identifying prostate aldolase by the sequence of specific analytical procedures presented in Fig. 4, this was an impractical means of identifying aldolase as a routine. Accordingly, a more direct immunological determination of the prostate aldolase formed in cell-free systems was adopted. The essential features of this specific assay are presented in Table 2. It should be stressed that there was a considerable enhancement of the incorporation of [³H]phenylalanine in the experiments presented in Table 2, on comparison with the studies described earlier in Table 1 and Fig. 1. This was achieved simply by increasing the amount of [³H]phenylalanine per assay tenfold. Considerable specificity was demonstrated in the precipitation of radioactive protein with anti-aldolase serum. Significant recovery of radioactivity over the amount precipitated by control rabbit serum was achieved only in syntheses done in the presence of prostate mRNA, and also to a much lower extent with muscle mRNA. From a comparison of the total radioactivity incorporated into protein with that selectively precipitated by anti-aldolase serum, it is evident that aldolase synthesis is a minor fraction (approx. 1.1%) of the total prostate protein formed *in vitro* in the presence of prostate mRNA. In view of the probable structural similarity between prostate and muscle aldolase, some cross reaction by the anti-aldolase serum is to be expected.

Time-course of the androgenic stimulation of prostate aldolase activity

Butler & Schade (1958) reported that the aldolase activity in the prostate gland of castrated animals was rapidly and dramatically enhanced by the administration of testosterone *in vivo*. Our experience was in accord with the extensive enhancement of aldolase activity by androgens (Fig. 5), but the response in enzyme activity was not as rapid as observed by Butler & Schade (1958), as it required some 16h of hormonal stimulation to attain a maximum and then declined. Nevertheless, in contrast with the androgenic regulation of the activity of another enzyme in the cell supernatant fraction of prostate homogenate, alkaline

Fig. 4. Identification of aldolase among the proteins formed by an ascites-tumour S-30 system in the presence of prostate mRNA

Protein synthesis was carried out in 100 μ l assays containing ascites-tumour S-30 system, 6 μ g of mRNA and [³⁵S]methionine (165 Ci/mmol). Duplicate assays were pooled and after supplementation with 120 μ g of prostate aldolase the labelled products were subjected to affinity chromatography on phosphocellulose (Gracy *et al.*, 1970). Labelled proteins specifically eluted with fructose 1,6-diphosphate were sequentially analysed, first, by isoelectric focusing, and finally, by sucrose-gradient ultracentrifugation. (a), (b) and (c) show aldolase activity (in arbitrary units) (—) and radioactive products of syntheses conducted in the presence of prostate mRNA (----) or globin mRNA (.....), or from S-30 preparations alone (controls) in the absence of mRNA (---). (a) Phosphocellulose chromatography. The hatched area represents radioactivity eluted in 50 mM-Tris-HCl buffer, pH 7.4, alone; no difference was found between the labelled products from incubations performed in the presence or absence of mRNA, nor was aldolase eluted in this buffer. Fructose 1,6-diphosphate (F) was applied where indicated by the arrow. (b) Isoelectric focusing. Proteins recovered within the bar in (a) were analysed in the pH range 3–10 in a supporting sucrose gradient (Mainwaring & Irving, 1973), anodic migration to the right. (c) Sucrose gradients. Proteins of pI values 8.4–9.1 recovered within the bar in (b) were dialysed against 50 mM-Tris-HCl buffer, pH 7.4, containing 0.5 mM-2-mercaptoethanol and analysed in linear 5–20% sucrose gradients, with the direction of centrifugation from right to left. Sedimentation markers were run separately; catalase, 11.4S (C); alkaline phosphatase, 6.4S (A); cytochrome c, 1.95S (Cyt). (d) Immunological analysis of the proteins made in the presence of prostate mRNA. Labelled proteins eluted from phosphocellulose by fructose 1,6-diphosphate (see a) were analysed directly in sucrose gradients with the direction of centrifugation from right to left; the marker, alkaline phosphatase (A), was run in parallel. Samples (100 μ l) from each gradient fraction were supplemented with 5 μ g of prostate aldolase and labelled proteins were precipitated with either anti-aldolase serum (●) or control serum (○).

phosphatase, the change in aldolase activity did precede the rise in general protein synthesis promoted by androgens, as measured in the synthetic activity of the isolated prostate microsomal fraction. However, from a comparison of the relative rate of change of prostate aldolase and form I RNA polymerase acti-

vities after androgenic stimulation (Mainwaring *et al.*, 1971), stimulation of aldolase activity must be considered a relatively late event in the hormonal response. This does not negate its present use as an indicator of the androgenic regulation of the synthesis of specific species of mRNA, but it should perhaps be

Table 2. Immunological determination of aldolase after the incubation of ascites-tumour S-30 preparations with prostate and other mRNA species *in vitro*

Prostate, muscle and liver mRNA were isolated from normal (non-castrated) animals. Ascites-tumour S-30 preparations (each assay volume, 50 μ l; 2.5 μ Ci of [3 H]phenylalanine, 12.5 Ci/mmol) were incubated with 2.5 μ g of mRNA or 10 μ g of poly(U); six assays were done with mRNA from each source. Portions (5 μ l) were removed at the completion of the incubations to determine the total incorporation of radioactivity into protein. The remainder of all samples was supplemented with 10 μ g of crystalline prostate aldolase; three samples were then treated with specific anti-aldolase serum and three with control serum. Immunologically precipitated protein was collected, washed and its radioactivity counted as described in the Materials and Methods section. Results are given as means $\pm$ S.E.M.

Added mRNA	Total incorporation of radioactivity (c.p.m.)	Precipitated radioactivity (c.p.m.)	
		Anti-aldolase serum	Control serum
None (controls)	8400 $\pm$ 290	55 $\pm$ 8	68 $\pm$ 6
Globin mRNA	30800 $\pm$ 1100	107 $\pm$ 11	99 $\pm$ 4
Prostate mRNA	20900 $\pm$ 400	329 $\pm$ 20	130 $\pm$ 21
Liver mRNA	17800 $\pm$ 380	149 $\pm$ 4	101 $\pm$ 11
Muscle mRNA	14100 $\pm$ 240	194 $\pm$ 2	100 $\pm$ 7
Poly(U)	31100 $\pm$ 550	60 $\pm$ 10	60 $\pm$ 6

Fig. 5. Time-course of changes in enzymic activities in the prostate gland after androgenic stimulation

Beginning 72h after bilateral orchidectomy, a large group of castrated animals was given 2.5mg of testosterone phenylpropionate subcutaneously. At timed intervals, groups of four animals were killed and the prostate glands were homogenized in a medium described by Mainwaring & Wilce (1973); microsomal and cell supernatant (105000g supernatant) fractions were prepared by differential centrifugation. The synthetic activity of the microsomal fraction was determined by the incorporation of [3 H]-phenylalanine into peptide linkage. Enzyme assays were determined in the cell supernatant fraction. All values are calculated with reference to activities in castrated animals that received an injection of oil only (controls). ●, Protein synthesis in the microsomal fraction; ○, alkaline phosphatase; ▲, aldolase; △, solubilized form I (nucleolar) RNA polymerase. Results on the latter enzyme are taken from Mainwaring *et al.* (1971), in which the experimental protocol was slightly different in terms of the duration of the period of castration before androgenic stimulation. The data are included simply to show the rapidity in the response of RNA polymerase to hormonal stimulation.

considered as part of the complex phenomena encompassed within the hormonal maintenance of the prostate gland, rather than a critical early event in the mechanism of action of androgens. The overall change in aldolase activity after androgenic stimulation was similarly extensive, irrespective of whether it was calculated with reference to prostate protein or DNA. In additional experiments, not presented in detail here, the enhancement of aldolase activity was impaired by the concomitant administration of the metabolic inhibitors, actinomycin D or cycloheximide (25 μ g/100mg body wt.), with testosterone *in vivo*. This provided evidence that continued RNA and protein synthesis plays an obligatory part in the androgenic induction of aldolase activity.

Androgen-mediated changes in aldolase mRNA synthesis monitored by procedures *in vitro*

At various intervals of time after the administration of testosterone to castrated rats *in vivo* poly(A)-rich mRNA was isolated from liver and prostate gland and added to the ascites-tumour S-30 system *in vitro*. The total incorporation of phenylalanine into peptide linkage was not dramatically influenced by the androgenic status of the animals from which the samples of mRNA were prepared. However, significant changes in aldolase synthesis were monitored by anti-aldolase serum (Table 3). These changes were tissue-specific, since androgens evoked a stimulation in aldolase synthesis in the presence of mRNA derived from prostate gland but not liver. An increase in aldolase synthesis was detectable after 4h of hormonal stimulation, reached a maximum at approx. 8h and then declined. The latter is an important observation, not only with regard to the reversibility of the induction of aldolase activity (see Fig. 5) but also with

Table 3. *Tissue-specific control of aldolase mRNA synthesis in the prostate gland by androgens*

Beginning 96h after castration, animals were given an injection of testosterone (2.5mg/animal) subcutaneously; eight animals served as controls and received an injection of oil only. At timed intervals, prostate glands and liver were pooled from eight animals and poly(A)-rich mRNA was isolated as described in the Materials and Methods section. A total of six ascites-tumour S-30 preparations (each assay volume, 50 μ l; 2.5 μ Ci of [3 H]phenylalanine, 12.5 Ci/mmol) was set up for each preparation of mRNA (2.5 μ g per assay). After incubation, the products of the reactions were assayed for total incorporation of labelled amino acid into protein and for the amount of labelled aldolase synthesized. For details see the legend to Table 2. Results are given as means $\pm$ S.E.M.

Source of mRNA	Testosterone treatment (h)	Total incorporation (c.p.m.)	Precipitated radioactivity (c.p.m.)	
			Anti-aldolase serum	Control serum
Prostate gland	None (controls)	17190 $\pm$ 200	132 $\pm$ 21	66 $\pm$ 10
	4	18490 $\pm$ 110	251 $\pm$ 17	120 $\pm$ 18
	8	22100 $\pm$ 270	349 $\pm$ 21	140 $\pm$ 13
	16	21000 $\pm$ 120	260 $\pm$ 17	111 $\pm$ 20
	24	20340 $\pm$ 240	167 $\pm$ 20	106 $\pm$ 17
Liver	None (controls)	15500 $\pm$ 190	170 $\pm$ 20	87 $\pm$ 8
	4	16200 $\pm$ 170	180 $\pm$ 17	94 $\pm$ 7
	8	16000 $\pm$ 240	168 $\pm$ 13	86 $\pm$ 6
	16	16400 $\pm$ 120	181 $\pm$ 17	94 $\pm$ 10
	24	16200 $\pm$ 140	174 $\pm$ 16	106 $\pm$ 6

reference to the retention time of androgens in the prostate gland *in vivo* of approx. 16h (Tveter & Attramadal, 1968; Mainwaring & Peterken, 1971). Meaningful changes in aldolase mRNA synthesis would thus be predicted to occur within this time scale. The relatively small amount of aldolase synthesis *in vitro* in the presence of prostate mRNA derived from castrated animals precludes the possibility that large amounts of pre-existing or stable aldolase mRNA are present in the prostate gland in the absence of androgens. Another particularly interesting facet of the temporal changes in aldolase mRNA synthesis is that attainment of the maximum and the subsequent decline occur somewhat sooner than the changes in aldolase activity *per se* determined in castrated animals after an identical regimen of testosterone injections (compare Table 3 and Fig. 5).

Steroid specificity of changes in prostate aldolase synthesis measured in polyribosomes in vivo

A concluding series of experiments was essentially directed towards the resolution of two problems. First, it was necessary to establish that the androgen-mediated regulation of aldolase mRNA measured *in vitro* reproduced the changes occurring in whole tissue with fidelity. Secondly, the establishment of the steroid specificity of the process was of paramount importance. A group of castrated animals was given a single injection of testosterone *in vivo*, and at timed intervals [35 S]methionine was injected directly into the prostate gland. Labelled aldolase in the isolated polyribosomes was then determined by immunological means (Table 4). The time-course of the induction of prostate aldolase measured under these conditions closely followed that of aldolase enzyme activity after androgenic stimulation (see

Fig. 5). Not only were prostate polyribosomes labelled to a greater extent after the administration of testosterone, but also a greater proportion of the complement of nascent polypeptide chains was precipitated by anti-aldolase serum. Since only testosterone was able to stimulate the amount of polyribosomes from a baseline content of 0.87–0.95 mg of rRNA/gland in the castrated controls to 1.44–1.50 mg of rRNA/gland after hormonal stimulation, the lack of effect of oestrogens and glucocorticoids on prostate aldolase synthesis in the prostate gland is even more striking than is indicated in Table 4. This result indicated that the changes in the amount of prostate aldolase mRNA determined *in vitro* were a valid assessment of the changes occurring within the intact gland *in vivo*. Once the time-course of the change in the activity of prostate polyribosomes had been delineated, an investigation into the steroid specificity of prostate aldolase synthesis could be undertaken. The absence of a change in aldolase synthesis in prostate polyribosomes after the administration of oestradiol-17 β or corticosterone indicated the marked steroid specificity in the regulation of aldolase synthesis.

Labelled prostate polyribosomes from castrated controls and testosterone-treated castrated animals were separated in sucrose gradients and individual fractions were analysed for aldolase by the immunological procedure (Fig. 6). Polyribosomes from the castrated animals were essentially recovered as ribosome monomers and dimers, as reported by Mainwaring & Wilce (1973); no conclusive evidence of aldolase synthesis could be detected, but it should be stressed that the low amount of radioactivity associated with the small proportion of heavy polyribosomes rendered the assay of aldolase very uncertain in these preparations. Heavy polyribosomes were a

Table 4. Time-course and steroid specificity of aldolase synthesis measured by labelling of prostate polyribosomes *in vivo*

Two experiments are summarized here. (a) Time-course of aldolase synthesis promoted by androgens. At 96 h after castration, 24 animals were given a subcutaneous injection of testosterone (2.5 mg/animal); six served as controls and received an injection of oil only. At timed intervals, 5 μ Ci of [35 S]methionine was injected directly into the prostate glands of six animals *in vivo*, under conditions of surgical anaesthesia. Precisely 15 min later, animals were killed and labelled prostate polyribosomes were isolated. Small samples of polyribosome suspensions were counted directly for radioactivity and assayed for rRNA. Additional samples (equivalent to 10 E_{254} units) were supplemented with 5 μ g of crystalline prostate aldolase; three were treated with anti-aldolase serum and three with control serum to measure immunologically-precipitable radioactivity. (b) Steroid specificity of aldolase synthesis in the prostate gland. Castrated animals were injected with various steroids (2.5 mg/animal) for 16 h, with six animals per experimental group. After labelling with [35 S]methionine *in vivo* labelled prostate polyribosomes were analysed as described in (a). Results are given as means $\pm$ S.E.M.

Experiment	Steroid treatment	Total incorporation (c.p.m./10 E_{250} units)	Precipitated radioactivity (c.p.m./10 E_{250} units)	
			Anti-aldolase serum	Control serum
(a) Time-course of androgenic response	None (controls)	4220 $\pm$ 300	120 $\pm$ 12	106 $\pm$ 6
	Testosterone, 4 h	5100 $\pm$ 210	171 $\pm$ 6	131 $\pm$ 11
	Testosterone, 8 h	7440 $\pm$ 320	240 $\pm$ 13	160 $\pm$ 7
	Testosterone, 16 h	9200 $\pm$ 420	307 $\pm$ 17	200 $\pm$ 20
	Testosterone, 24 h	7890 $\pm$ 240	234 $\pm$ 20	177 $\pm$ 17
(b) Steroid specificity of response	Testosterone	8290 $\pm$ 340	330 $\pm$ 21	179 $\pm$ 19
	Oestradiol-17 β	3140 $\pm$ 70	104 $\pm$ 7	120 $\pm$ 20
	Corticosterone	4520 $\pm$ 290	56 $\pm$ 21	71 $\pm$ 19

Fig. 6. Identification of the size of prostate polyribosomes synthesizing aldolase *in vivo*

At 72 h after bilateral orchidectomy, a group of eight animals was treated with testosterone (2.5 mg/animal) *in vivo* for 24 h. A further group served as controls, receiving injections of oil only. Prostate glands were then labelled with [35 S]methionine *in vivo* as described in the legend to Table 4, and polyribosomes were prepared. Two samples of labelled polyribosomes (each containing 20 E_{254} units of rRNA) were analysed in sucrose gradients, with the direction of centrifugation from left to right. Each gradient was subdivided into 0.25 ml fractions, with automatic monitoring of E_{254} . After pooling of gradient fractions to yield 0.50 ml of sample, 0.1 ml was counted directly for radioactivity. Of the remainder, 0.20 ml portions were supplemented with 5 μ g of crystalline prostate aldolase and a determination was made of the radioactivity precipitated with either anti-aldolase or control serum. (a) Polyribosomes from androgen-stimulated animals. (b) Polyribosomes from castrated animals (controls). —, E_{254} ; ○, total radioactivity per 0.1 ml of pooled fractions; ●, difference in radioactivity precipitated by anti-aldolase serum and control serum per 0.2 ml of pooled fractions. Because of the extreme differences in the amounts of radioactivity determined in these two procedures, the data are plotted on separate scales.

prominent feature of preparations derived from prostate glands of testosterone-treated animals. In these preparations, labelled aldolase was detectable and was specifically found associated with polyribosomes of sedimentation coefficient approx. 200S. The latter calculation is made with reference to the prominent peak in the upper regions of the gradients, especially in prostate polyribosomes derived from castrated animals. This internal sedimentation marker peak contains essentially the ribosome monomers, sedimentation coefficient 81S (Mainwaring & Wilce, 1973). The proportion of the nascent polypeptide chains recoverable in the presence of anti-aldolase serum was small, being of the order of 1% of the radioactivity associated with the heavy polyribosomes (approx. 200S). From a logarithmic plot of the migration of the aldolase-synthesizing polyribosomes relative to the 80S monomer marker, as recommended by Kuff & Roberts (1967), the polyribosomes of mean sedimentation coefficient 200S correspond approximately to ribosome tetramers, pentamers and hexamers.

Discussion

The translation of prostate mRNA in cell-free protein-synthesizing systems occurs under conditions similar to those used in the study of specific species of poly(A)-rich RNA (Mathews & Korner, 1970; Aviv *et al.*, 1971). Prostate mRNA interacts with the ascites-tumour S-30 system in a manner totally distinct from the behaviour of the synthetic mRNA, poly(U). This is not surprising, since it is widely accepted that synthetic and naturally occurring mRNA species are not bound similarly to free ribosome monomers (Gilbert, 1963; Takanami & Okamoto, 1963). There is also considerable evidence to suggest that different factors are necessary for the translation of poly(U) (Shafritz *et al.*, 1970) and naturally occurring mRNA (Pritchard *et al.*, 1970) in cell-free system derived from eukaryotic sources.

Certain proteins may be selectively removed from rabbit reticulocyte polyribosomes by washing in 0.5M-KCl (Miller & Schweet, 1968), and these serve as stimulatory factors for the synthesis of protein in cell-free systems derived from reticulocytes (Shafritz *et al.*, 1970; Pritchard *et al.*, 1970; Comstock *et al.*, 1972; Palmiter, 1973) and ascites-tumour cells (Metafora *et al.*, 1972; Schutz *et al.*, 1972). More recently, mixed cell-free systems containing components from reticulocytes and tumour cells have been advocated by Waldman & Goldstein (1973). Adequate incorporation of labelled amino acids into protein for our present purposes occurred without supplementation with such 'KCl-wash factors', and protein synthesis was carried out as originally described by Mathews & Korner (1970).

In general terms, the results strongly support the view that the synthesis of specific species of mRNA occurs *de novo* in the course of androgenic stimulation under conditions both *in vivo* and *in vitro*. This conclusion is essentially concordant with the findings from studies on other steroid-sensitive systems (Comstock *et al.*, 1972; Means *et al.*, 1972; Tuohimaa *et al.*, 1972; Beck *et al.*, 1972; Scott *et al.*, 1972). These results indicate that steroid hormones promote a major and fundamental change in transcription within steroid-sensitive cells. The changes in mRNA synthesis reported here are a more subtle manifestation of the androgenic response than the extensive changes in rRNA synthesis that are an accompanying feature of hormonal stimulation of the prostate gland (Mainwaring & Wilce, 1972, 1973). One particularly important distinction between the rate of aldolase mRNA synthesis measured in cell-free systems as compared with that measured in whole prostatic tissue and polyribosomes should be emphasized. From experiments based on the translation of purified prostate mRNA, the maximum production of aldolase mRNA occurs after 8 h of androgenic stimulation. This is considerably earlier than the time taken to attain maximum aldolase synthesis in whole tissue and polyribosomes. One plausible explanation of this important difference in response time is that the androgenic regulation of aldolase mRNA synthesis alone does not entirely account for the hormonal control of the synthesis of aldolase. It is reasonable to speculate that androgens control the synthesis of specific proteins by mechanisms operative at both the levels of transcription and translation. This is by no means a novel suggestion and experimental evidence favouring a considerable amount of translational control in the hormonal induction of proteins has been established in other systems (Means & O'Malley, 1971; Means *et al.*, 1971; Scott *et al.*, 1972). Such translational control could be implemented by various mechanisms, including the provision of specific initiation and translocation factors for protein synthesis or alterations in the supply of the amounts of rate-limiting species of tRNA.

The prostate gland is an alveolar structure essentially consisting of a single layer of cubical epithelial cells supported by a muscular stroma. The fact that the anti-aldolase serum was raised against purified aldolase of the muscle (type A) form raises the interesting question of whether the reported changes in aldolase mRNA occurred in the stroma or epithelium. Radioautographic evidence suggests that androgens are preferentially concentrated in the epithelial elements rather than the stroma (Tveter & Attramadal, 1969) but this does not necessarily raise difficulties in the overall interpretation of the present study. There is no reason *a priori* why the particularly androgen-responsive epithelial cells should not contain muscle

(type A) aldolase. Many non-muscular tissues contain muscle (type A) aldolase and indeed various hepatomas surprisingly contain this form of enzyme rather than liver (type B) aldolase (Gracy *et al.*, 1970; Ikehara *et al.*, 1970).

Note Added in Proof (Received 1 October 1974)

In preparing the manuscript, the authors overlooked the important paper by Schutz *et al.* (1973) on the glucocorticoid regulation of liver tryptophan 2,3-dioxygenase mRNA.

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