the Canadian Diabetic Association. All the cod islet experiments were carried out in Dr P. T. Grant's Department.

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Biosynthesis of an Insulin Precursor by Cod Islet Tissue and the Occurrence of Intermediates in the Conversion to Insulin

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Cod insulin contains four proline residues (Grant & Reid, 1968). The incorporation of [^{14}C]proline by slices of cod islet tissue at 15° into protein soluble in acid ethanol was approximately linear for 6 hr., and was inhibited by puromycin but slightly stimulated by actinomycin D. The radioactivity of protein fractions in these extracts was determined in view of the conclusion by Steiner, Cunningham, Spigelman & Aten (1967) that insulin is formed in rat and human islets from a single polypeptide chain precursor.

The radioactivity of an extract after 1 hr. was present in only one fraction (fraction P1). The molecular weight of this fraction was about 10200, and it was appreciably more basic than insulin as judged by gel filtration on Sephadex G50 and chromatography on DEAE-cellulose as described by Grant & Reid (1968). Longer periods of incubation increased the radioactivity of fraction P1 and after 1 hr. there was a detectable and progressive increase in the radioactivity both of insulin and of fractions intermediate in molecular size and basicity between fraction P1 and insulin.

Slices were incubated with [^{14}C]proline for 1 hr. to label fraction P1, *O*-*p*-nitrophenyl *O*-ethyl phenylpropylphosphonate (Becker, Fukuto, Boone, Canham & Boger, 1963) was added, and the incubation was continued for a further 4 hr. This tryptic and chymotryptic inhibitor had little effect on the biosynthesis of fraction P1, but there was a marked decrease in the radioactivity associated with insulin and the intermediate fractions. In a similar experiment, puromycin or an excess of unlabelled

L-proline resulted in the radioactivity of fraction P1 being less than that associated with insulin and the intermediate fractions.

Treatment of a radioactive preparation of the single-chain polypeptide, fraction P1, with trypsin (150:1), resulted in a progressive increase of biological activity in the digest as measured by the rat fat-pad bioassay, and of the radioactivity in intermediates and insulin fractions separated on DEAE-cellulose.

These results are consistent with the concept that insulin formation involves the biosynthesis of a precursor polypeptide, but the conversion of precursor into product in cod islet tissue appears to involve the cleavage of peptide bonds, resulting in the formation of a number of intermediates that in the present procedures are poorly separated from one another and to a certain extent from insulin.

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Rapid Effects of Testosterone on Prostatic Polyamine-Synthesizing Enzyme Systems

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The prostate glands and their secretions in many species contain high concentrations of spermidine and spermine (Mann, 1964; Tabor & Tabor, 1964). The ability of spermidine and spermine to interact with polynucleotides, ribosomes and phospholipids, and also to influence the enzymic synthesis of DNA, RNA and proteins (Tabor & Tabor, 1964; Raina & Telaranta, 1967) prompted us to examine the activity of some enzymes involved in polyamine biosynthesis in relation to androgen-induced growth of the ventral prostate in orchietomized rats. During the latter process, nuclear RNA polymerase activity is increased within 1 hr. after injection of testosterone (Liao, Leininger, Sagher & Barton, 1965), whereas DNA polymerase activity exhibits large but transitory elevations between the second and the fifth day after commencement of daily androgen treatments (Coffey, Shimazaki & Williams-Ashman, 1968).

Putrescine is formed in rat prostate by action of a pyridoxal phosphate-dependent L-ornithine decarboxylase (Pegg & Williams-Ashman, 1968a). Spermidine is synthesized from S-adenosyl-L-methionine (SAM) and putrescine. The decarboxylation of SAM by soluble prostatic extracts is markedly and specifically stimulated by putrescine;

in the presence of putrescine, there occurs a stoichiometric formation of spermidine and CO₂ (Pegg & Williams-Ashman, 1968b).

Rat ventral prostates were homogenized with 3 vol. of 0.25 M-sucrose-0.3 mM-EDTA, and centrifuged at 25000g for 20 min. The supernatant fluids were passed through columns of BioGel P-10 before assay of the two decarboxylase activities, which were expressed in terms of μ moles of ¹⁴CO₂ released/mg. of protein/hr. The putrescine-stimulated SAM decarboxylase activity declined from a normal value of 720 to 45 in animals castrated 7 days before death. Ornithine decarboxylase activity fell from 350 to 60 under the same conditions. Within 3 hr. after injection of 1 mg. of testosterone propionate into the castrated rats, the decarboxylation of SAM was doubled, and six- and ten-fold increases in activity were observed 24 and 48 hr. after administration of the hormone. Ornithine decarboxylase activities were significantly increased 6 hr. after injection of the androgen and were tripled after 24 hr. Daily injection of the androgen over a period of 2 weeks restored both activities to normal.

These findings suggest that RNA and polyamine biosynthesis may be coupled during the early phases of androgen-dependent prostatic growth. Several groups have recently found correlations between RNA and polyamine or putrescine formation during growth of other tissues (Dykstra & Herbst, 1965; Caldarera, Barbiroli & Morruzi, 1965; Kostyo, 1966; Raina, Jänne & Siimes, 1966; Jänne & Raina, 1968; Russell & Snyder, 1968).

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Testosterone Metabolism in the Rat Ventral Prostate

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The adult rat prostate is maintained in a functional state only in the presence of androgen. Castration results in atrophy of this organ, which is reversed by testosterone administration.

Effects similar to those resulting from castration follow administration of anti-androgens or oestrogens. Whether these effects are due to direct competition for active sites within the prostate or are mediated via other organs remains largely unknown. Androgens, in comparison with oestrogens, are effective only at higher concentrations and after longer time-intervals (Jensen, 1965). Also, testosterone action is associated with metabolism of the hormone in target organs. The possibility therefore exists that the action of testosterone depends on metabolism of the hormone, and that anti-androgens exert their effect by influencing this metabolism. For these reasons we have examined the metabolism of low concentrations of testosterone and have also studied the effects of anti-androgens on this.

A simple technique has been developed whereby the metabolites of testosterone can be extracted and estimated both by radioactivity determinations and by gas-liquid chromatography. Labelled testosterone was injected directly into the prostate in comparable studies *in vivo* and whole-organ studies *in vitro*. After 0-120 min. the prostate was homogenized at 0°, deproteinized with 95% ethanol and reduced to an aqueous residue, which was extracted with ether. The ether extract was concentrated, dried and applied to a thin-layer plate. Non-specific lipid moved to the solvent front on development with benzene. The steroids, which remained at the origin, were further separated from lipid material by developing the plates in cyclohexane-acetone (69:31, v/v). The developed plates were scanned for radioactivity. The area of silica gel containing the labelled metabolites was drawn into a pasteur pipette and eluted with chloroform. The total eluate was applied to a Pye 104 Gas Chromatograph by the solid-injection technique of Menini & Norymberski (1965).

The results of both gas-liquid chromatography and radioactivity determinations indicate that testosterone metabolism reaches a steady state after 60 min. and that 20-30% of the original testosterone is converted principally to androstane diol, androsterone and dihydrotestosterone. This is in agreement with the findings of Farnsworth & Brown (1963), using rat prostate minces.

Administration of 1 mg. of cyproterone acetate or