We have used the antibodies produced by this method to study the immunochemistry and biological role of small polypeptides (*11*). Future applications of this method may include synthesis of multivalent antigens for detection of antibodies to bradykinin or other haptens or for immunization against toxic compounds of low molecular weight, and for conjugation of antigens to insoluble resins.

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13. One rabbit was immunized with a conjugate of albumin and ACTH prepared with carbodiimide. Some of the antibodies produced by this rabbit were directed to ACTH, as shown by ACTH inhibition of complement fixation, and neutralization by the antiserum of the biological activity of ACTH. However, the antiserum also contained a considerable concentration of antibody to carbodiimide derivatives, and unequivocal interpretation of the serological data awaits further study. Porcine ACTH was the gift of A. Cohen, Abbott Laboratories.
14. Valuable assistance was given by Eleanor Wasserman and F. Castillo. Supported by grants from NSF, NIH, and the American Cancer Society, a fellowship from the Helen Hay Whitney Foundation (to T.L.G.), and an established investigatorship of the American Heart Association (to G.D.F.). This is publication No. 277 of the graduate Department of Biochemistry, Brandeis University.

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RNA Synthesis in Rat Seminal Vesicles: Stimulation by Testosterone

Abstract. *Within 70 minutes after the administration of testosterone to rats castrated 12 to 15 hours previously, the rate of synthesis of RNA in the seminal vesicle is increased by 50 percent and continues to rise until approximately 50 minutes after injection when a two- to threefold increase was attained. No further increase was detected for as long as 240 minutes after hormone administration. The base composition of the pulse-labeled RNA was intermediate between that of the total seminal vesicle RNA and DNA-like RNA. No change in this composition was detected at any interval after injection.*

It appears that the synthesis of RNA may be particularly involved in the action of androgenic steroids on accessory reproductive organs (*1*). RNA synthesis in the rat seminal vesicle was increased by testosterone propionate at a time when there was no detectable effect on the synthesis of protein (*1*). This observation suggests that the ultimate growth response of this and other organs on which androgens act may result from an initial enhancement of the synthesis of RNA and perhaps of specific classes of RNA. Liao and Williams-Ashman (*1*) suggested that testosterone governs the synthesis or utilization (or both) of messenger RNA in the ventral prostate. In our study we have determined the time course of the response of RNA synthesis in the seminal vesicle to the administration of testosterone. Incorporation of P^{32} into RNA during a brief interval (pulse labeling) was used as a measure of rate of synthesis, and also permitted analysis of the composition of the newly formed RNA (*2*).

Sexually mature Sprague-Dawley rats (400 to 450 g) were castrated 12 to 15 hours before the intraperitoneal injection of 10 mg of testosterone in saline; injection of saline alone was without effect. Treatment with the hormone took place for various periods of time, while carrier-free P^{32} was administered intraperitoneally 50 minutes before killing. Seminal vesicles were excised and frozen in liquid nitrogen. After the tissues were thawed, they were homogenized in 0.25*M* sucrose and trichloroacetic acid was added to a final concentration of 10 percent. The acid-soluble material was analyzed for inorganic phosphate (*3*) and radioactivity after centrifugation. The insoluble residue was washed several times with trichloroacetic acid and lipid solvents and then extracted with 10 percent NaCl at 100°C to obtain the nucleic acids (*4*). Radioactive contaminants were removed from the nucleic acid extract by the use of diethylaminoethyl cellulose paper in the chloride form (*5*). After hydrolysis of the RNA in 0.5*N* KOH at 37°C for 16 to 18 hours, perchloric acid was added at 0° to 5°C to precipitate any DNA or protein present. Radioactivity and optical density at 260 mμ of the clear supernatant fraction were determined. The specific radioactivity (counts per minute per milligram) of RNA calculated from these determinations was corrected for variations in the specific radioactivity of the acid-soluble phosphate pool. The specific radioactivity of the acid-labile phosphate fraction (obtained by adsorption onto charcoal and heating at 100°C for 7 minutes in 1*N* HCl) corresponded closely with that of the total acid-soluble phosphate fraction. The ribonucleotides in the remaining supernatant

Table 1. Effect of testosterone on composition of total and pulse-labeled seminal vesicle RNA. The composition of total RNA was determined by ultraviolet absorption of eluted nucleotides, while that of newly formed RNA was determined by liquid scintillation counting. Variation in observed values was less than 10 percent.

Time after testosterone (min)	Percentage*				Ratio: (AMP + UMP) / (GMP + CMP)	No. observations
	AMP†	GMP	UMP	CMP		
			Total RNA			
	19	33	20	28	0.65	(12)
			P^{32} *RNA*			
0	22	24	29	26	1.02	(6)
50	23	24	30	23	1.13	(2)
100	23	23	29	25	1.10	(2)
240	24	24	28	25	1.06	(2)
			DNA (ref. 9)			
	28	20	29(T)	23	1.35	

* Percentage of total micrograms of nucleotides or total counts. † AMP refers to adenosine-2′,3′ monophosphate. GMP, UMP, and CMP refer to the 2′,3′ monophosphates of guanosine, uridine, and cytidine.

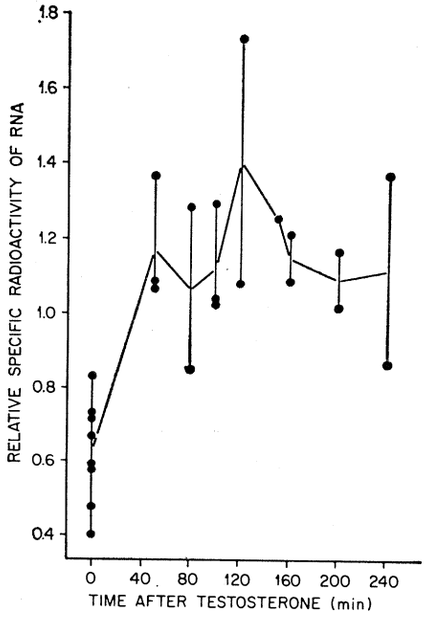

Fig. 1. Effect of testosterone on the rate of RNA synthesis in the seminal vesicle. Each point represents one determination. Relative specific radioactivity of RNA is the ratio: [(count/min per milligram of RNA) to (count/min per micromole of acid-soluble P_i)] times 100.

fraction were adsorbed onto a small charcoal column (*6*), and the nucleotides were eluted with ammoniacal ethanol (*7*). After evaporation, the samples were chromatographed on diethylaminoethyl cellulose paper in the formate form (*8*), and the ultraviolet spots were located. In all cases, the only radioactive regions on the chromatograms coincided with the four ultraviolet-absorbing spots corresponding to the four nucleotides. The nucleotides were eluted with 0.5*M* NH_4HCO_3 and, after evaporation, were analyzed for ultraviolet absorption and for radioactivity.

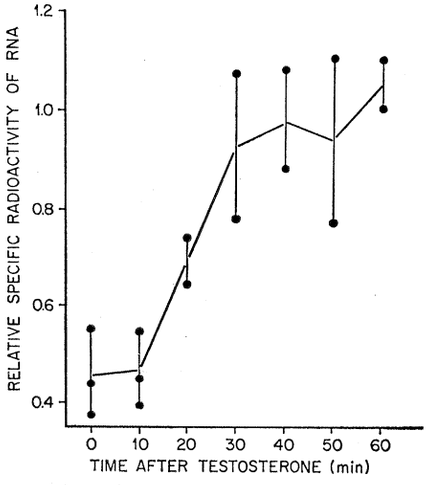

Fig. 2. Early effect of testosterone on the rate of RNA synthesis in seminal vesicle. For other details see Fig. 1.

The results indicate that testosterone is capable of inducing a rapid increase in the rate of RNA synthesis (Fig. 1). The specific activity of RNA at 50 minutes was nearly twice that of the untreated (zero time) controls. No further significant increase in specific activity was detected for as long as 4 hours after the hormone was given. Observations at earlier intervals revealed a 50 percent increase in the specific activity of RNA 20 minutes after injection of testosterone (Fig. 2), whereas no effect was observed at 10 minutes. In view of the time required for the hormone to reach the seminal vesicle and to interact with some cellular component, it appears that the synthesis of RNA is closely linked to the primary site of action of the hormone.

It is of some interest that the specific activity of the acid-soluble phosphate pool is also increased markedly by testosterone. This effect, however, does not occur until some 50 minutes after injection, 30 minutes after the first effect on RNA synthesis. The same effect was noted with the phosphate fraction adsorbed on charcoal and eluted with HCl at 100°C.

The distribution of P^{32} in the nucleotides of the newly synthesized RNA is unlike the base composition of either total seminal vesicle RNA (determined concomitantly with P^{32} composition) or the DNA of the rat (*9*) (Table 1). The observed composition suggests a mixture of ribosomal and DNA-like RNA's, similar to observations of Perry (*10*) and Georgiev *et al.* (*11*) on pulse-labeled RNA in other mammalian tissues and cells. Several intervals after hormone administration other than those listed in the table were also examined and no effect of testosterone on the P^{32} base composition was detected.

Attempts to fractionate this tissue into nuclear and cytoplasmic components have not been completely successful. In these experiments it does appear that an increase in the labeling of RNA occurs in both fractions 50 minutes after treatment with the hormone, but because of contamination of each fraction with the other, it is not known in which fraction the increase is initiated. In the other aspects examined, however, the early effect of androgen on the seminal vesicle is nearly identical to the effect of hydrocortisone on the liver (*5*). Thus these steroids cause an early and marked stimulation of RNA synthesis but do not alter the composition of the RNA formed. In each case this composition is indicative of a mixture of several kinds of RNA. These considerations suggest that the stimulation of RNA synthesis by these hormones is not limited to the synthesis of a single type of RNA.

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Taste Sensitivity to Phenylthiourea in Glaucoma

Abstract. *In a series of Caucasian patients over the age of 40 years, the number of individuals unable to recognize the bitter taste of phenylthiourea (8.1 mg/100 ml) was found to be 28 percent in a "normal" eye clinic population (446 individuals), 17 percent in a series of 155 patients with angle-closure glaucoma, and 53 percent in 211 patients with primary open-angle glaucoma.*

The primary glaucomas are divided into two categories based upon the anatomic appearance of the angle of the anterior chamber, namely primary angle-closure glaucoma and primary open-angle glaucoma. The hereditary nature of each variety of primary glau-