metabolic rate, (2) absorption is readily induced simply by saturating the cell with an easily exchangeable monovalent ion, (3) halide ions are not accumulated by the cells, and (4) equilibration kinetics are applicable to the absorption phenomenon.

Salt uptake by *Escherichia coli*[11] is similar in many details to that of *S. cervesiae*. The explanations advanced here are probably applicable to *E. coli* and might well be extended to other cells, including specialized cells of higher organisms as well as other microorganisms.

Summary.—Alkali ion absorption by Baker's yeast is associated with an exchange process largely mediated by H^+ which can either arise metabolically or be supplied by acid washing. The rate of cation entry into yeast cells is in agreement with a diffusion-limited passive exchange process.

* Participated in this work while on Postdoctoral Research Fellowship with Mineral Nutrition Laboratory. Present address: Department of Chemistry, Montana State College, Bozeman.

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AN EFFECT OF TESTOSTERONE ON AMINO ACID INCORPORATION BY PROSTATIC RIBONUCLEOPROTEIN PARTICLES

BY SHUTSUNG LIAO AND H. G. WILLIAMS-ASHMAN

BEN MAY LABORATORY FOR CANCER RESEARCH AND DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO

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This paper concerns an action of testosterone on the transfer of radioisotope from sRNA-valine-C^{14} to protein catalyzed *in vitro* by ribonucleoprotein particles isolated from the rat prostate gland. The incorporations were studied under conditions where they were proportional to the quantity of ribosomal material added, and where the levels of obligatory soluble protein factors were not rate-limiting. The rate and extent of such amino acyl transfers were markedly diminished with prostatic ribosomes prepared from adult rats which had been castrated 2–3 days previously. This effect of withdrawal of testicular hormones was entirely reversed by administration of testosterone to the orchiectomized animals, but not by addition of this hormone to the isolated enzyme systems. However, the conversion of radioisotope from sRNA-valine-C^{14} to acid-insoluble products by prostatic ribosomes

isolated from the castrates was enhanced as much as three-fold by addition of poly UG, whereas valine incorporation by prostatic ribosomes from testosterone-treated castrates was hardly affected by poly UG. A possible explanation for these findings is that the androgenic status of the host influences the levels of template RNA attached to prostatic ribosomes isolated by the stated methods.

Materials and Methods.—Materials: Uniformly labeled L-valine-C^{14} (100–200 mc/mmole) was obtained from the New England Nuclear Corporation. Nonradioactive amino acids, glutathione, and yeast RNA were purchased from the Nutritional Biochemicals Corporation. Crystalline pyruvic kinase, phosphopyruvate, and calf thymus DNA were supplied by the Sigma Chemical Company. ATP, GTP, UDP, and GDP were obtained from the Pabst Laboratories. Deoxycholic acid was converted to the potassium salt (pH 7.8) before use.

Isolation of enzyme fractions: The media used for preparation of various enzyme fractions had the following compositions: Medium A, 0.35 *M* sucrose, 0.025 *M* KCl, 0.01 *M* $MgCl_2$, 0.035 *M* Tris-HCl buffer, pH 7.5, 0.005 *M* β-mercaptoethanol; Medium B, identical with A except that β-mercaptoethanol was omitted; Medium C, 0.9 *M* sucrose, 0.025 *M* KCl, 0.01 *M* $MgCl_2$; Medium D, 0.01 *M* Tris-HCl buffer, pH 7.8, 0.01 *M* $MgCl_2$, 0.01 *M* KCl, 0.005 *M* β-mercaptoethanol. Approximately 10-week-old Sprague-Dawley rats (250–300 gm) were used. The ventral prostate glands (usually weighing between 250 and 350 mg per rat) were dissected away immediately after sacrifice by cervical fracture. The tissue was placed in ice-cold Medium A. After all of the tissue was collected, it was blotted with cheese cloth, minced with scissors on a watch glass placed on ice, and then homogenized with 3 volumes of Medium A at 0–2° for 1 min in an all glass apparatus. All further manipulations were carried out as close to 0° as possible. The homogenates were strained through a silk filter cloth (Kopp Scientific Inc., New York, N.Y.). Two methods were used for preliminary fractionation of the homogenates prior to isolation of the ribonucleoprotein particles. *Method I:* a suspension of microsomes was first isolated as follows. The homogenate was centrifuged at 500 × *g* for 15 min, and the precipitate of nuclei and cell debris discarded. The supernatant fluid was centrifuged at 9,000 × *g* for 15 min in order to sediment mitochondria and other heavy cytoplasmic particles. The sediment was discarded, and the supernatant fluid centrifuged for 1 hr at 105,000 × *g* in a Spinco preparative ultracentrifuge. The resulting microsomal pellets were combined, and suspended in Medium B (2 gm tissue equivalent per 1 ml). *Method II:* a suspension of nuclei, heavy cytoplasmic particles, and microsomes was first prepared by centrifugation of the whole homogenate at 105,000 × *g* for 1 hr. The precipitate was suspended in Medium B, and made up to the original tissue volume. Ribonucleoprotein particles were then isolated from the suspensions made by Method I or II by a procedure patterned on that described by Kirsch *et al.*[1] for liver ribosomes. The particle suspensions were diluted with 4 volumes of Medium C. To the mixture, one-ninth volume of 2.5% solution of deoxycholate was added slowly with stirring. The mixture was immediately centrifuged at 20,000 × *g* for 30 min, and the sediment discarded. The supernatant fluid was centrifuged at 105,000 × *g* for 90 min. After removal of the supernatant fluid by decantation, the sides of the tubes and the surfaces of the ribosomal pellets were rinsed 3 times with Medium B, and the ribosomes suspended in this medium (1 gm tissue equivalent per 1 ml). The suspension was centrifuged at 105,000 × *g* for 90 min, and the sediment resuspended in Medium B.

The supernatant fluid resulting from centrifugation of the original homogenate at 105,000 × *g* (i.e., after removal of microsomes in Method I, or after removal of combined cell particulate matter in Method II) was used as a source of "supernatant proteins." These solutions were dialyzed twice against 200 volumes of Medium D for 2 hr.

Preparation of sRNA-amino acids: Rat liver "pH 5 precipitates" were isolated from the soluble fraction of rat liver homogenates by isoelectric precipitation at pH 5.2 following addition of 1 *N* acetic acid.[2] The precipitates were collected by centrifugation, and suspended in Medium B (equivalent to one-third of the original volume). The material was reprecipitated in the same manner, and finally suspended in Medium B to give a solution containing approximately 20 mg of protein per 1 ml. Liver sRNA was isolated from liver pH 5 precipitates by the phenol method,[3] and was further purified by reprecipitation with ethanol from solutions of high ionic strength, followed by exhaustive dialysis against water. The transfer RNA in the sRNA preparations

was charged with amino acids as follows. A typical reaction mixture contained the following components (in μmoles unless otherwise stated) in a final volume of 20 ml: Tris-HCl buffer (pH 7.8), 2000; ATP, 200; $MgCl_2$, 200; phosphopyruvate, 200; pyruvic kinase, 100 μg; CTP, 2; liver sRNA, 10 mg; liver pH 5 precipitate (250 mg protein); L-valine-C^{14}, 0.2; and 0.2 μmole of the following nonradioactive L-amino acids: alanine, asparagine, arginine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and tyrosine. After incubation for 15 min at 35°, the reaction was terminated by addition of an equal volume of 90% phenol. The sRNA charged with amino acids was isolated from the aqueous layer of the phenol extract (obtained after shaking the mixture at 25° for 1 hr, and subsequent centrifugation) by the procedure of Ehrenstein and Lipmann.[4]

Synthetic polynucleotides: Poly U and poly UG (input UDP/GDP ratios 5:1) were prepared with the polynucleotide phosphorylase of *Micrococcus lysodeikticus*. The enzyme was purified from dried cells (Worthington) as far as step (ii) of the procedure of Steiner and Beers.[5] The 280/260 light absorption ratio of the polynucleotide phosphorylase preparations was in the neighborhood of 1.0. The reaction mixtures contained 0.08 *M* Tris-HCl buffer (pH 8.5), 0.006 *M* $MgCl_2$, 0.001 *M* trisodium ethylene diamine tetra acetic acid, 0.06 *M* KCl, 0.013 *M* UDP, 0.0025 *M* GDP (if added), and enzyme. The course of the reaction was followed by measurement of release of inorganic phosphate. After 8–10 hr incubation at 37°, the polymers were precipitated from the reaction mixture with ethanol, dissolved in 0.04 *M* NaCl–0.005 *M* sodium citrate, and then shaken for 1 hr at 25° with an equal volume of 90% phenol. After centrifugation, the aqueous phase was removed and extracted 5 times with peroxide-free ether. A gentle stream of nitrogen was passed through the solution to remove ether, and the polymers were then dialyzed exhaustively against water. The base composition of the samples of poly UG was determined by alkaline hydrolysis, followed by paper electrophoretic separation of the hydrolysis products.[6]

Measurement of amino acyl transfer from sRNA-amino acids to protein: The reactions were carried out in a final volume of 1.0 ml at 35°. The reaction mixtures contained the following components (in μmoles unless otherwise stated): sucrose, 35; Tris-HCl buffer (pH 7.8), 56; KCl, 52; $MgCl_2$, 13; β-mercaptoethanol, 11; ATP, 1; GTP, 1; phosphopyruvate, 10; pyruvic kinase, 50 μg; sRNA charged with valine-C^{14} and other C^{12}-amino acids; and supernatant protein. The quantities of the last two components, and the radioactivity of the sRNA-amino acids, are designated under the relevant protocols. It may be noted that the concentrations of many ingredients include contributions from the media used to suspend the ribonucleoprotein particles and the supernatant proteins. The reactions were initiated by addition of the ribosomal particles. The quantity of ribosomal material added to each vessel is expressed in terms of the total RNA associated with the particles. The reactions were terminated by addition of 5 ml of ice-cold 5% trichloracetic acid (TCA). The mixtures were stirred thoroughly, cooled on ice, and centrifuged at 2°. The precipitates were resuspended in 5 ml of 5% TCA, and then heated at 90° in a water bath for 20 min (hot TCA washing) in order to render acid-soluble the amino acids attached to the sRNA. After cooling and centrifugation, the precipitate was washed twice with 5 ml of 5% TCA, and washed a further two times with 5 ml of ethanol-ether (3:1 v/v). The washed precipitate was dissolved in concentrated formic acid[4] and transferred *in toto*, with suitable washings of formic acid, to aluminum planchettes (6.5 cm^2 in area). After drying the planchettes at 95–100°, the radioactivity was measured under conditions where errors due to self-absorption (not corrected for) were less than 10%. Sufficient counts were determined to reduce the standard error of counting to less than 3%. Corrections were applied for background counts and for zero-time controls (to which TCA was added prior to the ribosomal suspension). The zero-time values were invariably less than 20 cpm. Duplicate reaction mixtures used for study of amino acyl transfers always agreed within 10% of one another.

Other methods: Ribosomal RNA was estimated spectrophotometrically at 260 mμ after alkaline hydrolysis according to Hecht *et al.*,[7] assuming an extinction coefficient of 32.3 $cm^2\ mg^{-1}$ in 0.1 *N* NaOH. Protein was determined according to Warburg and Christian.[8] Orchiectomy was performed via the scrotal route under ether anesthesia. Testosterone propionate was administered by subcutaneous injection in sesame oil; the controls were injected with the same volume of oil. The doses of the hormone are shown in the protocols.

Results.—Incorporation of amino acids into protein by prostatic ribosomes: Ribo-

nucleoprotein particles isolated from the rat ventral prostate gland catalyzed the transfer of L-valine-C^{14} from added sRNA-valine-C^{14} to protein. The incorporation of valine necessitated the addition of a dialyzed soluble prostatic protein fraction (supernatant protein), and was stimulated by GTP (Table 1). A requirement for an energy source (phosphopyruvate and pyruvic kinase) was also manifest under these conditions. The incorporations were completely dependent upon the ribonucleoprotein particles. In various experiments, from 3–13% of the total radioisotope initially added as sRNA-valine-C^{14} was found to enter the protein fractions when the reactions were run to completion.

TABLE 1

TRANSFER OF L-VALINE-C^{14} FROM sRNA-VALINE-C^{14} TO PROTEIN

System	Radioactivity in protein fraction (cpm)
Complete	966
Omit phosphopyruvate, pyruvic kinase, and ATP	150
Omit ATP	840
Omit GTP	633
Omit supernatant protein	111

Components of the reaction mixtures are described in the text, except that only 1 μmole of β-mercaptoethanol was present, and 10 μmoles of glutathione were added. Other ingredients were: sRNA charged with L-valine-C^{14} and other amino acids, 0.40 mg (11,600 cpm); dialyzed supernatant protein fraction, 1.6 mg; prostatic ribonucleoprotein particles (prepared by Method I) equivalent to 3 gm fresh tissue. Incubated for 60 min.

In all of the following experiments, the sRNA charged with valine-C^{14} and other C^{12}-amino acids was manufactured from rat liver sRNA by the action of hepatic amino acid-activating enzymes. Other studies[9] showed that similarly labeled sRNA-amino acids were readily formed from sRNA and amino acid-activating enzymes present in "pH 5 precipitates" prepared from the soluble fraction of rat ventral prostate homogenates. Prostatic sRNA-valine-C^{14} formed in this way was isolated by the phenol method, and further refined by reprecipitation with ethanol. The rate and extent of radioisotope transfer to protein catalyzed by prostatic ribosomes were found to be similar with sRNA-valine-C^{14} prepared from either prostatic or liver sRNA.

Effect of orchiectomy: Initial experiments disclosed that 48–72 hr after castration of sexually mature rats, there was a marked decline in the ability of isolated prostatic ribosomes to catalyze the transfer of radioisotope from sRNA-valine-C^{14} to protein. Injection of testosterone into the orchiectomized animals completely reversed this effect of androgen deprivation. The magnitude of the changes (from 2- to 3-fold) was similar in experiments with prostatic ribosomes prepared by either Method I or Method II.[10] In the following studies, all of the animals were orchiectomized, and prostatic ribonucleoprotein particles were isolated at various time intervals thereafter. Some of the rats were treated with testosterone, and the valine incorporating activity of their prostatic ribosomes compared with that of particles isolated from castrates injected only with oil. This expedient obviated any possible effects of surgery *per se* on the prostates. The dialyzed supernatant protein fraction added to all vessels was derived from the prostates of the androgen-treated groups, and was present in excess. The typical experiment summarized in Figure 1 shows that both the initial rate and final extent of amino acyl transfer to protein was more than twice as great with ribonucleoprotein particles isolated from testosterone-treated castrates.

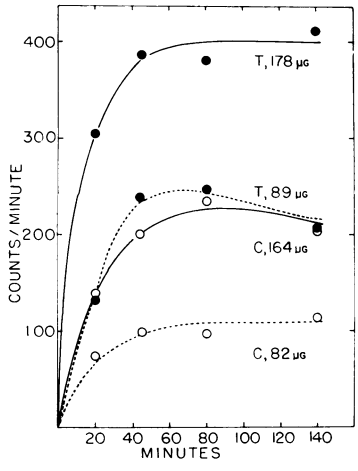

FIG. 1.—Influence of testosterone administration on incorporation of valine by prostatic ribosomes from castrated rats. Animals sacrificed 40 hr after surgery. One group injected with 1 mg testosterone propionate just prior to surgery, and 24 hr thereafter. Ventral prostates pooled from 65 castrates and from 45 testosterone-treated castrates. Ribosomes isolated by Method I. Ingredients of the test system as in the text, except that only 3 μmoles β-mercaptoethanol present, and 10 μmoles glutathione added. All vessels contained 0.97 mg dialyzed supernatant protein (from testosterone-treated castrates) and 0.40 mg sRNA charged with L-valine-C^{14} and other amino acids (11,640 cpm). The figures next to each curve represent the quantity of RNA attached to the ribosomes added to each vessel with particles derived from castrates (C) or testosterone-treated castrates (T). Ordinate: radioactivity of protein fraction. Abscissa: incubation time.

Effect of added polynucleotides: Attempts were undertaken to increase valine incorporation by isolated prostatic ribosomes by the direct addition of various polynuceotides. No consistent effects were obtained with yeast RNA, or with RNA prepared by the phenol method either from prostatic ribosomes, or from fresh unfractionated ventral prostate tissue. A similar lack of success attended efforts to form template RNA *in situ* by addition to the reactions mixtures of purified *E. coli* RNA polymerase (kindly provided by Dr. S. B. Weiss), ATP, UTP, GTP, CTP, and rat ventral prostate DNA. (Separate experiments showed that the same samples of prostatic DNA readily served as primers for the synthesis of polyribonucleotides by the bacterial RNA polymerase.) Valine incorporation by prostatic ribosomes was invariably depressed by addition of refined rat liver ribosomal RNA (Table 2).

Ochoa[13] and Nirenberg[14] observed that samples of poly UG with high U/G base ratios increased the entry of valine into protein catalyzed by bacterial ribosomes. Prostatic ribosomes from recently castrated animals exhibited a remarkable response to low levels of poly UG (U/G = 6.7). But radioisotope transfer from sRNA-valine-C^{14} to the protein fraction by prostatic ribosomes from testosterone-treated castrates was, in various experiments, only slightly increased (Figs. 2 and 3), or quite unaffected (Table 2) by addition of poly UG. Figure 2 shows that the response to poly UG was apparent under conditions where the incorporations were proportional to the amount of ribosomal material (expressed in terms of total RNA attached to the particles) added to the test systems. It may be noted that in the presence of poly UG, the radioactivity incorporated into the protein fractions by particles from untreated castrates was virtually the same as that observed with ribosomes from castrates injected with testosterone. The amounts of poly UG required to enhance the activity of the ribonucleoprotein particles were quite small, as shown in Figure 3. Puromycin, which seems to be a specific inhibitor for the transfer of amino acids from sRNA-amino acids to protein,[15] markedly depressed the incorporation of valine in the absence or presence of poly UG. This is evident from Table 2, which also shows that equivalent levels of poly U did not stimulate valine incorporation.

Direct action of testosterone: Amino acyl transfers catalyzed by prostatic ribosomes from both normal and androgen-deficient animals were consistently unaffected by the direct addition of testosterone (10^{-5} M). This steroid was also

TABLE 2

INFLUENCE OF POLYNUCLEOTIDES AND PUROMYCIN ON INCORPORATION OF VALINE INTO PROTEIN FRACTION

Additions	Radioactivity Incorporated into Protein Fraction (cpm) with Ribosomes Isolated from: Castrates	Testosterone-treated castrates
Nil	490	1,114
Poly UG (80 μg)	859	1,075
Puromycin (0.2 μmole)	31	110
Poly UG + Puromycin	182	171
Poly U (80 μg)	410	960
Calf thymus DNA (80 μg)	503	1,007
Yeast RNA (80 μg)	507	1,085
Rat liver ribosomal RNA (80 μg)	278	626

Other ingredients of the reaction mixtures are described in the text. Each tube contained 0.32 mg sRNA charged with L-valine-C^{14} and other C^{12}-amino acids (16,600 cpm) and 2.1 mg of dialyzed supernatant protein from the prostates of testosterone-treated castrates. The molar U/G ratio of the poly UG was 6.7. Ribonucleoprotein particles isolated from pooled prostatic tissue derived from 22 castrated rats (particles equivalent to 232 μg total ribosomal RNA added to each vessel) or from 10 testosterone-treated castrates (particles equivalent to 252 μg total ribosomal RNA added to each vessel). Animals sacrificed 46 hr after surgery. To one group, 2 mg of testosterone propionate was injected into each animal just prior to orchiectomy, and again 24 hr thereafter. Reaction mixtures incubated for 70 min. Ribosomes isolated by Method II.

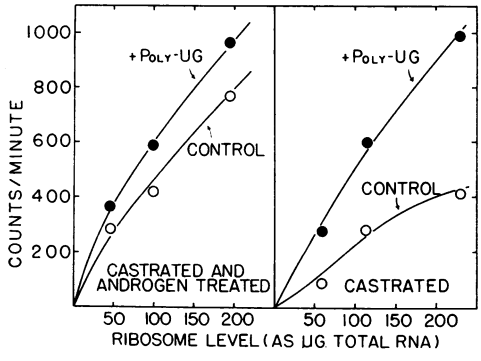

FIG. 2.—Effect of poly UG on valine incorporation by prostatic ribosomes from castrated rats, with or without treatment with testosterone. Animals sacrificed 72 hr after surgery. Testosterone propionate (2 mg) injected into one group just prior to orchiectomy, and also at 27 and 54 hr thereafter. Prostatic ribosomes isolated by Method II from pooled tissue derived from 20 castrates, and from 10 testosterone-treated castrates. Each vessel contained 2.2 mg dialyzed supernatant protein (from testosterone-treated castrates) and 0.35 mg sRNA charged with L-valine-C^{14} and other amino acids (19,250 cpm). Ordinate: radioactivity of protein fraction. Abscissa: quantity of ribosomal material added (expressed as μg of total RNA attached to the particles). Reaction mixtures incubated for 100 min.

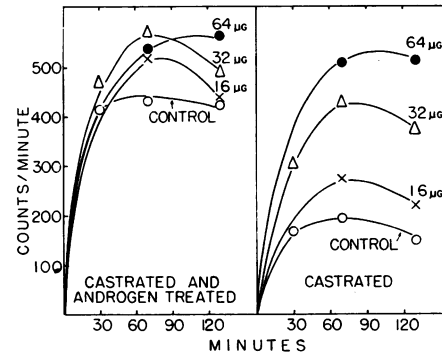

FIG. 3.—Effect of concentration of poly UG on valine incorporation by prostatic ribonucleoprotein particles from castrated rats. Animals sacrificed 64 hr after surgery. Testosterone propionate (2 mg) injected into one group just prior to surgery, and again at 40 hr thereafter. Prostatic ribosomes isolated by Method II from pooled ventral prostate tissue derived from 18 castrates, and from 12 testosterone-treated castrates. In every vessel, the total amount of RNA attached to the ribosomes from either group was 200 μg. Ordinate: radioactivity incorporated into protein fraction. Abscissa: time of incubation. The figures next to each curve represent the amount of poly UG (U/G = 6.7) added to the reaction mixtures. Each tube contained 0.35 mg sRNA charged with L-valine-C^{14} and 19 other C^{12}-amino acids (19,250 cpm) and 2.06 mg of dialyzed supernatant protein from prostates of testosterone-treated castrates.

without influence on the stimulation of valine incorporation by poly UG.

Discussion.—The foregoing observations show that the ability of prostatic ribosomes to incorporate valine from sRNA-valine into protein is greatly impaired within 2–3 days after orchiectomy. At such short intervals after castration of

adult rats, there is virtually no change in the relative proportion of various cell types in the ventral prostate gland, although there is a slight reduction in the height of the epithelial cells, and collapse of their apical ergastoplasmic sacs.[16, 17] In the present studies, the total amount of RNA attached to the particles was used as a measure of the quantity of ribonucleoprotein particles added to each reaction mixture, and sRNA-amino acids as such were used as the amino acyl donors. The findings are therefore uncomplicated by any possible effects of testosterone on the ribosomal population density, or on the levels of either sRNA or amino acid-activating enzymes,[18] in the ventral prostate gland. The effects of castration were clearly independent of soluble protein factors required for the incorporations.

There are many lines of evidence that only a small fraction of the total RNA associated with ribonucleoprotein particles derived from animal and microbial sources can serve a template function in protein biosynthesis.[19] If this is also the case with respect to prostatic ribosomes, then one explanation for the present findings is that prostatic ribonucleoprotein particles isolated from recently castrated rats are relatively deficient in template RNA. The remarkable increase in the valine incorporating activity of prostatic ribosomes from untreated castrates following addition of poly UG is consistent with this hypothesis. Under the stated conditions, prostatic ribosomes from testosterone-treated castrates showed little or no response to added poly UG, and in the presence of the latter polynucleotide, valine incorporation was nearly the same with ribonucleoprotein particles isolated from both the castrates injected only with oil, and from the testosterone-treated castrates. However, this does not necessarily imply that prostatic ribosomes prepared from the castrates injected with testosterone are nearly saturated with template RNA. It must be emphasized that what was measured in these experiments was merely the transfer of radioactivity from sRNA-valine-C^{14} to an acid-insoluble fraction which was resistant to hydrolysis by hot dilute trichloracetic acid, and which was insoluble in ethanol-ether. It is quite conceivable that some of the radioisotope originally added as sRNA-valine-C^{14} may have been converted (especially in the presence of poly UG) to peptides which might be, for example, sufficiently acid-soluble to have escaped detection under these conditions. Moreover, there is no assurance that the experimental conditions employed were optimal for the response of the valine-incorporating system to poly UG.

Some recent studies on the protein and polyribonucleotide metabolism of male accessory glands of reproduction may be germane to our findings. Butenandt *et al.*[20] reported that 6 hours after administration of testosterone to juvenile rats, there was a four-fold increase in the uptake of injected L-leucine-C^{14} by seminal vesicle proteins. From a study of the incorporation of labeled amino acids into proteins of seminal vesicle slices *in vitro*, Wilson[21] concluded that testosterone primarily accelerates the transfer of amino acids from sRNA-amino acids to ribonucleoprotein. Kochakian[22] has proposed that androgens regulate the biosynthesis of microsomal RNA in male accessory glands. The present studies are consistent with the view that testosterone governs the levels of template RNA attached to prostatic ribonucleoprotein particles. It has been suggested[23] that testosterone may control the synthesis and/or utilization of messenger RNA's which direct the assembly of proteins concerned with the androgen-dependent secretory functions of the prostate. Experiments in this laboratory[24] have delineated the

properties of an enzyme system in rat prostatic cell nuclei which resembles DNA-dependent RNA polymerases described in other mammalian tissues.[25] These considerations may be pertinent to the views of Karlson[26] and Zalokar[27] that sex hormones may function as specific gene activators.

Summary.—Ribonucleoprotein particles isolated from the rat ventral prostate gland readily catalyze the transfer of radioisotope from added sRNA-valine-C^{14} to protein. A large decrease in the rate and extent of such amino acyl transfers was found with prostatic ribosomes isolated from recently castrated adult rats, which was entirely reversed by administration of testosterone to the orchiectomized animals. Valine incorporation by prostatic ribosomes from the castrates was markedly increased by addition of poly UG, whereas valine incorporation by prostatic ribosomes from testosterone-treated castrates was little affected by poly UG.

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*RNA-INDUCED BIOSYNTHESIS OF SPECIFIC ENZYMES**

BY M. C. NIU, C. C. CORDOVA, L. C. NIU, AND C. L. RADBILL

DEPARTMENT OF BIOLOGY, TEMPLE UNIVERSITY, PHILADELPHIA

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It has long been known that development of function and of structure are intimately correlated. When function is expressed as the capacity for protein biosynthesis, one finds that specific protein formation most commonly appears prior to morphological differentiation. During the past decade, the senior author has engaged in the study of changes in development induced by RNA and/or RNA-containing compounds. Structures produced under these conditions can be highly specific.[1] Experiments have shown that RNA isolated from liver can dictate the type of protein to be synthesized intracellularly.[2] Serum albumin (S.A.) is one of the liver specific proteins which is not produced under experimental conditions by *in vitro*-cultured Nelson mouse ascites cells. However, after incubation with RNA isolated from mouse or calf liver, these cells acquire the ability to manufacture serum albumin. This finding has been extended to (*a*) the cells obtained from Lettre Ehrlich mouse ascites fluid and Novikoff rat hepatoma, and (*b*) the capability of these three strains of cancer cells to synthesize such liver specific enzymes as tryptophan pyrrolase (TPO) and glucose-6-phosphatase (G-6-Pase). The details of these experimental results are presented in this paper.

Material and Methods.—Mice and rats: Lettre Ehrlich ascites fluid was obtained from Sidney Weinhouse of this department and maintained through successive weekly transfers in the peritoneal cavity of Swiss Webster mice (10–15 gm) supplied by Pied Piper Farms. Rats carrying Novikoff hepatoma were generously supplied by Sergio DeCarvalho of the Rand Development Corporation, Cleveland, and also by Alex Novikoff of the Albert Einstein College of Medicine, New York City. Transfers (6 days) were carried out in Sprague-Dawley-Holtzman rats, weighing 150–200 grams each.

Isolation of RNA: RNA was prepared in the cold (2°C) from mouse, rat, and calf liver (L-RNA) and solid tumor (T-RNA) by a modified Kirby procedure.[3] To remove the contaminating phenol, the RNA solution was shaken twice with an equal volume of ether, the ether then being