Amino Acid Incorporation into Protein by Cell-free Systems from Rat Skeletal Muscle. IV. Effects of Animal Age, Androgens, and Anabolic Agents on Activity of Muscle Ribosomes*

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ABSTRACT: The activity of ribosomes isolated from rat thigh muscle in amino acid incorporation into protein was found to decrease with the age of the animal; this decrease could be correlated with a lower polyribosome content of isolated ribosomes. Although ribosome yield/g of muscle decreased as rats grew, the total yield from thigh muscles per animal remained constant.

Castration of immature male rats reduced the protein synthetic activity of isolated muscle ribosomes by one-half within 9 days. Androgen replacement brought the activity back to control levels; this effect was blocked by low doses of actinomycin D. Treatment of castrate rats with 17α-ethyl-19-nortestosterone also tended to restore muscle ribosome activity to normal. Administration of testosterone propionate or its analog produced dose-dependent decreases in amino acid incorporation by isolated muscle ribosomes when injected into normal rats of the same age. Incorporating activity was correlated with polyribosome content as measured by sucrose gradient centrifugation. These results may be interpreted on the basis of gonadal-pituitary interrelationships during the rapid growth stage of the rat.

Secondary effects of androgens in promoting muscle growth have been known for 30 years. These effects are in addition to the primary stimulation of accessory sex organs by these steroids (Kochakian, 1935; Kochakian and Murlin, 1935). Androgens appear to be involved in the regulation of skeletal muscle growth, although differences in responsiveness to these hormones between individual muscles and among various species have been reported (Kochakian, 1946; Kochakian *et al.*, 1956; Kochakian and Tillotson, 1957).

The site of action of androgens in muscle has not been established. It has been reported that castration decreases the level of RNA in responsive muscles and that testosterone stimulates a return to normal levels (Kochakian *et al.*, 1964). This is similar to effects on target organs such as prostate and seminal vesicles, where testosterone has been linked to stimulation of messenger RNA synthesis (Liao and Williams-Ashman, 1962; Wicks and Kenny, 1964).

The previous papers in this series (Florini, 1962; Breuer *et al.*, 1964; Florini and Breuer, 1965) have been concerned with mechanisms of protein synthesis in rat skeletal muscle; we have now begun to investigate possible effects of androgens and anabolic agents on the components of the muscle system. We have found evidence that one limiting factor in the activity of muscle ribosomes is their content of messenger RNA (Florini and Breuer, 1965). It seemed possible that the level of messenger RNA in muscle might be hormonally controlled; we now find that castration and androgen replacement do indeed affect both protein synthesis and polyribosome content, and therefore presumably the content of messenger RNA in muscle.

During the course of these experiments we observed that ribosome concentration in skeletal muscle varied with age. Muscle from young rats has been shown to contain a high concentration of RNA when compared to muscle from adults (Devi *et al.*, 1963). We inferred that muscle from weanling rats should also be more active in amino acid incorporation; our studies show that ribosomes from muscles of young rats possess a greater intrinsic capacity for protein synthesis which can be directly correlated with their polyribosome content.

Experimental

The procedures for isolation and purification of rat thigh muscle ribosomes, transfer enzymes, and liver or muscle [^{3}H]leucyl-s-RNA were described in a previous paper (Breuer *et al.*, 1964). They were modified for the hormone studies as follows: Treatment of resuspended microsomal pellets with Lubrol WX and deoxycholate was performed directly in Potter-type homogenizers rather than stirring the mixture in beakers. The same homogenizer was used for microsomes from each ex-

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perimental group in order to standardize treatment of the preparations. The suspensions were then centrifuged through a layer of 1.0 *M* sucrose medium for 2 hours at 105,000 × *g* to pellet the ribosomes.

In the hormone studies, male rats weighing 50–60 g were castrated under ether anesthesia. Animals were then assorted randomly into groups of 12 and were given food and water *ad libitum*. On the following day and daily thereafter for a total of 7 days, experimental groups received subcutaneous injections of 0.1 ml of 0.5% carboxymethyl-cellulose containing the designated hormone; controls, both intact and castrated, were injected with the vehicle alone. All injections were given at approximately the same time each day. Body weights were recorded at the start and completion of each experiment to determine the effectiveness of the anabolic agents and the possible toxicity of higher dose levels. Rats were sacrificed on the ninth day after castration, and ribosomes were prepared from each group.

For the assay of amino acid incorporation into ribosomal protein, the reaction mixture consisted of ribosomes containing 0.8 mg of RNA, 0.8 mg of transfer enzyme protein, 2.5 μmoles of ATP, 0.6 μmole of GTP, 10 μmoles of GSH, 90 μmoles of KCl, 16 μmoles of $MgCl_2$, 80 μmoles of Tris-HCl, *p*H 7.6, and 0.1 mg of [^{3}H]L-leucyl-s-RNA (specific activity 2–10 μcuries ^{3}H/mg RNA), in a total volume of 1.0 ml. Under these conditions the amount of ribosomes was the limiting factor in the assay. The assays were performed in triplicate and tubes were incubated for 15 minutes at 37°. Protein was isolated for radioactivity measurements and for ninhydrin determinations as described in the previous paper (Breuer *et al.*, 1964). Results are expressed in terms of total dpm of [^{3}H]leucine incorporated/mg of ribosomal RNA input. Figures are given with standard errors of the means of triplicate determinations using ribosomes isolated from muscle of 12 rats in each group.

RNA concentrations of isolated ribosome preparations were determined by absorbancy at 260 mμ using an extinction coefficient of 20 cm^2/mg. Values obtained using this figure agreed within 3% of those found by determining RNA by the orcinol reaction. Only preparations with a 260/280 mμ absorbancy ratio greater than 1.75 were used in these studies; this corresponds to an RNA/protein ratio of 1.23 as found by direct orcinol and Lowry determinations.

Sucrose gradient centrifugation was performed in the SW 39 rotor of the Spinco Model L ultracentrifuge. Profiles of absorbancy at 260 mμ were recorded as described previously (Breuer *et al.*, 1964).

TABLE I: Effect of Rat Age on Ribosome Content of Skeletal Muscle.[a]

Average Body Weight (g)	μg Ribosomal RNA/g Muscle	μg Ribosomal RNA in Thigh Muscles/Rat
40	74.4	125
50	73.1	183
120	19.4	155
140	19.1	197
200	11.4	113
250	9.8	156
350–500	3.6	99

[a] Ribosomal RNA concentrations were determined from the isolated resuspended pellets as described in the text. Figures given for each average body weight represent determinations from pooled muscle of 25–50 rats per group.

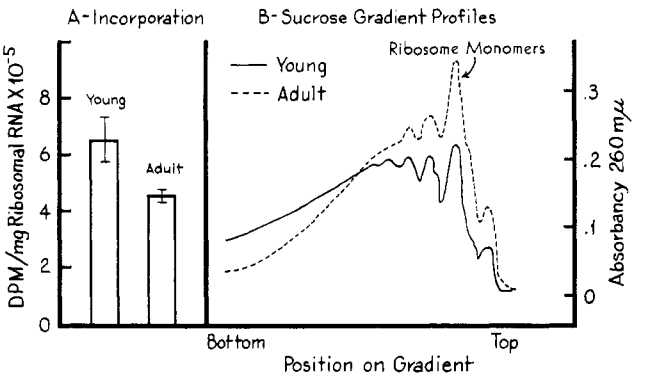

FIGURE 1: Activity and sucrose gradient profiles of ribosomes from thigh muscle of weanling and adult rats. (A) Assay of ribosomes for their ability to incorporate [^{3}H]leucine from leucyl s-RNA into ribosomal protein with transfer enzymes (0.8 mg) from liver. The incubation mixture and conditions are as described in the text. Each bar represents the mean of six determinations with standard error; the experiments were performed on ribosomes isolated from pools of thigh muscles isolated from two groups of 50 weanling rats and two groups of 25 adult animals. (B) The absorbancy profiles at 260 mμ following centrifugation through sucrose gradients of ribosomes (containing 0.3 mg RNA) from pooled muscle of weanling and adult rats.

Results

Age, Ribosome Content, and Activity. Table I lists the yield of ribosomes expressed as ribosomal RNA from the thigh muscles of rats, and their body weights. Although the isolation of ribosomes is not a quantitative procedure, results similar to those shown were consistently obtained in many studies conducted during the past year. The ribosomal RNA content of muscle/g of tissue decreased strikingly as the rats increase in age, although the thigh muscle total ribosomal RNA/rat did not change significantly.

The activity of these ribosomes in amino acid incorporation into protein and their fractionation by sucrose gradient centrifugation are shown in Figure 1. Ribosomes isolated from muscle of older rats were 67% as active in amino acid incorporation as those from

TABLE II: Effect of Castration and Administration of Testosterone Propionate (TP) on Activity of Muscle Ribosomes in Amino Acid Incorporation and on Body Weight Gain.[a]

Condition	Treatment	Body Weight Gain for 8 Days (g)	dpm/mg Ribosomal RNA $\times 10^{-5}$
Castrated	None	30.5 ± 0.81 (2)[b]	1.95 ± 0.36 (2)[c]
Castrated	0.1 mg TP/day	33.7 ± 1.27	4.06
Castrated	0.5 mg TP/day	38.3 ± 0.97 (2)	4.92 ± 0.25 (2)
Castrated	2.5 mg TP/day	27.8 ± 0.69	3.26
Intact	None	35.4 ± 1.05 (4)	4.05 ± 0.21 (4)
Intact	0.1 mg TP/day	43.3 ± 1.32	3.61
Intact	0.5 mg TP/day	41.6 ± 1.22	3.13
Intact	2.5 mg TP/day	37.6 ± 1.20	1.76

[a] Incubation mixtures and conditions for incorporation of [^{3}H]leucine from leucyl s-RNA into protein are as described in the text. [b] Figures are given as mean body weight gains with standard errors for the number of experiments specified in parentheses. All studies were performed using 12 rats per group except untreated castrates in which two groups of 24 and 12 animals were used. [c] Figures are given as mean ± standard error for the number of experiments specified in parentheses. Each experiment was performed on ribosomes prepared from a pool of thigh muscle from 12 rats. Triplicate incubations were done in all cases.

weanling rats. This difference could be correlated with the polyribosome content of the ribosome preparations by comparing the 260 mμ absorbancy profiles as seen in Figure 1B. Areas under the curves of ribosome monomers and polyribosomes to the left of the monomer peak were measured with a Keuffel and Esser compensating planimeter (Model W4236M); the polysome fraction of total ribosomes from young muscle was 83% while that in "old" muscle ribosomes was 72%. The most striking difference between the two preparations was the larger amount of 260 mμ absorbing material near the bottom of the gradient of the ribosomes isolated from muscle of weanling rats. Figure 1 is a composite of two sets of experiments; sucrose gradient profiles represent the analysis of four samples from each experimental preparation.

Castration, Androgen Replacement, and Ribosome Activity. The results of studies with immature castrate and intact rats treated with testosterone propionate are summarized in Table II. In two separate experiments skeletal muscle ribosomes from untreated castrates were 52% as active in amino acid incorporation as were control ribosomes. Injection of 0.1 mg/day of testosterone propionate for 7 days completely reversed this decrease in activity; the 2.5 mg/day dose was less effective in increasing ribosome activity, but incorporation was still significantly greater than in untreated castrates ($p < 0.005$). In contrast to the results obtained with testosterone propionate in castrated rats, injection of the same levels of this androgen for 7 days in unoperated animals produced a graded reduction in the capacity of isolated muscle ribosomes to transfer leucine from s-RNA to protein; 0.1 mg/day effected a 13% decrease, 0.5 mg/day 25%, and 2.5 mg/day 58% less incorporation than that of controls.

Preliminary studies had shown no significant changes in activity of enzymes involved in the transfer of leucine from s-RNA to ribosomal protein. The ribosomes from all experimental groups were therefore assayed with enzyme prepared from control muscle.

Sucrose gradient centrifugation analyses were performed to supplement the data on amino acid incorporation in ribosomes isolated from the muscles of castrated and treated animals. Figure 2A shows that castration reduced the proportion of "heavy" polyribosomes with a corresponding increase in single ribosomes and breakdown products. In contrast, ribosomes from castrated rats treated with 0.1 mg of testosterone propionate/day for 7 days show a 260 mμ absorbancy profile identical with that of control muscle ribosomes (Figure 2B). The difference in the appearance of profiles between Figure 2A and 2B is a reflection of the difference in time of the centrifuge runs.

Testosterone propionate, tested in concentrations ranging from 10^{-4} to 10^{-8} M, had no effect on the incorporation of amino acid into ribosomal protein *in vitro* when assayed with muscle ribosomes from either castrated or unoperated rats.

Transfer reaction assays were performed using equal amounts of ribosomes from castrates and controls mixed together, and incorporation was found to be slightly greater than additive in all cases. Similar additivity was observed when ribosomes from muscle of androgen-treated rats were mixed with control ribosomes. These results indicate that orchiectomy produced an effect on the messenger RNA content of ribosomes from skeletal muscle and that the lowered amino acid incorporation observed using ribosomes from castrated rats was not due to the production of an inhibitor of protein synthesis.

In order to test the assumption that the action of testosterone propionate on muscle protein synthesis involved RNA synthesis, actinomycin D was injected into castrated rats which also received testosterone. Castrated males were injected daily for 7 days with 250 μg of testosterone propionate; on the fifth, sixth and seventh days these animals were injected intraperitoneally with 10 μg of actinomycin D. Another group of 15 rats received only the three actinomycin injections at the same time. The following morning the animals were sacrificed, and ribosomes were prepared from pooled thigh muscles in the usual manner. The results are summarized in Figure 3. In Figure 3A the incorporation data is expressed on the basis of per cent incorporation comparing the activity of ribosomes isolated from actinomycin-treated castrated rats with similar data from earlier experiments (Table II) using normal control and testosterone-treated castrate rats. The usual action of testosterone propionate in returning the activity of ribosomes from castrated rats to control levels was prevented by actinomycin. Rats which received actinomycin alone showed even greater reduction in activity of muscle ribosomes. The sucrose gradient profiles in Figure 3B indicated that injection of actinomycin even at the low levels used induced almost a complete shift of ultraviolet-absorbing material from the polyribosome to the ribosome monomer area of the gradient.

Anabolic Agents and Ribosome Activity. In another series of experiments the effects of a steroid possessing a relatively high ratio of anabolic to androgenic activity were investigated. 17α-Ethyl-19-nortestosterone has been reported to meet this requirement (Saunders and Drill, 1956; Perrine, 1961). Subcutaneous injection of this compound daily for 7 days in castrate and intact rats produced the results shown in Table III. The response of amino acid incorporation into protein by ribosomes was the same as that observed with testosterone propionate, i.e., steroid treatment at low levels caused a partial restoration of incorporating activity in ribosomes from castrates and reduced the activity of ribosomes from muscle of unoperated rats. Under our conditions the anabolic steroid was not as effective as testosterone propionate in elevating castrate muscle ribosomes to the control level, and a more marked decrease in activity in muscle ribosomes of normal rats was found with 17α-ethyl-19-nortestosterone.

Analysis of ribosome preparations from these studies by sucrose gradient centrifugation again showed that activity in protein synthesis could be correlated with 260 mμ absorbancy profiles. In each case in which incorporating activity was decreased or increased from control values there were corresponding fluctuations in the amount of polyribosomal material in the ribosome preparations.

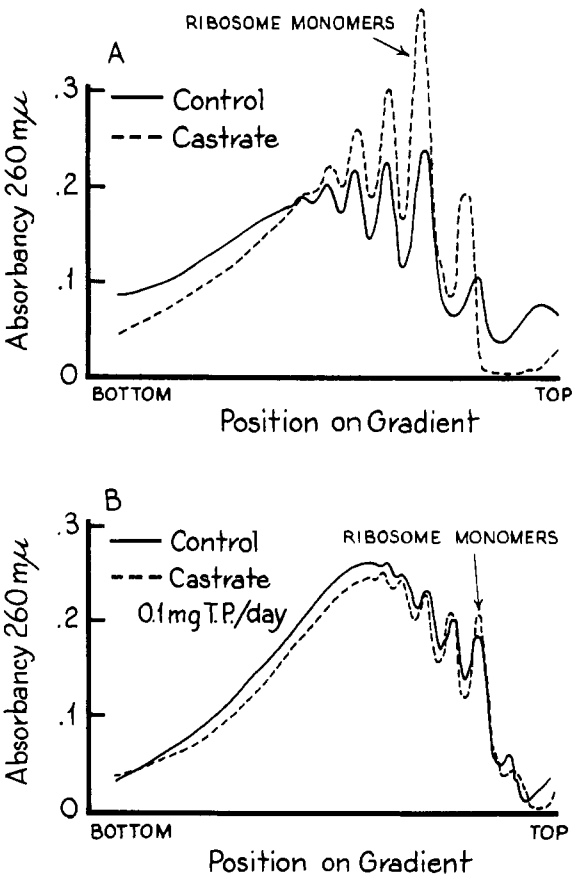

FIGURE 2: The effect of castration and administration of testosterone propionate on sucrose gradient profiles from rat skeletal muscle. (A) Ribosomes (containing 0.3 mg RNA) from muscle of unoperated controls and from castrates centrifuged on a linear 15–30% gradient for 1 hour at 35,000 rpm. (B) Ribosomes (containing 0.3 mg RNA) from muscle of unoperated controls and from castrated rats treated with 0.1 mg testosterone propionate per day for 7 days, centrifuged for 45 minutes at 35,000 rpm.

Discussion

Although a great deal is known about the physical characteristics of muscle proteins and their physiological function, information on the mechanisms of their biosynthesis remains limited. Several authors have indicated that actin and myosin appear in embryonic life (Ogawa, 1962; Csapo and Herrmann, 1951; Ebert, 1953). It may be inferred that the principle deposition of these major muscle proteins occurs early in the development of mammals; the turnover of myosin in the adult is very slow compared to that of other muscle proteins and of liver proteins (Velick, 1956; Bidinost, 1951). Since it has become increasingly evident that cytoplasmic ribonucleoprotein particles are involved in myosin synthesis (Ebert, 1959; Heuson-Stiennon, 1964; Breuer *et al.*, 1964), differences both qualitative and quantitative should be found in ribosomes from animals of different ages.

The data presented in Table I show that aging involves a dilution of muscle ribosomes with increasing muscle mass. The interpretation is complicated by the fact

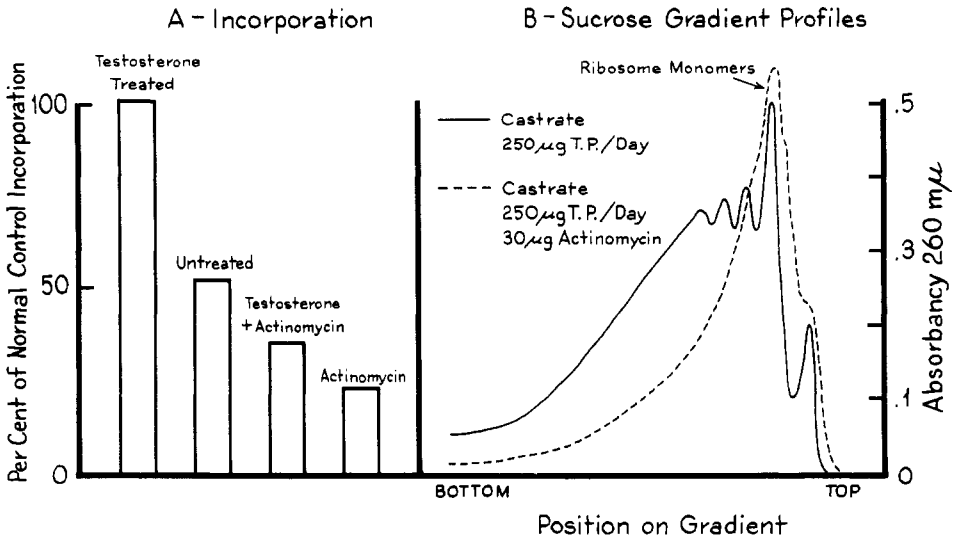

FIGURE 3: The effect of actinomycin D on the response to testosterone of amino acid incorporation into protein by ribosomes from rat muscle. (A) Incorporation of [^{3}H]leucine from leucyl s-RNA to protein in muscle ribosomes isolated from rats injected with testosterone propionate, actinomycin D, or both. Incubation mixtures and conditions are as described in the text, as are injection schedules and dosages. (B) Sucrose gradient profiles of muscle ribosomes (containing 0.35 mg RNA) centrifuged on a linear 15–30% sucrose gradient for 45 minutes at 35,000 rpm.

TABLE III: Effect of Castration and Administration of 17α-Ethyl-19-nortestosterone on Activity of Muscle Ribosomes in Amino Acid Incorporation and on Body Weight Gain.[a]

Condition	Treatment	Body Weight Gain for 8 Days (g)	dpm/mg Ribosomal RNA $\times 10^{-5}$
Castrated	None	30.5 ± 0.81 (2)[b]	1.95 ± 0.36 (2)[c]
Castrated	2.5 mg/day	36.6 ± 0.84	3.76
Castrated	5.0 mg/day	34.3 ± 1.66	3.05
Intact	None	35.4 ± 1.05 (4)	4.05 ± 0.21 (4)
Intact	1 mg/day	31.8 ± 0.82	2.39
Intact	2.5 mg/day	31.3 ± 0.65	0.43
Intact	5 mg/day	28.2 ± 0.78	1.49

[a] Incubation mixtures and conditions for the incorporation of [^{3}H]leucine from leucyl s-RNA into protein are as described in the text. [b] Figures are given as mean body weight gains with standard errors for the number of experiments specified in parentheses. All studies were performed using 12 rats per group except untreated castrates in which two groups of 24 and 12 animals were used. [c] Figures are given as mean ± standard error for the number of experiments specified in parentheses. Each experiment was performed on ribosomes prepared from a pool of thigh muscle from 12 rats. Triplicate incubations were done in all cases.

that there is much more connective tissue in the muscle of older animals, making its homogenization more difficult. This may lead to greater breakdown of ribonucleoprotein particles during the isolation process which could affect yields, incorporating activity, and sucrose gradient profiles of these ribosomes. However, we can say that within rather wide limits the amount of ribosomal RNA in thigh muscle is constant despite a 20-fold difference in muscle mass between weanling and adult rats. Despite the complications arising from mechanical difficulties in disrupting muscle of adult rats, our data are consistent with the findings of Devi *et al.* (1963) that total RNA extractable from rat muscle decreases with increasing age. Since their procedure involved a chemical extraction of muscle tissue, the degree of disruption was ostensibly not a factor in their results. Figure 1B shows that the ribosomes from the two developmental stages of growth are also qualitatively different. The ribosomes from muscle of young rats possess a greater content of "heavier" polyribosomes

than do those from adult muscle. The extremely long polyribosomes capable of synthesizing myosin subunits (Breuer *et al.*, 1964) are isolated from this portion of similar sucrose gradients. Further studies would be necessary to establish the sites of synthesis of specific proteins on muscle ribosomes in order to define the differences observed during the development of muscle tissue.

The responsiveness to androgen of rat muscle has been shown to be a general phenomenon, with increased sensitivity occurring only in the perineal region (Kochakian, 1964). The observations in Table II which indicate that castration of only 9 days duration reduced amino acid incorporation by ribosomes from thigh muscle some 50%, and that testosterone propionate restored this activity, serve to illustrate a dependency of muscle protein synthesis upon androgen.

Administration of actinomycin D prevented the stimulation of ribosome activity usually seen after injection of testosterone propionate in castrated rats (Figure 3). These data may be interpreted as evidence that testosterone stimulates the synthesis of messenger RNA in muscle, in agreement with current theories concerning the site of action of many hormones (Karlson, 1963; Kenney and Kull, 1963; Feigelson *et al.*, 1963; Korner, 1964). However, the extreme toxicity of actinomycin in immature, castrated rats makes this interpretation less certain. The animals used in this study lost an average of 25 g body weight after 3 days of actinomycin injection with a total of only 30 μg of the drug. In addition, the recently proposed alternate explanation that actinomycin causes increased fragility of polyribosomes independent of its effects on RNA synthesis (Revel *et al.*, 1964) has not yet been investigated in the muscle system. Nevertheless, we feel that our results provide indirect evidence for turnover of messenger RNA in muscle; studies now in progress on RNA synthesis in isolated muscle nuclei may be expected to provide better clarification of the relationship between testosterone propionate and RNA metabolism in muscle.

The results obtained with testosterone administration in unoperated rats are not easily explained. However, their validity is reinforced by the duplication of effect shown by 17α-ethyl-19-nortestosterone injection in normal rats. It has been known for some time that androgen administration to immature or adult male rats causes testicular atrophy which was attributed to inhibition of pituitary gonadotrophin secretion (Moore and Price, 1937; Korenchevsky and Hall, 1939). Kochakian and Webster (1958) showed that testosterone propionate decreased the growth rate of intact rats although it did not cause weight loss. In studies with intact adult rats Kochakian *et al.* (1950) found that testosterone produced a smaller body weight gain and a smaller decrease in urinary nitrogen excretion than seen with the steroid in castrates. It is possible that pituitary involvement in the androgen responsiveness of muscle has been a factor in our results. The rats used in these studies were at an age in which a rapid growth would normally have been observed. Exogenous androgen given to these rats may have had a suppressive effect on the total pituitary secretion in addition to specific gonadotrophin inhibition, which would not have been evident in the castrated animals. Further experiments with hypophysectomized and hypophysectomized-castrated animals treated with androgen are required to resolve these questions.

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Biosynthesis of Cell Wall Polysaccharide in Mutant Strains of *Salmonella*. III. Transfer of L-Rhamnose and D-Galactose*

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ABSTRACT: A rough mutant of *Salmonella typhimurium*, TV 208, originally isolated by Stocker and Subbaiah [T. V. Subbaiah and B. A. D. Stocker, *Nature 201*, 1298 (1964)], lacks enzyme(s) converting thymidine diphosphate 4-keto-6-deoxy-D-glucose into thymidine diphosphate L-rhamnose (TDPrhamnose). Because of this defect it synthesizes an incomplete cell wall lipopolysaccharide of rough, or, more precisely, R_{II} type. When a particulate fraction from the sonic extract of this strain was incubated with [^{14}C]TDPrhamnose, the sugar was transferred to the incomplete lipopolysaccharide contained in the particulate fraction. The incorporation was greatly stimulated by adding nonradioactive uridine diphosphate D-galactose (UDPGal), and nonradioactive TDPrhamnose in turn stimulated the incorporation of [^{14}C]D-galactose from UDPGal. However, the addition of guanosine diphosphate D-mannose had little effect on the incorporation of either [^{14}C]L-rhamnose or [^{14}C]D-galactose. Periodate oxidation showed that all the rhamnose incorporated in the presence of UDPGal occupied a nonreducing terminal position. Partial acid hydrolysis of lipopolysaccharide containing the incorporated galactose and rhamnose yielded an oligosaccharide which appeared to be rhamnosylgalactose. From these results we propose that D-galactose is first attached to the incomplete lipopolysaccharide of R_{II} type, then followed by the transfer of L-rhamnose. These reactions are believed to represent steps in the biosynthesis of the complete lipopolysaccharide produced by smooth strains.

Much work has already been done on the chemical composition of the cell wall lipopolysaccharides (LPS)[1] from enteric bacteria, such as *Salmonella*. When LPS is treated with acid, it is dissociated into a lipid (lipid A) and a water-soluble polysaccharide (Westphal and Lüderitz, 1954). The polysaccharides obtained from the LPS of many *Salmonella* species are often very complex in composition; for instance, the one from *Salmonella typhimurium* would contain an aldoheptose, phosphate, D-glucose, D-mannose, D-galactose, L-rhamnose, abequose (Kauffmann *et al.*, 1960; Krüger *et al.*, 1962), 2-keto-3-deoxyoctonate (Heath and Ghalambor, 1963; Osborn, 1963), and *N*-acetyl-D-glucosamine (I. W. Sutherland, O. Lüderitz, and O. Westphal, to be published; Osborn *et al.*, 1964).

The biosynthesis of these complex polysaccharides, as that of less complicated polysaccharides, probably involves two distinct stages: first, the "activated" form of each constituent sugar is synthesized, usually as nucleoside diphospho sugar (Leloir, 1953), and then the activated sugars are transferred to the acceptor molecule. If a mutation results in the loss of ability to make an "activated" sugar, the cell would synthesize the cell wall polysaccharide up to the point where the sugar in question would be normally transferred, and then the synthesis would stop there, leaving an incomplete polysaccharide.

Using UDPGal-4-epimerase-less mutants of *Salmonella enteritidis* and *S. typhimurium*, which cannot make activated D-galactose (UDPGal), we have shown that they indeed synthesized an incomplete polysaccharide containing only heptose, glucose, and phosphate (Fukasawa and Nikaido, 1960; Nikaido, 1961; Nikaido, 1962a). These results indicated that the central "core" of cell wall polysaccharides from these *Salmonellae* consists of these three components, that D-galactose is then transferred onto this core, and that L-rhamnose, D-mannose, and abequose are transferred later than the galactose moiety in question. The postu-

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[1] Abbreviations used: LPS, lipopolysaccharide; UDPGal, uridine diphosphate D-galactose; UDPG, uridine diphosphate D-glucose; TDPrhamnose, thymidine diphosphate L-rhamnose; TDPG, thymidine diphosphate D-glucose.