cleolar DNA of *X. laevis* can be separated from most of the DNA of the cell as a heavier peak (G•C satellite) (*12–14*). Saturation hybridization (*12, 14*) indicates that about 40 percent of the G•C satellite codes for rRNA (*15*). Annealing experiments with fractionated satellite DNA show that the stretches of DNA coding for 18*S* and 28*S* rRNA are alternating, closely adjoining, but separated by stretches of DNA higher in G•C content and not homologous to rRNA (*13, 14*). The latter observations agree with the evidence that each rRNA precursor molecule consists of one 18*S* and one 28*S* rRNA molecule, plus a portion that is degraded during the formation of the two rRNA molecules (*13, 16*).

We propose that the redundant structural arrangement of the rRNA precursor genes and intergene segments seen in isolated nucleolar cores visually confirms the biochemical nature of nucleolus organizer DNA in amphibians. Thus, the DNA axis of the matrix-covered segments corresponds to the satellite portion that is homologous to the entire precursor rRNA molecule (that is, homologous to one 18*S* and one 28*S* rRNA molecule plus the degraded part of the precursor rRNA molecule), and the DNA in the intergene regions corresponds to the remaining portion of the satellite. Measurements of relative lengths of matrix-free and adjacent matrix-covered units in *X. laevis* show that the mean length of intergene segments is about two-thirds the length of a precursor rRNA gene. This indicates that approximately 40 percent of the G•C satellite is inactive nucleolar DNA and about 60 percent consists of genes coding for precursor molecules.

Although the structure of chromosomal loci synthesizing RNA has already been documented, we believe ours are the first observations of the structure of individual genes and associated transcription products whose specific function is known—namely, the extrachromosomal nucleolar genes on which rRNA precursor molecules are synthesized.

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5. Details of techniques are given in legends for Figs. 2 and 3. Oocytes of *Xenopus laevis,* the African clawed toad, and *Triturus viridescens,* the spotted newt of eastern North America, were used in these studies. Limited examinations in two other genera, *Rana* and *Plethodon,* indicate that these observations probably extend to all amphibians. Earlier reports of these results are found in: O. L. Miller, Jr., and B. R. Beatty, *J. Cell Biol.* **39**, 156a (1968); ———, in *Handbook of Molecular Cytology,* A. Lima-de-Faria, Ed. (North-Holland, Amsterdam, in press); ———, *Genetics,* in press.
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Anabolic Steroid: Effects on Strength Development

Abstract. *Twelve matched pairs of subjects, fed a high protein diet, were trained with weights for 6 weeks. In the final 3 weeks twelve subjects received 5 milligrams of methandrostenolone (Dianabol) twice daily. Maximum weight lifting, thickness of skin folds, oxygen uptake, blood chemistry profile, and concentration of blood lipids were determined. Also used were cable tensiometry and anthropometric measurements. The strength of treated subjects increased significantly; their mean weight gain was 2.48 kilograms with no significant change in skin fold thickness. Several anthropometric measurements increased significantly, as did oxygen uptake ability and nitrogen retention by the blood.*

Use of anabolic steroids by athletes attempting to develop strength has become increasingly widespread, especially by those in activities where strength is the prime factor for successful performance. Although many instances of extraordinary and rapid improvement have been reported, the evidence appears entirely empirical. Little is known about possible long-term side effects on adults. However, physically immature individuals can expect irrevocable and irreversible developments. Possible acceleration of epiphyseal ossification (*1*) and manifestations resembling macrogenitosomia precox (*2*) are two of the severe contraindications in the use of steroids by teen-agers.

Anabolic agents have been used for some time for patients recovering from illness or after surgery, for treatment of osteoporosis, fracture healing, severe burns, and muscular dystrophy, for protein tissue building, and for myotrophism. Anabolic agents have also been credited with having an important effect in stimulating the appetite and imparting a feeling of well-being (*3*). Although there is little data on the use of anabolic steroids by athletes, there is sufficient clinical evidence that the anabolic potencies of these drugs should, on theoretical grounds, stimulate muscle hypertrophy and strength increases in normal healthy men. Androgens do exert a positive effect on muscle growth in various animal species; for example, hypertrophy of certain muscles in immature guinea pigs accompanied administration of testosterone propionate (*4*), and weekly intramuscular injection of 1 mg of testosterone per kilogram of body weight resulted in increased weight gains in steers (*5*).

It was difficult to find volunteers who were willing to take the steroid. Many considered participation but were apprehensive because of the paucity of knowledge concerning side effects. There appears to be a widespread rumor that steroid treatment reduces the sexual drive. Thus we changed our

Table 1. Effects of steroid treatment on strength measured in kilograms with cable tensiometry.

Exercise	Treatment group			Control group			Mean difference (control and treated)
	Before	After	Difference	Before	After	Difference	
Shoulder flexion	80.04	101.11	21.07	81.94	88.97	7.03	14.04*
Elbow flexion	76.49	92.65	16.16	72.44	73.30	0.86	15.30*
Elbow extension	53.39	70.25	16.86	53.35	61.24	7.89	8.97†
Knee flexion	81.45	95.75	14.30	88.14	91.45	3.31	10.99†
Knee extension	124.01	144.09	20.08	135.54	145.66	10.12	9.96

* Significant $P = .05$. † Significant $P = .01$.

original plan for a double-blind study and told the subjects in advance that they definitely would or would not be taking the steroid. Twelve men who volunteered to take the steroid were paired with volunteers unwilling to take the steroid, according to age, weight, approximate strength, and training background (ages ranged from 19 to 39 years). The duration of the experiment was 6 weeks; in the first 3 weeks subjects became accustomed to the weight training regime and an adequate degree of strength fitness was incurred; in the last 3 weeks, one group was given one 5-mg tablet of methandrostenolone (Dianabol) (*6*) twice daily. Throughout the experiment all subjects supplemented their diets with 15 g of 92 percent protein powder twice daily.

Subjects trained three times per week for approximately 1 hour, according to a program designed to work the major muscle groups of the body through the implementation of the progressive overload principle. The program was as follows: bench press and squat, four sets of four to six repetitions; seated dumbbell curls, three sets of 10 to 12 repetitions. Maximum weights were handled for the number of sets and repetitions required. Weights were increased as rapidly as possible to maintain the training at near maximum effort at all times. Without exception each pair trained together, performing the same number of sets and repetitions. The pairs also participated together in physical education activities other than the specified weight training program. The training programs of the treatment and the control subjects were therefore identical.

The effectiveness of the 3-week steroid treatment on the development of strength and muscle hypertrophy was determined by seven measurements made at the end of the first 3 weeks when no treatment was administered and at the end of the 3-week treatment period. (i) Dynamic strength was measured by one repetition maximum on the bench press and squat (*7*); (ii) static strength was measured with cable tensiometry (*8*); (iii) subcutaneous adipose tissue was assessed by measuring skin folding with the Harpenden caliper at six sites; (iv) anthropometric measurements were taken at five sites; (v) oxygen uptake was measured by the Astrand oxygen uptake test; (vi) blood chemistry profile was examined; and (vii) blood lipids were analyzed.

Dynamic strength and static strength increased significantly in the treated individuals (Tables 1 and 2). Increase in knee extension approached significance. Thus under the conditions in this study, treatment with anabolic steroids is apparently effective in developing muscular strength. The increase in strength of the control group was typical.

Reports from athletes have described empirically significant muscular hypertrophy resulting from the use of anabolic steroids; our data support these claims. Weight gain of 2.48 kg for the treatment groups, and of 0.29 kg for the control, was significant ($P = .01$).

There was no significant change in deposition of subcutaneous adipose tissue in the two groups. Since treated subjects had a significant weight gain, it can be assumed that this gain represents an increase in lean body mass and not adipose tissue.

The statistically significant increase in oxygen uptake ability in treated individuals (Table 3) was unexpected because the training program was not designed to develop the cardiovascular system. At this time we have not found evidence that athletes whose success depends upon great cardiovascular endurance have used steroid treatment. If these findings are substantiated by other studies with training programs designed to develop the cardiovascular system, the implication would be that the use of anabolic steroids increases oxygen uptake ability and thereby could improve performance in events requiring endurance.

In treated subjects, the total bilirubin and alkaline phosphate in the blood significantly decreased. There was significant decrease in the free cholesterol

Table 2. Effects of steroid treatment on strength measured in kilograms by maximum weight lifting.

Exercise	Treatment group			Control group			Mean difference (control and treated)
	Before	After	Difference	Before	After	Difference	
Bench press	107.07	118.80	16.37	96.90	103.30	6.40	10.33*
Squat	128.51	152.48	23.97	129.55	143.80	14.25	9.72*

* Significant $P = .01$.

Table 3. Effects of steroid treatment on anthropometric measurements, body weight, and oxygen uptake.

Measurement	Treatment group			Control group			Mean difference (control and treated)
	Before	After	Difference	Before	After	Difference	
Biceps, right	37.64	39.01	1.37	36.80	36.78	0.02	1.35*
Forearm, right	31.90	33.06	1.16	32.56	33.07	0.51	0.65
Waist	85.32	86.59	1.27	87.20	87.06	0.14	1.41
Thigh, right	59.28	60.68	1.40	60.40	60.66	0.26	1.14
Calf, right	36.50	38.56	2.06	40.03	39.73	0.30	2.36*
Weight (kg)	77.48	79.96	2.48	80.70	80.99	0.29	2.19*
O_2 uptake (liter min^{-1})	3.48	4.34	0.86	3.87	3.99	0.12	0.74*
O_2 (ml kg^{-1} min^{-1})	45.64	52.68	7.04	48.18	49.50	1.32	5.72†

* Significant $P = .01$. † Significant $P = .05$.

in controls. Both lactate dehydrogenase and serum glutamic oxalacetic transaminase increased in treated subjects. It has been demonstrated repeatedly that anabolic steroids exert a positive effect on nitrogen retention and that the degree of nitrogen retention is partially dependent upon caloric and protein intake (*9*). The extent to which anabolic steroid treatment favorably influences protein synthesis has not been satisfactorily answered (*10*). In our study the combination of steroid treatment, high protein intake, and heavy muscular stress apparently accelerated protein synthesis in the muscle tissue, with this change being manifested by increased static and dynamic strength and body weight.

There were no consistent or apparently significant physiological side effects. A few individuals indicated an increase in urine production, and some felt a degree of tension which may have resulted from the strenuous training program. The near absence of normal muscle soreness and stiffness following the training sessions was noted by some. It appears possible to train at near maximum five or six times a week during the treatment. No attempt was made to analyze possible psychological implications. No reduction in sex drive was reported.

It appears that anabolic steroids can accelerate the acquisition of muscular strength and muscular power and simultaneously permit training at or near maximum capacity with greater frequency. Treatment should be used cautiously until more information is available on physiological effects on humans.

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Herpesvirus in Marek's Disease Tumors

Abstract. *Intranuclear and cytoplasmic virus particles of the herpes type were located in epithelial cells that line the kidney collecting tubules obtained from a chick with Marek's disease. The chick had contracted the disease by direct contact transmission. The virus was not observed in any of the invading tumor cells in the same kidney.*

The epizootiology of Marek's disease, a lymphoproliferative disease of chickens, suggests that it is of an infectious nature; and a cell-associated, herpes-type virus (HTV) has been demonstrated in cell cultures derived from tumors induced by various isolates (*1–3*). Although successful transmission of Marek's disease with a cell-free virus inoculum remains to be accomplished, circumstantial evidence has been presented that implicates HTV as the etiological agent (*4, 5*).

Although this virus replicates in cell cultures from kidneys of infected chicks and will also grow when transferred to fibroblast monolayers originating from normal chick or duck embryo (*1, 3, 4, 6*), HTV has not been shown to occur in Marek's disease tumors prior to cultivation. Our own efforts had failed to demonstrate the virus in tumors directly (*3*). Recently, however, we have observed typical HTV in the kidney tumor of a chick that had contracted Marek's disease by natural transmission, as a result of being caged with birds infected with the GA strain of this disease. We now describe the types of cells, as judged by electron microscopy, that harbor the virus particles before and during cultivation of Marek's disease tumors.

A group of 2-day-old Athens-Canadian chicks (*7*) was kept for 6 days with other chicks carrying Marek's disease. The exposed chicks were then separated and kept in isolation. Three chicks were killed weekly for diagnosis on the basis of gross and microscopic pathology, development of a characteristic cytopathic effect in chick kidney cell cultures, and the direct electron microscopic observation of gonads, liver, spleen, and kidney tissue. The tissues and cell cultures for electron microscopy were prepared as described (*3*). The observations reported here were made on chicks that had been held in isolation for 4 weeks after exposure. At autopsy, these birds showed enlargement of gonads and kidneys.

Figure 1 illustrates the massive enlargement of the kidneys due, as shown in Fig. 2, to infiltration with lymphoid cells. However, parenchymal elements with epithelial cells are easily found, and a tangential aspect of the wall of a distal uriniferous tubule still delineated by its basement membrane and surrounded by invading lymphoid cells can be seen (Fig. 2). The epithelial nature of the virus-bearing cells in the tumor is demonstrated by the presence of adhesion zones (Fig. 3) as well as their location with respect to the basement membrane and the lumen of the tubule (Fig. 2). The intercellular adhesion zones are of the intermediate junction type (*8*). Empty and nucleated HTV particles are observed in the nucleus and cytoplasm, but no enveloped virus particles are evident (Fig. 4). Small nuclear particles, as reported previously in virus-containing cultures originating from chicks with Marek's disease (*3*), are also apparent. The nuclear envelope does not show the "reduplication" phenomenon often seen in herpesvirus-infected cells; it appears normal except for a few unusual outpocketings, suggesting some activity perhaps related to the envelopment of virus particles (Fig. 4). It is of particular interest that no virus was seen in the lymphoid infiltrating cells of the same tumor.

Unexposed control chicks housed in another building did not develop Marek's disease, and HTV was not present in tissues or the cell cultures derived from their kidneys. The direct electron-microscopic examination of tissues or tumors of chicks killed 1, 2, 3, 5, and 6 weeks after exposure to the disease were also negative for HTV. However, HTV was recovered in cell cultures derived from kidneys of chicks held in isolation 2 weeks or more after exposure to the disease whether they developed gross tumors or not (*9*). All samples were screened by electron microscopy for an approximately equal time on the same number of sections.

Upon cultivation of the chick kidney tumor by conventional techniques, the epithelial and interstitial cells grew, formed a monolayer, and produced in 6 days a cytopathic effect consisting of clusters of refractile, rounded cells (Fig. 5). Each of these cells contained HTV, and the morphology was like that de-