South Central and Southeast Asia. *Anopheles balabacensis balabacensis* is distributed from Indochina through Thailand and into the island of Borneo; it is considered to be a serious vector of human malaria in the inland hills of Viet Nam, Cambodia, Thailand, and northern Malaya (*4*).

Field studies of human and simian malaria were conducted in the monsoon forests of the state of Perlis in the northern Malay States in December 1964 and January 1965. The relative attraction of man and monkeys for anophelines in the area was determined by trapping mosquitoes in monkey-baited net traps on platforms in the forest and in human-baited net traps on the ground. Mosquitoes were collected every 2 hours from 6 p.m. until 6 a.m. and taken to a nearby field laboratory each morning, where they were identified and dissected. The salivary glands were dissected in serum-saline, and the sporozoites from positive specimens were inoculated intravenously into malaria-free rhesus monkeys.

Of 42 *A. b. balabacensis* dissected, five had sporozoites in their salivary glands. Sporozoites from three of the mosquitoes were inoculated into one monkey (R464), and sporozoites from a fourth were inoculated into another (R466); the material from the fifth infected mosquito was preserved for morphological studies. The two monkeys became positive for malaria 10 and 14 days after inoculation. The parasite in one (R464) reached a peak density of 685,000 per cubic millimeter on the 6th day of patency and was identified as *P. cynomolgi*. The second monkey (R466) developed a *P. inui* infection with a maximum parasitemia of 113,000 per cubic millimeter on the 11th day of patency.

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DNA-Dependent Synthesis of RNA Is Not Implicated in Growth Response of Chick Comb to Androgens

Abstract. *Local application of actinomycin D does not detectably modify growth response of the chick comb to androgens. The mode of action of androgens on chick comb (a connective-tissue organ) appears to differ from their action on epithelial-tissue organs such as seminal vesicles and prostate.*

Much recent experimental evidence indicates that estrogens (*1–4*) and androgens (*5*) exert their growth-promoting action on specific target tissues initially by stimulating synthesis of RNA. Kinetic studies show that this activation of RNA synthesis precedes in time the phase of increased synthesis of protein (enzyme) that leads to proliferation of the target tissue (*2*). It has also been demonstrated that inhibition by actinomycin D of DNA-dependent synthesis of RNA blocks the ultimate physiological and cytological manifestations of the effects of some of these steroid hormones (*3*). These observations have led to the postulate that stimulation of synthesis of RNA in the target tissue is an important and determining event in the mode of action of these hormones.

Available data on the action of androgens on prostate, seminal vesicles, and muscles seem to accord with this model (*6*). Another system has been known since the birth of endocrinology to depend on androgens. Berthold in 1849 reported regression of the rooster comb on castration and its reappearance after implantation of testes (*7*). Subsequent studies have shown that the weight and size of the comb are linearly related to the amount of androgen hormone directly applied to the comb of capon or chick (*8*); the response is so exactly proportional

Table 1. Effect of androgens on the growth of chick comb, with and without topically applied actinomycin D(AMD). The number of birds in each test follows (in parentheses) the test designation. AST, androsterone; TSP, testosterone propionate.

Treatment	Comb (mean ± S.E.)	
	Length + height (mm)	Weight/body wt (mg/g)
	Experiment 1	
(*a*, 6) 0.05 ml ethanol	11.67 ± 0.192	0.193 ± 0.041
(*b*, 5) 3 μg AST*	15.80 ± 0.970	0.643 ± 0.104
(*c*, 6) 0.25 μg AMD†, 3 μg AST*	15.50 ± 0.955	0.716 ± 0.112
	Experiment 2	
(*d*, 10) 0.05 ml ethanol	10.80 ± 0.291	0.180 ± 0.017
(*e*, 9) 1.14 μg TSP*	14.40 ± 0.821	0.640 ± 0.032
(*f*, 5) 0.3 μg AMD†, 1.14 μg TSP*	13.80 ± 0.313	0.895 ± 0.117
	Statistical significance	
Length + height: $a \sim b$, $P < .01$	Comb wt/body wt:	$a \sim b$, $P < .001$
$a \sim c$, $< .01$		$a \sim c$, $< .01$
$b \sim c$, $< .90$		$b \sim c$, $< .90$
$d \sim e$, $< .001$		$d \sim e$, $< .001$
$d \sim f$, $< .001$		$d \sim f$, $< .001$
$e \sim f$, $< .700$		$e \sim f$, $< .01$

* Applied daily. † Applied twice daily.

Table 2. Effect of actinomycin D (AMD, topically applied twice daily) on the growth of chick comb under the influence of endogenous androgns. The number of birds tested follows (in parentheses) the test designation.

Application	Comb (mean ± S.E.)		
	Length + height (mm)		Weight/body wt (mg/g)
	Day 15	Day 28	
	Experiment 1		
(*a*, 8) 0.05 ml saline	10.75 ± 0.36	21.10 ± 1.29	0.77 ± .09
(*b*, 8) 0.3 μg AMD	11.27 ± 0.83	21.87 ± 1.23	1.06 ± 0.17
	Experiment 2		
(*c*, 14) 0.1 ml saline		22.93 ± 1.35	0.63 ± .132
(*d*, 14) 0.2 μg AMD		21.30 ± 0.995	.54 ± .134
	Statistical significance		
Length + height: $a \sim b$, $P < .70$		Comb wt/body wt:	$a \sim$ b, $P < .20$
$c \sim d$, $< .40$			$c \sim$ d, $< .70$

Table 3. Effect of locally intradermally injected (twice daily from day 2 to day 10) actinomycin D (AMD; sterile isoosmotic saline for the control group) on the growth of chick comb under the influence of topically and daily applied testoterone propionate (TSP; ethanol for the control group). The number of birds in each test follows (in parentheses) the test designation.

Treatment	Comb (mean ± S.E.)	
	Length + height (mm)	Weight/body wt (mg/g)
*Experiment 1**		
(*a*, 8) 0.05 ml ethanol		0.32 ± .01817
(*b*, 8) 0.04 ml saline		.305 ± .0179
(*c*, 8) 1.14 μg TSP		.68 ± .0648
(*d*, 8) 0.2 μg AMD, 1.14 μg TSP		.68 ± .01
Experiment 2†		
(*e*, 7) 0.02 ml ethanol	11.4 ± 0.689	.364 ± .037
(*f*, 10) 0.02 ml saline	12.8 ± 0.6632	.379 ± .022
(*g*, 8) 1.14 μg TSP	16.7 ± 1.31	1.547 ± .0212
(*h*, 8) 0.2 μg AMD, 1.14 μg TSP	15.3 ± 0.65	1.348 ± .114

Statistical significance

Length + height: $e \sim g$, $P < .001$; $f \sim h$, $< .001$; $g \sim h$, $< .7$

Comb wt/body wt: $a \sim c$, $P < .001$; $b \sim d$, $< .001$; $c \sim d$, not significant; $e \sim g$, $< .001$; $f \sim h$, $< .001$; $g \sim h$, $< .5$

* TSP supplied by L. Light and Co., Ltd., Colnbrook, England. † TSP supplied by Sigma Chemical Co., St. Louis, Mo.

that the system has been proposed as a method for bioassay of androgens (*9*).

We now report experiments designed to determine whether the stimulation of synthesis of RNA is essential for the growth of the comb under the influence of androgens. One-day-old, male, White Leghorn chicks (*10*) were divided into three groups. Group 1 received topical application of 95-percent ethanol (0.05 ml) daily. Group 2 received androsterone or testosterone propionate, dissolved in 0.05 ml of ethanol, topically on the comb; the daily dose was 3 μg of androsterone or 1.14 μg of testosterone propionate. Group 3 received actinomycin D topically in addition to the androgen steroid; actinomycin D dissolved in isoosmotic saline was applied twice daily: 1 hour before application of the androgen and 8 hours after. All chicks were thus variously treated from day 2 to day 10 (*9*) when the chicks were weighed. Combs were measured (*11*) before removal and individual weighing (Table 1).

Obviously the size and weight of the combs of birds treated with androgens were significantly greater than those of the control group. Application of actinomycin D along with androgens did not influence the response of the comb to the androgens. In order to rule out possible interaction of actinomycin D and externally applied steroid, another series of experiments was performed in which the growth of the comb was measured at an age at which the endogeneous hormone is active. Here again actinomycin D did not prevent normal growth of the comb (Table 2).

It may be argued that actinomycin D is not absorbed into the tissue when applied topically on the comb. Control experiments were performed, in which actinomycin D was injected subcutaneously into the comb with a microsyringe. The results (Table 3) were essentially unchanged.

A point that remains open is the possibility of nonsaturating levels of actinomycin in the tissue. No precise data are available for calculation of the optimal concentrations of actinomycin to be used for such studies. The investigator is faced with the problem of stepping in ranges which may be toxic and at which aspecific effects may begin to appear. In our experiments we applied daily and individually as much as 0.3 μg of actinomycin D, which amount did not noticeably inhibit stimulation by testosterone of the growth of the comb. Amounts as great as 0.6 μg have been tried on certain animals with no effect. Much smaller amounts of the antibiotic effectively prevent the action of estradiol on vagina or of human chorionic gonadotrophin on immature testis (*3*).

Our results suggest that development of the chick comb under the influence of androsterone and testosterone may not require the prior stimulation of synthesis in the comb of DNA-dependent RNA. If this is so, the chick comb differs in this respect from epithelial-tissue organs such as seminal vesicles and prostate.

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Color Vision in the Antelope Ground Squirrel

Abstract. *Antelope ground squirrels* (Citellus leucurus) *were able, after conditioning, to respond correctly to a port illuminated with light at a wavelength of 460 nanometers. This color, randomly presented at one of two positions, was correctly selected in reference to a second port illuminated with light at 500, 520, 569, and 600 nanometers, or with white light of varying intensity. Luminosity was not a factor in the discrimination.*

The study of color vision in animals is fraught with numerous difficulties which have limited development of the subject. One problem, and not an unimportant one, is the choice of the most