young are transferred as a group to a separate quart jar. Cysts are filtered, dried, and resuspended in sea water so that emergence may be determined.

Eight 3-liter subcultures of our diploid amphigonic stock No. 3 were begun on the same day. After 3 weeks each jar had a population of 250 ± 5 adult shrimp. One jar received only acetone and served as a control. The other seven jars each received 1 ml of one of a series of dilutions of DDT in acetone. Geigy's 99.9 percent *p,p'*-DDT was used.

That knockdown occurs within an hour after 0.1 part of DDT per million was added to the culture (Table 1) is consistent with the Beltsville bioassay procedure (*2*). In lower concentrations of DDT, spasmodic swimming at irregular rates was punctuated by jerky flexing of the body axis. Settling to the bottom was gradual. Immobilization and death proceeded for a period of days.

Within 5 days all adults in the jar containing 10^{-4} parts of DDT per million were dead, although a few small larvae persisted. These, too, died before reaching maturity, and except for those given the two lowest doses, populations became extinct within 3 weeks. Even in solutions with 10^{-5} parts per million a majority of adults died within 3 weeks or before the next generation had matured. The first generation produced after treatment was noticeably smaller, while that produced by the controls experienced no difficulty in maintaining population size.

Table 2 characterizes the mated pairs of adults and their reproductive performance. A summary of data on mated pairs from stock 3 is included for comparison because exposure to the organic solvent, acetone, decreases survival time and shortens the opportunity to deposit broods. Mean survival times for pairs removed from the treated mass cultures approach those of acetone controls. With standard errors of 1 to 2 days, differences between the means are not statistically significant. The frequency of brood deposit was unaltered and more broods were deposited by animals given the higher dose of DDT. Furthermore, proportionately more of the zygotes from treated adults were deposited as cysts. Presumably this is a characteristic response to environmental stress because it has also occurred in experiments with radioisotopes (*1*).

Among the various aspects of reproductive performance tabulated, only the average number of zygotes per brood shows a decrease correlated with exposure to a toxic agent. However, 108.5 ± 27.8 is not impressively different from 155.0 ± 25.9 when the variability reflected in the associated standard errors is considered. Nevertheless, young shrimp evidently profited from the relaxed crowding because more larvae survived to adulthood in the smaller broods. Such compensating tendencies are reflected in a trend toward achieving the top carrying capacity of the mass culture. More than 250 shrimp are maturing in the second generation after treatment, and the populations appear to be on the way to numerical recovery.

A decrease in the number of zygotes can be accounted for by somatic debility of the mothers. Induced dominant lethality must be discounted since embryos and larvae have good viability. Furthermore, sex ratios favoring females, the heterogametic sex, are normal and reveal no induced recessive sex-linked lethality. Therefore we have no evidence of direct damage to cells of the germ line. The only studies of this kind on animals other than insects have been with birds. There, too, poor reproduction can be accounted for by the poor condition of the adult females (*3*); however, the fact that the eggs incorporate DDT further complicates the matter.

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2 December 1966

Antiandrogen Implanted in Brain Stimulates Male Reproductive System

Abstract. *Chronic intrahypothalamic implantation of cyproterone, an antiandrogen, in male rats resulted in specific stimulation of testes, seminal vesicles, and prostates. Implantation of cholesterol-filled or empty tubes in the median eminence in controls was ineffective. We conclude that decreases in the amount of testosterone reaching specific receptors in the median eminence or in nearby regions activate a mechanism that produces increased gonadotropin secretion.*

It is well known that a negative-feedback relation exists between the gonadal steroids and the pituitary gonadotropic hormones, such that the circulating level of steroids tends to be inversely related to the gonadotropin output (*1*). It has been shown that increased local concentration of estrogen (*2*) or androgen (*3*) in the medial basal hypothalamus (median-eminence region), by implantation of small amounts of the crystalline hormones, results in inhibition of gonadotropin output in both sexes. Since, in those studies, similar implantation in the pituitary had little or no inhibitory effect, it was concluded that steroid-sensitive structures in the hypothalamus respond to increased circulating levels of steroids by initiating a signal that results in decreased gonadotropin secretion, the pituitary itself being insensitive (*4*). The converse experiment—production of regional decreases in steroid concentration in the brain or pituitary—is much more difficult to perform, and there is no direct evidence of the site of receptors for reduced circulating steroid levels.

The recent discovery of a potent and specific antiandrogenic compound suggested an experimental approach to this problem. It is reported that cyproterone, a synthetic steroid (*5*), blocks the action of testosterone on the reproductive systems of rats and other species and shows no progestational, androgenic, or estrogenic activities (*6*). The finding that cyproterone prevents differentiation of the male reproductive system by fetal or neonatal androgen (*6*) suggests that it inhibits the action of testosterone on the brain as well as on its peripheral target organs, since it is generally accepted that this early effect of androgen is exerted on the central nervous system —probably on the hypothalamus (*7*). We reasoned that, if this conclusion is correct, intrahypothalamic implantation of crystalline cyproterone should prevent the action of testosterone on the area surrounding the implant. Such prevention would permit testing of the

Table 1. Effects of intrahypothalamic (median-eminence) implantation of cyproterone (Cyp) or cholesterol (Cho) on weights (means ± S.E.) of reproductive and endocrine organs in immature and adult male rats. Weeks after implantation.

Implant	N	Body weight gained (g)	Organ weight (mg)					
			Testis	Seminal vesicles	Ventral prostates	Pituitary	Adrenal	Thyroids
Experiment 1: Adults, after 3 weeks								
Cho	10	31.0±4.7	1256±45	267±13	351±22	7.2±0.4	27.0±3.3	15.3±0.7
Cyp	10	33.0±4.7	1336±52	336±10*	378±41	7.4±0.3	26.0±1.5	14.4±0.8
Experiment 2: Immature rats, after 2 weeks								
Cho	12	63.0±3.3	692±24	29.0±2.2	56.0±4.8	4.0±0.2	13.9±0.7	7.6±0.5
Cyp	12	76.0±3.3*	756±18†	45.0±4.2*	77.0±7.0‡	4.5±0.3	13.7±0.4	7.9±0.5

Two-tailed probabilities: * $p < .01$. † $p < .05$. ‡ $p < .02$.

hypothesis that this area responds to decreases in circulating testosterone by providing a stimulus for increased gonadotropin secretion.

In the initial experiment sexually mature male rats of the Long-Evans strain, 83 to 86 days old, were implanted stereotaxically with pellets of crystalline cyproterone in the median-eminence region of the hypothalamus. The pellets were produced by tamping cyproterone into the ends of lengths of 20-gauge stainless-steel tubing; their weights were determined by differential weighing on a microbalance *(8)*. During implantation they were protected by a thin coating of sugar, and subsequently were ejected from the carrier tubes into the basal hypothalamus. The tubes were then fixed to the skull with dental cement and screws. At autopsy 3 weeks later, all testes, seminal vesicles, ventral prostates, pituitaries, adrenals, and thyroids were removed and weighed. In this and subsequent experiments, the locations of the implants were carefully verified at autopsy, and only rats in which the implants were clearly visible in the median-eminence region were included in the study. To control for possible effects of operative trauma and mechanical damage to the brain, another group of animals of the same age were simultaneously implanted with similar pellets of cholesterol.

The weights of seminal vesicles proved to be significantly greater in cyproterone-implanted animals than in cholesterol-implanted controls (Table 1). Although testicular and prostatic weights also were higher in the former group, the differences were not statistically significant; and there was no significant difference between the two groups in any of the other parameters. The increased weights of seminal vesicles suggested increased secretion of gonadotropin by the pituitary, but the evidence was not sufficiently convincing in the absence of significant effects on testes and prostates. However, testicular weight in adult animals does not readily respond to increased levels of endogenous or exogenous gonadotropin *(9)*. Accordingly, we performed a second experiment, using 30-day-old (prepuberal) male rats, whose reproductive system is considerably more sensitive to stimulation by gonadotropins.

Experimental procedures were the same as in the first experiment, except that we implanted only half as much cyproterone *(8)* and the animals were killed 2 weeks later. The results (Table 1) provided clear-cut evidence of higher levels of gonadotropin secretion in the cyproterone-implanted than in the control rats, the testicular, prostatic, and seminal-vesicle weights being all significantly greater in the former group. As in the first experiment, there were no significant differences in pituitary, adrenal, or thyroid weights, but the gain in body weight was significantly greater in the cyproterone-implanted animals.

Before conclusion that implantation of cyproterone resulted in increased gonadotropin levels, an alternative interpretation had to be excluded: implantation of cholesterol may have reduced gonadotropin secretion. This possibility was considered in a third experiment in which three groups, each of eight 30-day-old males, received either median-eminence implants of cholesterol or empty tubes, or no treatment. Although both operations reduced final body weight, there were no significant differences between the groups in weights of testes, ventral prostates, or seminal vesicles.

The stimulatory effects of intracerebrally implanted cyproterone on the male reproductive system cannot be due to diffusion of the compound into the systemic circulation, since the effect of systemically injected cyproterone is to depress rather than stimulate the accessory sexual glands—to some extent the testes also *(6)*. Since, however, the effects of implants in different regions of the brain and in the pituitary have not yet been studied, the possibility remains that the implants were acting by diffusion of cyproterone to the pituitary or to neighboring regions of the hypothalamus.

The negative-feedback effects of crystalline testosterone implanted in the median-eminence region are, however, not explicable on the basis of diffusion to the pituitary *(4)*. Furthermore, cyproterone is considerably less soluble than testosterone. Thus one may justifiably conclude that the antiandrogen acts on the hypothalamo-pituitary axis (and probably on the hypothalamus) to initiate processes that result in increase in gonadotropin secretion by the pituitary. This effect appears to be specific to the pituitary-gonadal axis, since no effects on the adrenals or thyroids were observed.

In the light of previous work pointing to the median eminence as the site of the inhibitory action of testosterone on gonadotropin secretion *(3)*, our results are best explained by a blockage by cyproterone of this inhibitory action on the basal hypothalamus. Thus the median-eminence region may be assumed to be capable of responding to both increases and decreases in circulating levels of testosterone by initiating, respectively, decreases and increases in gonadotropin secretion.

Another implication of our findings is that there are certain similarities in the mechanisms of action of androgen on its peripheral and on its central target tissues. The nature of these similarities should be made clearer by further elucidation of the mechanism of the cyproterone-testosterone antagonism.

Note added in proof: Preliminary experiments show no effect of intrapituitary implantation of cyproterone on the weights of testes, seminal vesicles, or prostates in 30-day-old rats.

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24 October 1966

Neuromuscular Transmitter Substance in Insect Visceral Muscle

Abstract. *Stimulation of the nerves innervating the proctodeum (hindgut) of the cockroach* Periplaneta americana *(L.) causes a slow-type, graded contraction of the longitudinal muscles. An unidentified substance, or substances, present in the foregut and hindgut, the specific activity of which is highest in the nerves innervating these organs, effects a similar contraction. This "gut-factor" is depleted from the hindgut after surgical section of the proctodeal nerves. None of Factors P_1 and P_2, 5-hydroxytryptamine, acetylcholine, adrenaline, noradrenaline, α-aminobutyric acid or glutamate duplicates the pharmacological behavior of this substance. The active factor is associated with subcellular particles that require centrifugal forces of approximately 1,000,000 g-min for sedimentation. The substance is inactivated in homogenates of gut tissue in the absence of suitable precautions. It is proposed that the "gut-factor" functions as an excitatory neuromuscular transmitter substance in insect visceral muscle.*

Until recently, the characterization of excitatory neuromuscular transmitter substances in insects has been unsuccessful. However, glutamate may function as an excitatory neuromuscular transmitter in some arthropod muscle, including the somatic muscle of the cockroach *Periplaneta americana* L. (*1*). This report deals with a potential neuromuscular transmitter in the striated, visceral muscle of *Periplaneta americana* L.

Two peptides, Factors P_1 and P_2, that have pharmacological activity on the isolated hindgut of the cockroach have been extracted from the *corpus cardiacum* gland of the roach (*2*). In a study of the distribution of these peptides in various tissues of the cockroach, a substance (or substances) was extracted from the foregut (stomodeum) and hindgut (proctodeum) which also had pharmacological activity on the hindgut. This factor differs from Factors P_1 and P_2 in its action on the hindgut and in the fact that it is not inactivated by chymotrypsin. The substance is heat stable and dialyzable. The active substance appears to function as a neuromuscular transmitter in the longitudinal muscles of the proctodeum and probably elsewhere in visceral muscle of *Periplaneta*.

The "gut-factor" was extracted from foreguts or hindguts washed free of their contents with physiological saline. The viscera were homogenized in 1*N* acetic acid, and the homogenate was dialyzed against 10 volumes of water; both procedures were performed at 0° to 1°C. The dialyzate was dried by rotary evaporation at 40°C under reduced pressure. Traces of acetic acid were removed by extraction of the dry residue with ethyl acetate. For pharmacological assay, the residue was dissolved in water or .05*M* phosphate buffer, *p*H 7. The pharmacological technique with the roach hindgut has been described previously (*2*). Quantitative results in terms of "rectum equivalents" were obtained by a comparison of the heights of contractions caused by unknowns with those caused by a standard extract of rectums. Specific activities express the content of the active substance in a tissue in terms of rectum equivalents per milligram of tissue protein. Protein was determined by the Lowry method (*3*) on duplicate aliquots of extract before dialysis.

As in many insects, the roach proctodeum is divided by a constriction into the anterior intestine and the posterior intestine, or rectum. The arrangement of the longitudinal muscles is different in the two regions, but the muscles of both are innervated by the same two nerves; these nerves arise bilaterally from the two large cercal nerves just posterior to the sixth abdominal ganglion (*4*). Stimulation of these proctodeal nerves (1 volt, 1-msec duration) at frequencies exceeding 5 to 10 impulses per second results in contraction of the longitudinal muscles in the whole hindgut (the anterior intestine and the rectum) (Fig. 1A). The response of the rectum only is shown in Fig. 1B. In both cases, the contraction is of the slow type and is graded, increas-

Table 1. Rectum equivalents, protein, and specific activities of "gut-factor" (rectum equivalents per milligram protein) in various tissues of the cockroach. Determinations were performed on tissue pooled from 20 to 40 roaches.

Tissue	Rectum equivalents per organ	Protein per organ (μg)	Specific activity
Stomodeum	1.89	1332	1.4
Stomodeal nerve	0.35	9.4	37.3
Stomodeal nerve	.28	8.6	32.6
Proctodeum	1.52	1557	1.0
Proctodeal nerve	0.032	2.0	16.0
Proctodeal nerve	.029	2.9	10.0
Nerve*	.014	57.8	0.2
Ganglia†	.16	159	1.0

* Thoracic peripheral nerve. † Pro-, meso- and metathoracic ganglia.

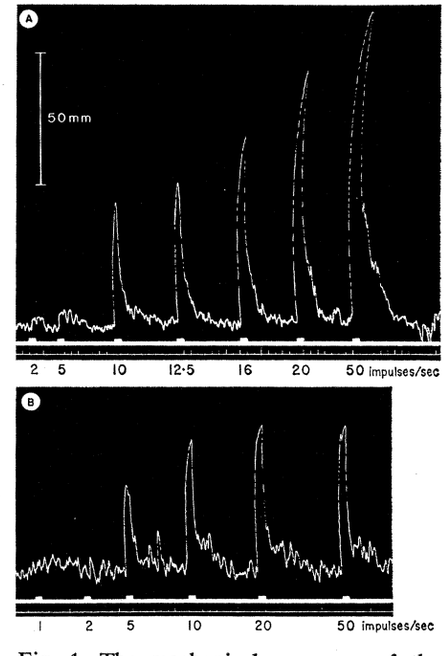

Fig. 1. The mechanical responses of the whole hindgut (A) and of the rectum only (B) to stimulation of the proctodeal nerves. Stimulation (1 volt and 1-msec duration) with platinum electrodes for 10 seconds at the frequencies indicated. Time marker is 10 seconds.