Table 1. Stimulation of histone phosphorylation by cyclic AMP. In addition to the components listed in the text, the reaction mixtures contained 0.66 μg of histone kinase and, where indicated, 0.0025 μmole of cyclic AMP (Sigma); the specific activity of the ^{32}P-ATP was 6930 count/min per nanomole of β,γ-phosphate. The incubation time was 5 minutes.

Substrate	^{32}P Transferred (pmole)		Stimulation (%)
	+ Cyclic 3′,5′-AMP	Control	
f1 Histone	124	23	530
f2b Histone	364	92	400
Protamine	62	57	110
None	5	8	

cooled, protein and filter debris were precipitated by addition of 0.5 ml of 4*N* HCl, 1*N* in H_2SO_4, followed by 0.1 ml of 0.1*M* silicotungstic acid, 0.1*N* in H_2SO_4. To 2.0 ml of the supernatant, 0.5 ml of 5 percent ammonium molybdate, 4*N* in H_2SO_4 was added, and the resulting phosphomolybdate complex was extracted with 2.5 ml of isobutanol-benzene, 1:1 (*16*). Portions (2.0 ml) of the extracts were plated and counted in a thin-window GM counter.

The effect of cyclic AMP on the phosphorylation of histones and protamine is presented in Table 1. In other experiments, the increased histone phosphorylation observed under these conditions ranged from four- to sixfold (400 to 600 percent). There was little or no increase in protamine phosphorylation. Half-maximum stimulation of histone phosphorylation is produced by approximately $3 \times 10^{-8}M$ cyclic AMP, and the maximum effect is obtained at $10^{-7}M$ (Fig. 1). The 5′- and mixed 2′- + 3′-monophosphates of adenosine, uridine, guanosine, and cytidine ($10^{-5}M$) did not replace cyclic AMP.

Cyclic AMP affects the initial rate of histone phosphorylation, and this rate remains approximately constant under the conditions of incubation for 10 minutes in both its presence and absence (Fig. 2). The greatest effects of cyclic AMP are obtained at low (less than saturating) concentrations of histone. For example, raising the histone concentration from 0.1 to 1.0 mg/ml increases the rate of phosphorylation in control reactions fivefold; the stimulation produced by cyclic AMP at these two concentrations of histone was 4.6- and 3.3-fold, respectively.

Cyclic AMP does not change the extent of phosphorylation of histones. The maximum amount of phosphate that can be transferred to f1 histone on prolonged incubation with large amounts of enzyme and excess ATP is 33 to 40 nmole/mg, regardless of the presence of cyclic AMP. These data indicate that the site of phosphorylation of f1 histone, which in the absence of cyclic AMP is mainly a single specific serine residue (*17*), is the same in the presence of the cyclic nucleotide.

Huang and Bonner and Allfrey *et al.* (*18*) have shown that histones can inhibit RNA synthesis in cell-free systems. Their findings have caused interest in the possibility that histones may act as repressors of DNA template activity in eukaryotic cells. The additional possibility that acetylation (*19*) or phosphorylation (*20*) of histones may modify DNA-histone interactions and cause derepression of template activity has also been considered. In this context, the observation that cyclic AMP stimulates histone phosphorylation suggests a mechanism for the induction of RNA synthesis by those hormones that cause increases in the concentration of cyclic AMP.

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10. I thank Dr. G. H. Dixon for communicating these results prior to publication and Dr. E. G. Krebs for providing a manuscript of the paper quoted in (*5*).
11. Prepared by homogenizing calf liver in a Waring blender with ten volumes of chilled acetone (—15°C) and washing three times with five-volume portions of acetone.
12. Prepared according to Keilin and Hartree [in *Meth. Enzymol.* **1**, 98 (1955)].
13. Histone kinase activity is assayed under the conditions described with the following exceptions: histone concentration, 1 mg/ml; cyclic AMP omitted; final volume, 0.25 ml; incubation time, 20 minutes. For routine assay, acid-insoluble ^{32}P was determined as described in the legend to Fig. 1.
14. Calf thymus histones were prepared according to the methods of E. W. Johns, *Biochem. J.* **92**, 55 (1964). Protamine was prepared from salmon sperm by the method of M. J. Callanan, W. R. Carroll, E. R. Mitchell, *J. Biol. Chem.* **229**, 279 (1957).
15. β,γ^{32}P-Adenosine triphosphate was prepared with a photophosphorylation system adapted from B. Petrack, A. Craston, F. Sheppy, F. Farron [*J. Biol. Chem.* **240**, 906 (1965)] and purified by chromatography on DEAE-cellulose with NH_4HCO_3 buffer, *p*H 8.6.
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Hypothalamic Stimulation of Growth Hormone Secretion

Abstract. *Stimulation of the ventromedial hypothalamic nucleus in rats resulted in increased plasma growth hormone levels within 5 minutes, as measured by radioimmunoassay. Stimulation of the cerebral cortex was without effect. These observations confirm previous results involving destructive lesions and establish the ventromedial nucleus as a hypothalamic locus involved in the control of growth hormone secretion.*

Evidence for the hypothalamic control of growth hormone secretion has been based primarily on experiments involving destructive hypothalamic lesions. In most studies, however, large areas of the ventral hypothalamus, including the median eminence, have been destroyed (*1*), thus precluding the specific localization of the site of control of growth hormone secretion. Recent studies from our laboratory (*2*) have demonstrated that destruction of the ventromedial hypothalamic nucleus (VMN) in weanling rats results in impaired growth associated with decreased growth hormone levels in both the pituitary and plasma. Since the median eminence was undamaged in these animals, we suggested that the hypothalamic control of growth hormone secretion resides in the VMN. The present report offers evidence of a different nature to support this hypothesis.

Female Holtzman rats weighing 180 to 220 g were anesthetized with pento-

barbital, tracheotomized, and a catheter was inserted in the carotid artery. The animals were then positioned in a Horsley-Clarke stereotactic instrument. Burr holes were made, and a 0.25-mm unipolar stainless steel electrode, insulated with spar varnish except at the tip, was inserted in the VMN. The coordinates have been previously described (*3*). Stimulation was in the form of biphasic square wave pulses of 1 ma intensity, 5 msec duration, and 50 cycle/sec. The stimulation was applied for 3 minutes, and the current intensity was monitored with an oscilloscope. The stereotactic instrument served as the indifferent electrode. Control animals were stimulated in a similar manner except that the tip of the electrode was positioned in the cerebral cortex.

Heparinized blood samples (0.2 ml) were removed through the catheter immediately prior to stimulation and at several intervals after stimulation was begun (3, 5, 10, and 15 minutes). Plasma was separated at 4°C and stored at −20°C for the subsequent assay of growth hormone.

After the last blood sample was obtained, an anodal direct current of 1.0 ma intensity and of 5 seconds duration was used to produce a lesion, thereby identifying the site of the electrode tip. The rats were then killed. The brains were fixed in formalin, serially sectioned, and stained with cresyl violet, and the location of the lesions (stimulation sites) was identified.

Plasma growth hormone was measured by a double antibody radioimmunoassay method previously described (*2*); rat growth hormone-I^{131} and guinea pig anti-porcine growth hormone serum were used. No immunologic cross-reaction has been observed with rodent thyroid-stimulating hormone or prolactin, porcine adrenocorticotropin, or bovine follicle-stimulating hormone or luteinizing hormone. Plasma levels in hypophysectomized rats range from under 2 to 9 mμg/ml. In rats with transplantable pituitary tumors producing growth hormone, plasma levels as high as 40 μg/ml have been observed and correlate well with bioassay results (*4*).

Thirteen of 14 rats in which stimulation of the VMN was performed had elevated plasma growth hormone levels within 15 minutes of the start of stimulation, as shown in Fig. 1. No elevation of plasma growth hormone occurred by the end of the 3-minute stimulation period, but a rise occurred in over half of the group of rats 2 minutes later. With the exception of the one animal which did not respond, all plasma samples measured at 10 and 15 minutes contained growth hormone levels greater than in the prestimulation samples.

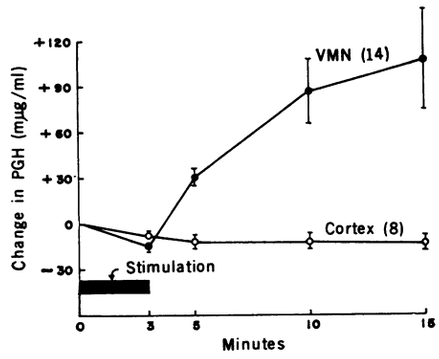

Fig. 1. Changes in plasma growth hormone (PGH) levels after stimulation of either ventromedial nucleus (VMN) or cerebral cortex in rats. Shown are the mean ± standard error. The number of animals in each group is shown in parentheses.

There was a tendency for the prestimulation growth hormone level to influence the response to stimulation. The animals with initial levels of less than 50 mμg/ml (seven rats) had a twofold (absolute) and a 4½-fold (percentage) greater rise following stimulation than those (seven rats) with initial values of greater than 50 mμg/ml. The one nonresponder had the highest prestimulatory growth hormone value (143 mμg/ml). The difference in the initial growth hormone levels of the VMN-stimulated (65 ± 9) and cortex-stimulated (38 ± 7) rats was not significant. A similar phenomenon has been reported in human subjects where the growth hormone response to several stimuli (insulin, arginine, endotoxin) was impaired by elevated prestimulatory values (*5*). This appears to be a manifestation of a general biological phenomenon, Wilder's Law of the Initial Value (*6*). Control rats which were stimulated in the cerebral cortex showed a gradual decline in plasma growth hormone levels.

Figure 2 illustrates the electrolytic lesion in a rat in which the tip of the electrode was in the VMN. Elevations of plasma growth hormone were observed only in those rats in which the electrode tip was within the VMN. Rats which received stimulation in the lateral hypothalamus or the fornix failed to show any rise in plasma growth hormone levels after stimulation. In preliminary studies four rats were stimulated in the VMN for 1 minute. Only one showed a rise in growth hormone levels.

These observations give support to the previous study (*2*) which identified the VMN as the hypothalamic locus responsible for the control of growth hormone secretion. Whether growth hormone releasing factor is formed in the VMN and transported into the

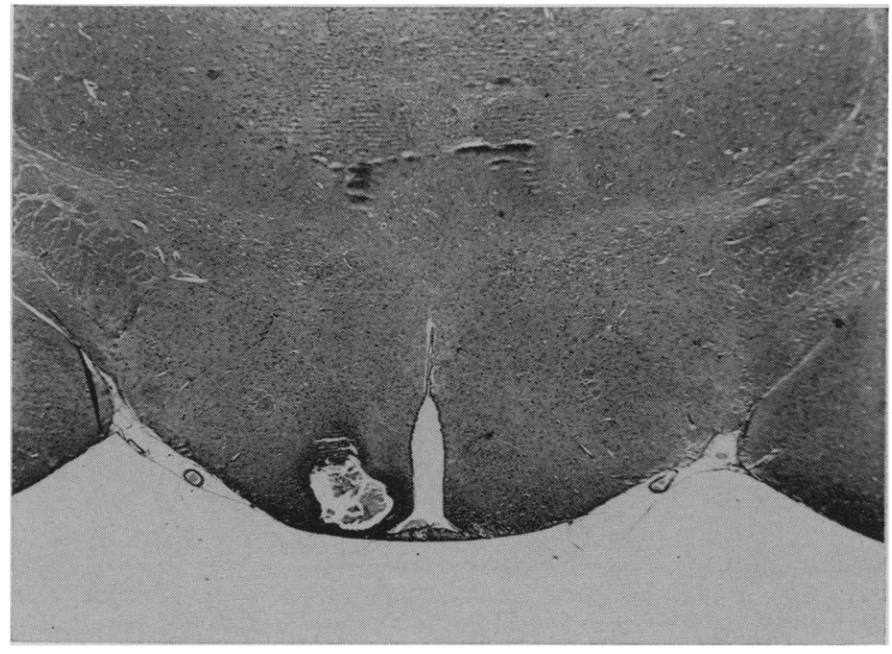

Fig. 2. Photomicrograph showing a coronal section of the hypothalamus of a rat which was stimulated in the ventromedial nucleus. The electrolytic lesion identifies the site of stimulation.

portal hypophyseal capillary plexus which originates in the median eminence or whether neural connections from the VMN stimulate formation of growth hormone releasing factor in the median eminence is not yet known.

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Insect Hormone Activity of p-(1,5-Dimethylhexyl)benzoic Acid Derivatives in Dysdercus species

Abstract. *Derivatives of* p-*(1,5-dimethylhexyl)benzoic acid are juvenile hormone analogs with selective action on the hemipteran insects of the family Pyrrhocoridae. Their juvenile hormone activity is constant on five species of* Dysdercus; *it is about ten times lower on* Pyrrhocoris, *and no activity has been detected on hemipterans of some other families. Absence of profound species-specific variations in the activity suggests that the most active compounds of this type can be used as selective pesticides against all species of* Dysdercus.

Substances with juvenile hormone activity on Pyrrhocoridae occur in the wood of certain evergreen trees (*1*). They have been identified as the terpenes juvabione (*2*) and dehydrojuvabione (*3*). We have described the juvenile hormone effects of *p*-(1,5-dimethylhexyl)benzoic acid derivatives which are structurally related to dehydrojuvabione, and contain an aromatic ring in the molecule (*4*). Some of these synthetic derivatives are about 100 times more active than the original natural products, retaining their specific action on Pyrrhocorid bugs.

We have prepared some new derivatives and tested them, in addition to those mentioned previously (*4*), for species-specific variations in juvenile hormone activity in *Dysdercus*. The experiments were performed on larvae of African species *Dysdercus* (*Neodysdercus) intermedius* Distant and *Dysdercus* (*Dysdercus*) *superstitiosus* (Fabr.); South American species *Dysdercus* (*Dysdercus*) *fulvoniger discolor* (Walker) and *Dysdercus (Dysdercus) chaquensis* Freiberg; and an Indian species *Dysdercus* (*Paradysdercus*) *cingulatus* (Fabr.). They were reared in glass jars at 25°C with an 18-hour light, 6-hour dark cycle, and fed with cottonseeds, and drinking water in cotton-plugged vials.

The juvenile hormone activity was tested by topical assay of freshly moulted larvae of the last instar (*5*). Test substances were applied on abdominal tergites as acetone solutions in 1-μl drops. Hormone activity was evaluated from the degree of morphogenetical change induced. The effects are expressed in activity units indicating the amount of the substance in micrograms per specimen which caused formation of a half-larval, half-adult intermediate. Dose-response experiments on large numbers of larvae suggest that the range of juvenile hormone effect from zero (formation of perfect normal adults) to the maximum activity (formation of perfect supernumerary giant larvae) corresponded approximately to a tenfold change in concentration (*5*). For example, when the activity unit is 0.05 μg per specimen, the substance will show first signs of activity when applied at approximately 0.01 μg per specimen, medium activity at 0.05 μg per specimen, and maximum activity when more than 0.1 μg per specimen is applied.

Initially, we determined the range of activity of each compound by testing it in a series of tenfold dilutions on five or ten specimens at each concentration. The most active concentrations were then utilized to determine the activity unit more precisely.

Preparation of the compounds (Fig. 1) has been described (*4*). Purity of the compounds was checked by elemental analysis and infrared spectroscopy.

The most active compounds are the

Table 1. Juvenile hormone activity units of test substances on several species of *Dysdercus*. The unit value is the amount of the substance in micrograms per specimen that caused formation of half-larval, half-adult intermediates after topical application to freshly moulted last-instar larvae. Structural formulas of the compounds I through XII are shown in Fig. 1.

Compound	Activity units				
	D. intermedius	*D. discolor*	*D. chaquensis*	*D. cingulatus*	*D. superstitiosus*
I	0.3	0.05	0.05	0.1	0.3
II	3	.5	.5	1	
III	0.7	.4	.3	0.5	
IV	.8	.8	.4	.8	
V	.08	.08	.08	.08	
VI	.3	.4	.1	.1	0.4
VII	.05	.02	.01	.04	.05
VIII	.05	.06	.01	.06	
IX	.05	.03	.007	.04	
X	20.0			7.0	
XI	100.0			30.0	
XII	4.0			4.0	
Juvabione	5.0	0.5	1.0	0.5	1.0
Dehydrojuvabione	3.0	.1	0.5	.5	0.8

Table 2. Average juvenile hormone activity units for compounds I to IX (Table 1) in several species of *Dysdercus* and in *Pyrrhocoris apterus* L.

Species	Average unit per specimen (μg)	Freshly moulted last-instar larvae (mg)	Average unit per gram of larvae
D. intermedius	0.59	38.8	15.2
D. discolor	.26	17.5	14.9
D. chaquensis	.16	15.7	10.2
D. cingulatus	.30	26.9	11.2
P. apterus	1.57	16.0	98.0