as by penetration of the integument and may account for the increased activity of the 20-hydroxyecdysone and the variable response to the *trans,trans*-10,11-epoxyfarnesenic acid methyl ester.

Endocrine activity regulates growth and metamorphosis in insects, and the hormones involved, such as 20-hydroxyecdysone, have been isolated from insects (*9*) and from crustacea (*10*). In addition, the juvenile hormone or its analogs terminate photoperiodically induced diapause in insects (*2–4*). My results demonstrate that molting hormones can be used to terminate larval diapause in a tick and are the first to demonstrate this phenomenon in an arthropod other than an insect. These results, added to evidence cited above, strongly suggest that there are similar hormonal systems among the Acarina, Crustacea, and Insecta.

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References and Notes

1. J. E. Wright, in preparation.
2. W. S. Bowers and C. C. Blickenstaff, *Science* **154**, 1673 (1966).
3. G. K. Bracken and K. K. Nair, *Nature* **216**, 483 (1967).
4. R. V. Connin, O. K. Jantz, W. S. Bowers, *J. Econ. Entomol.* **60**, 1752 (1967).
5. U. S. Srivastava and L. I. Gilbert, *Science* **161**, 61 (1968).
6. P. Karlson, *Vitamins Hormones* **14**, 227 (1956).
7. J. N. Kaplanis, M. J. Thompson, W. E. Robbins, B. M. Bryce, *Science* **157**, 143 (1967).
8. O. H. Graham, in *Methods of Testing Chemicals on Insects*, H. H. Shepard, Ed. (Burgess, Minneapolis, Minn., 1960), vol. 2, pp. 200–16.
9. J. N. Kaplanis, M. J. Thompson, R. T. Yamamoto, W. E. Robbins, S. J. Louloudes, *Steroids* **8**, 605 (1966).
10. F. Hampshire and D. H. S. Horn, *Chem. Commun.* **2**, 37 (1966).
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Nicotine Hydrogen Tartrate: Effect on Essential Fatty Acid Deficiency in Mature Pigs

Abstract. *Nicotine (as the acid tartrate) prevented the development of essential fatty acid deficiency symptoms in animals receiving a linoleate-deficient diet.*

Uncastrated male pigs, 6 months old (grown on a normal commercial pig diet) and weighing about 100 kg, were used to study the effects of nicotine on tissue and serum lipid concentration and composition (*1*).

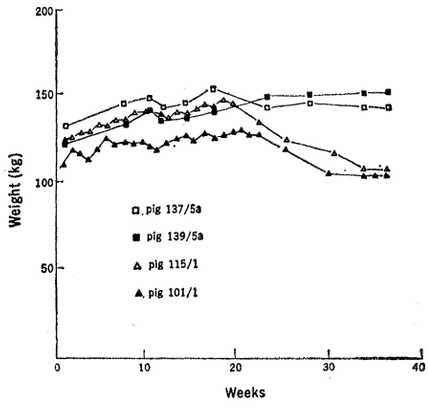

Fig. 1 (left). Weight of four pigs. Numbers 137/5a and 139/5a were fed a low fat diet with nicotine supplement; 115/1 and 101/1 had no supplement. The other animals are omitted for reasons of clarity.

Table 1. Composition of isocaloric diet.

Substance	Composition (%)
Barley meal	70.0
Fine Millars Offal	20.0
Extracted soya bean meal	7.5
Salt	0.5
Ground limestone	0.5
Sterilized bone meal	1.0
"Eves" No. 32 (totally digestible)	0.25

Animals were kept in individual pens with concrete floors; all received an isocaloric diet containing 0.3 percent linoleate calories and yielding 4500 calories per day (Table 1). Twenty-two animals received this diet alone. Fifteen other animals received a daily injection (before feeding) of an aqueous solution of nicotine hydrogen tartrate equivalent to 1 milligram per kilogram of body weight, administered subcutaneously at the top of the forehock, in addition to the control diet. All animals were weighed weekly. Animals were killed at intervals for morphological study; thus only a relatively small number of animals remained for long-term observation.

All animals thrived for the first 6 months of the study, after which eight pigs on the low fat diet (without nicotine) remained and these lost weight steadily although they continued to consume their entire ration (Fig. 1). These pigs suffered from severe skin irritation with scaling, and seven of them died. Autopsy revealed no recognizable organic disease. The eighth pig was saved by isocaloric substitution of maize oil (28 g/day), from the age of 20 months, and gained 14 kg in the 21st month.

The changes observed are those produced by essential fatty acid deficiency (*2*). This is supported by the reversal of the condition by increasing linoleate calories to 2 percent with the maize oil supplement. In contrast, none of the 15 pigs receiving nicotine hydrogen tartrate showed either loss of weight or skin irritation, and all of them continued to thrive. By 35 weeks there were marked differences in the weight of the eight pigs receiving a low fat diet alone and the five receiving a low fat diet with injections of nicotine hydrogen tartrate (Fig. 1), and marked physical differences were observed between animals of the two groups.

The manner in which the nicotine salt produces this striking effect is not clear, but it may act by sparing the polyunsaturated fatty acid stores of the body.

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References and Notes

1. N. Woolf, J. W. P. Bradley, T. Crawford, K. C. Carstairs, *Brit. J. Exp. Pathol.* **49**, 257 (1968).
2. W. M. Witz and W. M. Beeson, *J. Anim. Sci.* **10**, 112 (1951); E. G. Hill, E. L. Warmanen, C. L. Silbernick, R. T. Holman, *J. Nutr.* **74**, 335 (1961); W. M. F. Leat, *Brit. J. Nutr.* **16**, 559 (1962).
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Histone Structure: Asymmetric Distribution of Lysine Residues in Lysine-rich Histone

Abstract. *Structural studies on a very lysine-rich histone show that the carboxyl-terminal half of the molecule is enriched in lysine (and proline), which suggests that it is a site for binding to DNA. The amino-terminal half, containing most of the acidic residues, resembles small, nonhistone proteins and so might have specificity for factors other than DNA.*

The lysine residues in lysine-rich histones are not uniformly spaced (*1*). We now present evidence that most of the lysine residues are packed within the carboxyl-terminal half of the polypeptide chain and that other amino acids are clustered within certain regions of the histone molecule (*2*).

Table 1. Amino acid compositions (moles of amino acid per mole of peptide) of peptides derived from rabbit thymus lysine-rich histone, which has a molecular weight of about 21,000 (*8*). Abbreviations are: NBS, *N*-bromosuccinimide-treated; Asp, aspartic acid; Gly, glycine; Leu, leucine; Lys, lysine; Phe, phenylalanine.

Amino acid	Rabbit thymus peak 3	NBS peptides		Degradation peptides		Chymotryptic peptides			
		N_1	N_2	A	B	III	VI	VII_b	VII_c
Lysine	61.2	52.6	12.2	11.1	46.7	45.0	8.3	2.9	4.6
Histidine	0	0	0	0	0	0	0	0	0
Arginine	2.8	1.1	1.7	1.9	0	0	1.6	0	1.1
Aspartic acid	4.7	4.0	1.1	4.0	1.6	1.2	1.0	0.5	3.0
Threonine	8.0	6.2	1.9	4.1	5.0	3.3	2.0	2.7	0.6
Serine	12.0	7.3	4.1	8.1	5.4	3.9	4.3	3.8	2.1
Glutamic acid	7.6	3.7	3.9	7.0	2.0	1.7	4.9	1.4	2.1
Proline	21.8	16.7	6.2	6.9	15.9	16.3	5.8	0	0
Glycine	15.5	9.2	5.4	7.7	6.9	5.5	3.7	4.5	3.4
Alanine	56.0	37.2	19.2	20.8	32.3	33.8	14.2	1.5	3.9
Cystine/2	0	0	0	0	0	0	0	0	0
Valine	9.0	6.6	2.3	4.5	4.7	3.8	2.1	1.9	1.3
Methionine	0	0	0	0	0	0	0	0	0
Isoleucine	1.8	0.9	0.9	1.8	0	0	1.0	0.1	1.0
Leucine	9.0	5.0	4.0	6.2	1.7	1.7	1.7	2.8	2.2
Tyrosine	0.8	0	0	0.9	0	0	0	0	1.0
Phenylalanine	1.0	0.8	0	0.3	1.0	0	0	0.9	0
NH_2-terminal	None	Asp	None			Lys	None	Gly	Lys
COOH-terminal	Lys	Lys	None			Lys	Leu	Phe	Leu

The histone chosen for study was one fraction (peak 3) of the complement found in thymus glands from young rabbits (*3*). This fraction appeared molecularly homogeneous when studied by chromatography, electrophoresis, and amino acid analysis (*4*). Investigation of terminal amino acids failed to indicate the presence of an amino-terminal amino acid and revealed the carboxyl-terminal sequence to be lysyl-lysine.

The molecule has no tryptophan, histidine, or sulfur-containing amino acids, but has a single tyrosine residue (*3*). Oxidative cleavage at the tyrosyl residue with *N*-bromosuccinimide (*5*) broke the molecule into two pieces. The two peptides, designated N_1 and N_2, were obtained by chromatography on Sephadex G-100 (Table 1). The amino-terminal residues were investigated by the dansyl chloride method (*6*); carboxyl-terminal residues were determined by digestion with carboxypeptidase A and B.

Peptides resulting from proteolytic degradation (*7*) of peak 3 after storage in water at 4°C for a long time were also analyzed. Two peptides, designated as A and B, were isolated by IRC-50 chromatography (Table 1). Terminal amino acids were not determined.

Peak 3 was also subjected to chymotryptic cleavage (10 minutes; enzyme to substrate ratio, 1:450). The four major peptides released were isolated by chromatography on Sephadex G-100 and by paper electrophoresis and were designated III, VI, VII_b, and VII_c (Table 1).

The various sets of peptides were aligned as shown in Fig. 1. The order of the peptides in each set was determined in the following manner; peptide N_2 yielded no amino- or carboxyl-terminal amino acid upon analysis, and it contained the spirolactone derivative of tyrosine, as determined by the absorbancy of peptide N_2 at 260 nm. Since oxidative cleavage occurs on the carboxyl side of tyrosine, peptide N_2 must have originated from the amino terminus of the histone molecule. Analysis of terminal amino acids of N_1 and N_2 confirmed this placement.

Peptide A originates from the amino-terminal portion of the molecule since it contained the single tyrosine residue, whose derivative was at the carboxyl terminus of peptide N_2. Peptide B contained the single phenylalanine, and must represent the carboxyl-terminal portion of the molecule.

The four chymotryptic peptides were aligned in a similar manner, their amino acid compositions and amino- and carboxyl-terminal residues being taken into account.

In each method the fragments obtained account for all the amino acid residues of peak 3. The small differences observed can easily be attributed to experimental error in amino acid analyses of the various components. In accounting for various portions of the molecule, all the data are internally consistent. For example, compare peptide N_2 with peptide A, peptide III with peptide B and peptide N_1; each set of peptides from one method confirms the information derived from another.

Striking differences in the lysine distribution are apparent from Table 1. The carboxyl-terminal region of the molecule (N_1, B, III) is particularly enriched in lysines whereas the amino-terminal region (N_2, A, VI) is depleted in lysines. The amino-terminal region has an amino acid composition not unlike that of small proteins such as ribonuclease and lysozyme.

Distributions of other amino acids are also unusual (Table 1). The amino-terminal peptides (N_2, A, VI, VII_b, VII_c) contain almost all (about three-fourths) of the acidic residues and a large majority of the hydrophobic residues. In contrast, the carboxyl-terminal half (see peptide III) has very few hydrophobic residues. One interesting region is the quarter of the molecule represented by peptides VII_b and VII_c. This region is completely devoid of proline, a remarkable feature in a protein with such a high content of proline.

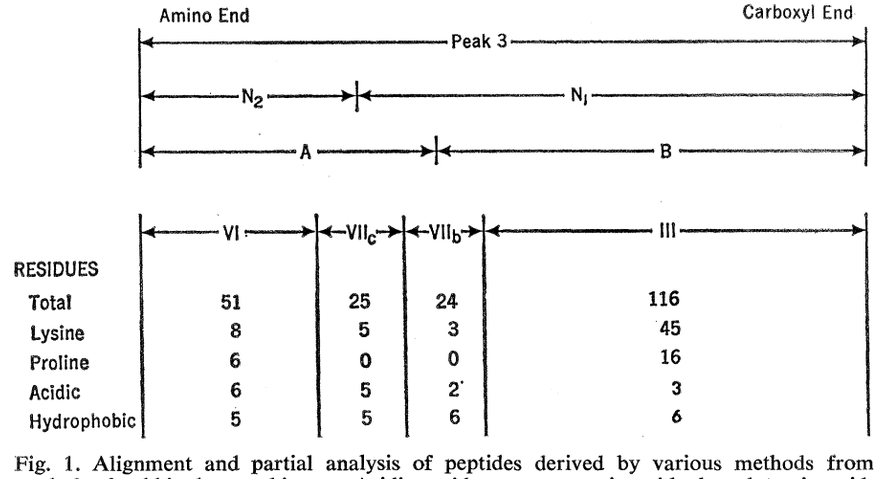

Fig. 1. Alignment and partial analysis of peptides derived by various methods from peak 3 of rabbit thymus histone. Acidic residues are aspartic acid plus glutamic acid; hydrophobic residues are valine, isoleucine, leucine, tyrosine, and phenylalanine.

This arrangement of amino acid residues within this histone raises the possibility that the carboxyl-terminal region might be designed to interact strongly with DNA, whereas the amino-terminal half might be designed for specific interaction with some other agent, for example, one which is involved in processes of repression and derepression.

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References and Notes

1. D. M. P. Phillips, in *The Nucleohistones*, J. Bonner and P. Tso, Eds. (Holden-Day, San Francisco, 1964), p. 46.
2. M. Bustin, S. C. Rall, R. D. Cole, in preparation.
3. M. Bustin and R. D. Cole, *J. Biol. Chem.* **243**, 4500 (1968).
4. M. Bustin, thesis, University of California, Berkeley (1968).
5. L. K. Ramachandran and B. Witkop, *Meth. Enzymol.* **11**, 283 (1967).
6. W. R. Gray, *ibid.*, p. 139.
7. D. M. P. Phillips and E. W. Johns, *Biochem. J.* **72**, 538 (1959).
8. D. Teller, J. M. Kinkade, R. D. Cole, *Biochem. Biophys. Res. Commun.* **20**, 739 (1965).
9. Supported by PHS grants AM-02691, AM-06482, and GM-00031 and by the Agricultural Experimental Station.

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Amino Acid Incorporation into Rat Brain Proteins during Spreading Cortical Depression

Abstrast. *Unilateral spreading cortical depression was elicited by applying potassium chloride solutions to the dura of conscious, freely moving rats. Incorporation of ^{3}H-leucine into soluble cortical proteins was decreased in the depressed hemisphere relative to the control side, while soluble brainstem proteins from both sides had the same specific activity. Various subfractions of soluble cortical proteins were affected to equal degrees.*

Spreading cortical depression (SCD) (*1*) consists of repeated waves of depressed electrocortical activity that can be induced by topical application of KCl to a small area of the cerebral cortex. The depression spreads to the rest of the cortex of the hemisphere on the side to which KCl has been applied, but not to the other hemisphere or to subcortical areas. Ruščák (*2*) has shown that SCD in anesthetized rats is accompanied by decreased incorporation of ^{35}S-methionine into total brain proteins. We now report experiments which extend his findings to conscious, freely moving animals, and which suggest that none of the soluble proteins is preferentially affected.

Male Sprague-Dawley rats (250 to 350 g) were used; we implanted bilateral epidural cannulas on either side of the sagittal suture just anterior to the lambdoid suture (*3*). The cannulas were filled with sterile, 0.9 percent saline. Between 24 and 48 hours after surgery, unilateral SCD was induced in conscious rats by rinsing one cannula with 10 percent KCl, and the existence of SCD was verified by observing the loss of the placing response in the contralateral hind limb (*4*). Leucine-4,5-^{3}H (5 c/mmole, Tracerlab, Waltham, Mass.) was then injected intraperitoneally in a dose of 3.5 μc per gram of body weight.

After the period designated for the incorporation of the tracer, the animals were killed by cardiac perfusion with cold saline under sodium pentobarbital anesthesia, in order to ensure the removal of blood proteins from the brain. The brain was exposed and sectioned transversely through the posterior colliculus and the longitudinal fissure, and the cerebrum (minus olfactory lobes) was quickly removed. The left and right halves were separated, rinsed in cold saline, and immediately frozen in a mixture of dry ice and acetone. Each hemisphere was divided roughly into "cortex" and "brainstem" (*5*).

Extracts of the cortex and brainstem of each hemisphere were prepared in sodium-potassium phosphate buffer (*p*H 7.4, ionic strength 0.1) by homogenizing in a Ten Broeck tissue grinder at 0° to 4°C, with 10 ml of buffer per gram of tissue (wet weight). The homogenates were centrifuged for 1 hour at 50,000*g* in the No. 40 rotor of a Spinco model L centrifuge. The pellets were discarded, and the clear supernatants containing the soluble proteins were analyzed. The proteins soluble at *p*H 7.4 in the extraction buffer were separated into two classes, designated as *p*H 5–insoluble and *p*H 5–soluble proteins. The specific activity of each of these two fractions was determined by first bringing a portion of the extract to *p*H 5. About half of the protein precipitated. After centrifugation, the soluble fraction was removed, and trichloroacetic acid (TCA) was added to precipitate the remaining protein. A portion of each brain extract at *p*H 7.4 was also added directly to half the volume of 30 percent TCA in order to precipitate all the protein. The washed *p*H 5 precipitate and the two TCA precipitates were dissolved in 97 percent formic acid. The formic acid solution (0.2 ml) was added to 20 ml of scintillation fluid (*6*). Radioactivity was determined by counting in a liquid scintillation counter equipped with an external standard and corrected for quenching (*6*). A portion of each TCA supernatant was also counted by adding it directly to the scintillation fluid. The protein concentration of each extract and each *p*H 5–soluble supernatant was determined (*7*), with bovine serum albumin as a standard. Specific activity (count min^{-1} mg^{-1}) was calculated from the radioactivity (count min^{-1} ml^{-1} in protein) and the protein concentration (mg ml^{-1}) of the extract.

We first determined the kinetics of

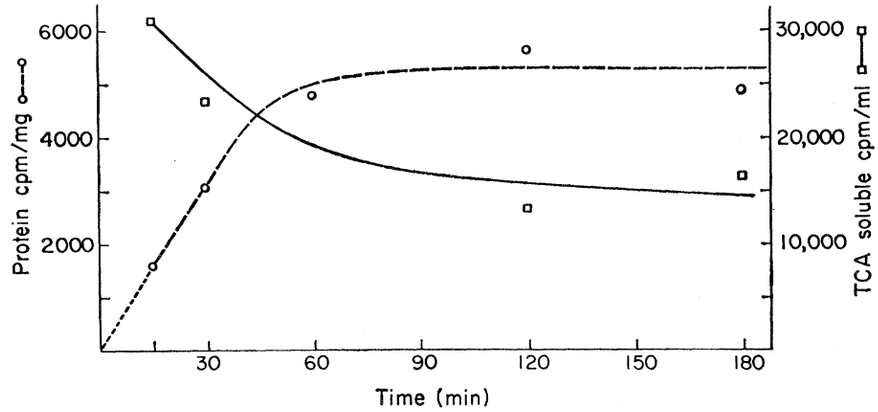

Fig. 1. Change with time in specific activity of total soluble proteins (○—○) and radioactivity of the acid soluble fraction (□—□) of rat cortex, after intraperitoneal injection of ^{3}H-leucine (3.5 μc per gram body weight). Each point represents the average of determinations on two or more animals.