6. G. Tucker, R. E. Gagnon, M. R. Haussler, *Arch. Biochem. Biophys.* **155**, 47 (1973).
7. M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673 (1972).
8. D. R. Fraser and E. Kodicek, *Nat. New Biol.* **241**, 163 (1973).
9. R. G. Larkins, K. W. Colston, L. S. Galante, S. J. MacAuley, I. M. A. Evans, I. MacIntyre, *Lancet* **1973-II**, 289 (1973); L. S. Galante, K. W. Colston, S. J. MacAuley, I. MacIntyre, *ibid.*, p. 985.
10. L. Galante, K. W. Colston, I. M. A. Evans, P. G. H. Byfield, E. W. Matthews, I. MacIntyre, *Nature (Lond.)* **244**, 438 (1973).
11. Y. Tanaka and H. F. DeLuca, *Arch. Biochem. Biophys.* **154**, 566 (1973).
12. P. F. Brumbaugh, D. H. Haussler, R. Bressler, M. R. Haussler, *Science* **183**, 1089 (1974); P. F. Brumbaugh, D. H. Haussler, K. M. Bursac, M. R. Haussler, *Biochemistry* **13**, 4091 (1974).
13. J. E. Wergedal, *Calcif. Tissue Res.* **3**, 55 (1969).
14. Serum samples from seven to ten rats were pooled for assay of $1\alpha,25\text{-}(OH)_2D_3$; 10- to 20-ml samples were usually used. In rats with enhanced circulating concentration of $1\alpha,25\text{-}(OH)_2D_3$, such as calcium-deficient intact, phosphate-deficient intact, and phosphate-deficient TPTX, only 3 to 4 ml of serum was required.
15. The method of extraction and chromatographic purification of $1\alpha,25\text{-}(OH)_2D_3$ described by P. F. Brumbaugh *et al.* [*Biochemistry* **13**, 4091 (1974)] was modified as follows. Silicic acid column chromatography was deleted from the isolation scheme. After adding [$^3$H]-$1\alpha,25(OH)_2D_3$ (6 c/mmole; 2000 count/min) and extraction of sterols, $1\alpha,25\text{-}(OH)_2D_3$ was purified by two successive columns of Sephadex LH-20 (1 by 15 cm; 5 g). Elution was performed with 65 percent chloroform in hexane and the hormone emerged between 40 and 95 ml elution volume. Final purification of the hormone was effected by Celite liquid-liquid partition chromatography [M. R. Haussler and H. Rasmussen, *J. Biol. Chem.* **247**, 2328 (1972)]. The solvent system was 10 percent ethyl acetate in hexane (mobile) and 45 percent water in ethanol (stationary). Micro-Celite columns (0.8 by 8.5 cm) were packed into 10-ml pipets fitted with glass wool plugs, and eluted with mobile phase and $1\alpha,25\text{-}(OH)_2D_3$ emerged when the elution volume was between 7 and 22 ml. The final sample was evaporated under nitrogen and solubilized in 400 μl of ethanol. After determination of yield by counting 50 μl of each sample for tritium (yields ranged from 50 to 75 percent), radioreceptor assays were performed on 100- μl portions.
16. The radioreceptor assay was modified slightly from P. F. Brumbaugh *et al.* [*Biochemistry* **13**, 4091 (1974)] as follows. Intestinal mucosa (6 g) from two rachitic chicks was homogenized in 25 ml of 0.25*M* sucrose in 0.05*M* tris-HCl (*p*H 7.4), 0.025*M* KCl, 0.005*M* $MgCl_2$. The cytosol fraction was obtained by centrifugation at 100,000*g* for 1 hour. Chromatin was prepared from crude nuclei (isolated from homogenate at 1,000*g* for 10 minutes) by homogenizing successively in one 25-ml portion of 0.8 m*M* EDTA and 25 m*M* NaCl, *p*H 8; one 25-ml portion of 1 percent Triton X-100 and 0.01*M* tris-HCl, *p*H 7.5; and one 25-ml portion of 0.01*M* tris-HCl, *p*H 7.5. The chromatin was harvested by sedimentation at 30,000*g* for 10 minutes after each washing. The entire chromatin pellet from 6 g of mucosa was reconstituted with the cytosol fraction by homogenization to create a cytosol-chromatin system for the competitive binding assay. The assay was carried out as described, except that 20 μl of distilled ethanol was added to each assay tube (containing nitrogen-dried sterol) just before the addition of 200 μl of reconstituted receptor system. The ethanol aided in solubilizing the sterols and reduced nonspecific binding. Triplicate assays were performed on all samples; the variation between assays was 10 to 15 percent.
17. M. Stauffer, D. Baylink, J. Wergedal, C. Rich, *Am. J. Physiol.* **225**, 269 (1973).
18. H. Rasmussen, M. Wong, D. Bikle, D. B. P. Goodman, *J. Clin. Invest.* **51**, 2502 (1972).
19. M. R. Haussler, in preparation.
20. Supported by PHS grants AM 15781, AM 09096, and DE 02600; PHS training grant GM 01982; and by a research career development award DE 19108 to D.J.B. We thank E. Feist and K. Bursac for technical assistance.

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## Organophosphorus and Methyl Carbamate Insecticide Teratogenesis: Diminished NAD in Chicken Embryos

Abstract. *Studies with 36 organophosphorus and 12 methyl carbamate compounds establish a correlation between reduction in nicotinamide adenine dinucleotide (NAD) levels and severity of teratogenic signs in chicken embryos, a relation supported by reversal of these effects by nicotinamide derivatives. Diminished NAD occurring at organophosphorus and methyl carbamate concentrations as low as 0.6 to 2.0 parts per million in the egg constitutes a newly recognized biochemical lesion induced by the two most important classes of insecticide chemicals.*

Organophosphorus (OP) and methyl carbamate (MC) inhibitors of acetylcholinesterase have gradually replaced the chlorinated insecticides so that they are now the insect control agents used most frequently and in the largest amounts. It is therefore important to define the nature and mechanism of any deleterious effect of OP and MC compounds not attributable to disruptions in the cholinergic system. This report concerns such an effect, the mechanism of teratogenesis in chicken embryos, and establishes that over various test conditions the severity of the teratogenic signs is related to the degree of reduction in the embryo nicotinamide adenine dinucleotide (NAD) level.

Many investigations (*1–7*) with chicken embryos establish that about 20 different OP compounds (including dicrotophos, Diazinon, malathion, and malaoxon) and several methyl carbamates (including eserine and carbaryl) produce similar or identical developmental defects (involving some or all of micromelia, abnormal beak, reduced body size, retarded down development, gross edema, and wryneck) when injected into the yolk sac at day 0 to 5 of incubation. No definite relation between structure and teratogenic activity has emerged from these studies; that is, dicrotophos (as the *cis*-crotonamide isomer) and eserine sulfate are teratogenic at 0.6 to 2.0 ppm in the egg, whereas closely related compounds such as tri-*o*-cresyl phosphate and the *trans*-crotonamide isomer of dicrotophos are not, even at 200 ppm. The teratogenic signs are almost completely alleviated, except for the malformation of the neck, when dicrotophos, Diazinon, malathion, malaoxon, eserine sulfate, and carbaryl are supplemented with nicotinamide or nicotinic acid; on a similar basis, the teratogenesis induced by dicrotophos is alleviated with any one of many precursors or derivatives of nicotinamide or nicotinic acid including the NAD cofactors (NAD, NADH, NADP, NADPH) (*1, 3–5*). These observations impose strict limits on any proposal for the biochemical mechanism involved in induction of this type of teratogenesis. Such proposals include (i) no involvement of acetylcholinesterase or the cholinergic system (*4, 5*); (ii) reduced uptake of endogenous tryptophan from the yolk (*5*); (iii) reduced sulfated mucopolysaccharide and RNA content and increased glycogen storage and calcification in the developing tibiotarsus (*6*); and (iv) phosphorylation or carbamoylation of a yolk sac membrane site initiating a sequence of events leading to embryonic abnormalities (*7*). However, not any one of these observations or hypotheses adequately explains all aspects of the phenomenon.

Several of the OP- and MC-teratogenic signs (micromelia, abnormal beak, reduced body size, and retardation of down development) resemble those produced by 6-aminonicotinamide (6-AN), whereas 3-acetylpyridine (3-AP) yields different embryonic abnormalities (muscular hypoplasia, most noticeable in the legs) (*8*). Both 6-AN and 3-AP are nicotinamide antagonists that are stated to act, at least in part, by diminishing the NAD levels in chicken embryos (*8, 9*), chicken limb mesodermal cell cultures (*10*), and mammals (*11*). Studies with embryonic chicken limbs and cultures of their mesodermal cells indicate the importance of embryo NAD levels in the control of muscle and cartilage development in that myogenic cells are observed when NAD levels are high and chondrogenic cells are seen when NAD levels are low (*12*).

These interrelations suggest the possibility that the OP and MC teratogens may act in part by diminishing the embryo NAD. This new hypothesis was subjected to a series of tests, correlating the embryo NAD at day 12 with the severity of teratogenic signs at day 19, after injection of teratogens on day 4 of incubation.

Fertile, white Leghorn eggs incubated at 37.5°C and 73 percent relative humidity were treated under sterile techniques by injection into the yolk sac with a methoxytriglycol solution (30 μl) of an OP or MC compound (*13*) in the presence or absence of a candidate-alleviating agent. Embryo NAD was assayed spectrophotometrically against a standard curve for authentic NAD after addition of yeast alcohol dehydrogenase and ethanol to form reduced NAD (NADH) (*14*). Other eggs from the same treated group were used to observe and record the teratogenic signs, rated

from – (no effect) to ++++ (most severe manifestation) (*1*). The original rating system was modified in two respects. (i) The teratogenic signs produced by 0.1 and 0.3 mg of dicrotophos are rated + + and + + + instead of + + + and + + + +, respectively, to more closely differentiate the effects of the dose-related teratogenesis. (ii) A new rating of * is used to designate embryos with wryneck and sometimes also tibiotarsal arthrogryposis, but not the other teratogenic signs normally associated with OP and MC teratogenesis. The carrier solvent, methoxytriglycol, alone did not alter the normal embryo development or NAD level.

Several features of the relation between embryo NAD levels and the teratogenic signs following treatment with OP and MC compounds are shown in Fig. 1. Dicrotophos and eserine sulfate are almost equally potent in producing a dose-dependent reduction in NAD levels and increase in the severity of the teratogenic signs. Treatment with nicotinamide at 0.8 μmole per egg completely reverses the effect on NAD levels and the resulting embryos are nearly normal with the exception of wryneck and tibiotarsal abnormalities at high teratogen doses.

Three other types of tests further establish the excellent correlation between the embryo NAD levels and the teratogenic signs. (i) The activity of 36 OP and 12 MC compounds was compared after injection at 1 mg per egg. The potency decreased in relation to both the effect on NAD and the teratogenic signs in the following order. For OP compounds: dicrotophos, monocrotophos, and Diazinon > parathion, methamidophos and phosphamidon > 30 other commercial OP compounds (*15*). For MC compounds: eserine sulfate > carbaryl, carbofuran, Meobal, and metalkamate > seven other commercial MC insecticides (*16*). (ii) Cotreatment with nicotinamide (0.8 μmole per egg) partially or completely alleviates the abnormalities (except for the wryneck and sometimes the tibiotarsal arthrogryposis) produced by each of these teratogenic compounds. (iii) In tests with dicrotophos at 1 mg per egg and candidate-alleviating agents at 0.8 μmole per egg (except for tryptophan administered to each egg at 5.0 μmole in 0.01*M* $NaHCO_3$), it was found that four compounds (NAD, nicotinamide, nicotinic acid, and tryptophan) that partially or completely reverse the reduction in NAD levels are effective in alleviating the teratogenic signs whereas four other compounds (β-picoline, isonicotinic acid, picolinic acid, and 3-pyridylacetic acid) that do not affect the NAD levels fail to alleviate the teratogenic signs. Thus, the correlation of diminished NAD levels with teratogenesis extends over a sufficient number of test conditions to propose a cause and effect relationship.

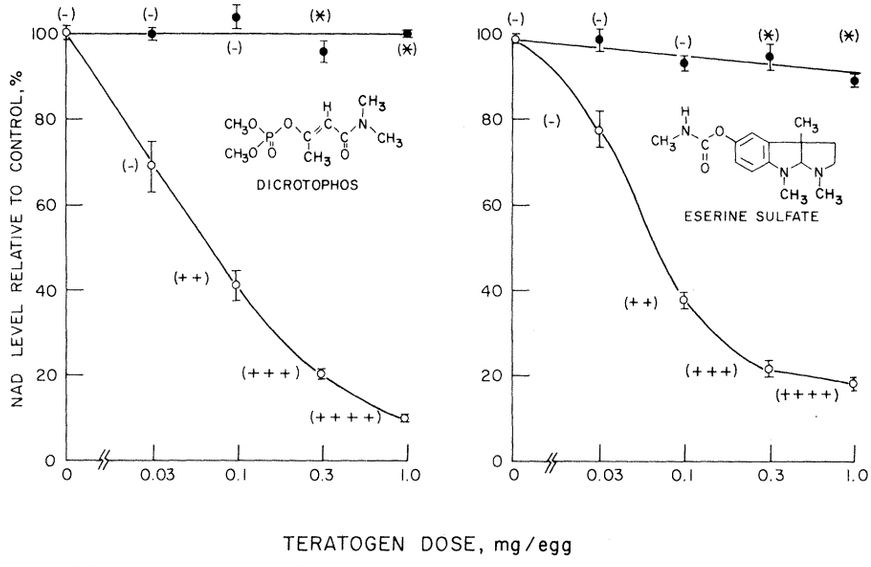


Fig. 1. Effect of varying doses of dicrotophos and eserine sulfate injected alone (○) or in combination with 0.8 μmole of nicotinamide (●) at day 4 of incubation on the NAD levels at day 12 and the teratogenic signs at day 19 of incubation. The NAD results are the averages and standard errors obtained in a typical experimental test series with six individual embryos. The level of NAD in embryos from typical control eggs was 185 ± 4 nmole per gram of embryo (fresh weight); this value was increased by no more than 10 percent on injection of nicotinamide. Ten embryos were used for determination of the teratogenic signs (–, + +, + + +, + + + +, *) as specified in the text.

It is now important to define the biochemical lesion with OP and MC compounds that leads to diminished NAD levels and teratogenesis. Nicotinamide antagonists such as 6-AN and 3-AP are utilized in place of nicotinic acid by some organisms in the synthesis of adenine dinucleotides which are not suitable replacements for NAD in all of its functions (*8–11*). The structures of the OP and MC teratogens are such that they cannot be incorporated into adenine dinucleotides, as with 6-AN, even though there is a similar end effect, that is, teratogenic signs. Both OP and MC compounds inhibit acetylcholinesterase and other sensitive hydrolases by phosphorylation and carbamoylation, respectively, of a serine residue in the active site of the enzyme (*17*). By analogy, the OP and MC teratogens may phosphorylate or carbamoylate an enzyme important in generating or maintaining adequate levels of NAD. If this is the case, the structure-activity relationships for inhibition of this enzyme differ considerably from those for acetylcholinesterase inhibition.

Carbaryl is not only teratogenic in the chicken embryo test, but at high doses it also produces embryonic abnormalities in mice, guinea pigs, and dogs but not in hamsters, rabbits, and swine (*18, 19*). The teratogenic effects of OP insecticides in mammals are less clearly defined or they do not occur (*19, 20*). A better understanding of the mechanism by which OP and MC compounds diminish embryo NAD levels has an important bearing on the use of teratogenic tests in the chicken embryo to evaluate possible effects in mammalian systems. It may also be relevant to other types of biological activity of OP and MC pesticides not associated with inhibition of acetylcholinesterase.

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### References and Notes

1. J.-C. Roger, D. G. Upshall, J. E. Casida, *Biochem. Pharmacol.* **18**, 373 (1969).
2. I. P. Agarwal, *J. Anim. Morphol. Physiol.* **3**, 63 (1956); J.-P. Marliac and M. K. Mutchler, *Fed. Proc.* **22**, 188 (1963); J.-P. Marliac, *ibid.* **23**, 105 (1964); J. F. Dunachie and W. W. Fletcher, *Nature (Lond.)* **212**, 1062 (1966); K. S. Khera, *Toxicol. Appl. Pharmacol.* **8**, 345 (1966); _____ and S. Bedok, *Food Cosmet. Toxicol.* **5**, 359 (1967); J. Greenberg and Q. N. LaHam, *Can. J. Zool.* **47**, 539 (1969).
3. W. Landauer, *J. Exp. Zool.* **136**, 509 (1957); J.-C. Roger, H. Chambers, J. E. Casida, *Science* **144**, 539 (1964); J.-P. Marliac, M. J. Verrett, J. P. McLaughlin, O. G. Fitzhugh, paper presented at the Society of Toxicology 4th annual meeting, Williamsburg, Va., 8 to 10 March 1965; W. Landauer and N. Salam, *Dev. Biol.* **28**, 35 (1972).
4. D. G. Upshall, J.-C. Roger, J. E. Casida, *Biochem. Pharmacol.* **17**, 1529 (1968); N. E. Walker, *Toxicol. Appl. Pharmacol.* **19**, 590 (1971).
5. J. Greenberg and Q. N. LaHam, *Can. J. Zool.* **48**, 1047 (1970).
6. M. Ho and M. A. Gibson, *ibid.* **50**, 1293 (1972).
7. I. R. Flockhart and J. E. Casida, *Biochem. Pharmacol.* **21**, 2591 (1972).
8. W. Landauer and E. M. Clark, *J. Exp. Zool.* **151**, 253 (1962).
9. I. H. Krakoff, C. R. Lacon, D. A. Karnofsky, *Nature (Lond.)* **184**, 1805 (1959).
10. A. I. Caplan, *J. Exp. Zool.* **180**, 351 (1972); *Exp. Cell Res.* **70**, 185 (1972).
11. W. J. Johnson, in *Metabolic Inhibitors*, R. M. Hochster and J. H. Quastel, Eds. (Academic Press, New York, 1963), vol. 2, chap. 16, pp. 1–21.
12. M. J. Rosenberg and A. I. Caplan, *Dev. Biol.* **38**, 157 (1974).

13. The chemical designations for OP and MC insecticides referred to by their common names are given in E. E. Kenaga and C. S. End [*Entomol. Soc. Am. Spec. Publ. 74-1* (1974)] and D. E. H. Frear, Ed. [*Pesticide Index* (College Science, State College, Pa., ed. 4, 1969)]. The compounds used were analytical reference standards obtained from the Pesticides and Toxic Substances Effects Laboratory (Environmental Protection Agency, Research Triangle Park, N.C.) or the manufacturers.
14. M. M. Ciotti and N. O. Kaplan, *Methods Enzymol.* **3**, 890 (1957).
15. The less active OP compounds were Abate, azinphosmethyl, carbophenothion, chlorpyrifos, coumaphos, crufomate, dichlorvos, dimethoate, dioxathion, disulfoton, ethion, fenitrothion, fenthion, fonofos, leptophos, malathion, methidathion, methyl parathion, mevinphos, naled, oxydemetonmethyl, phorate, phosalone, phosmet, ronnel, Salithion, TEPP, trichlorfon, EPN, and DEF.
16. The less active MC compounds were aldicarb, dimetilan, isolan, methomyl, mexacarbate, propoxur, and pyramat.
17. G. B. Koelle, Ed., "Cholinesterases and anticholinesterase agents," in *Handbuch der Experimentellen Pharmakologie* (Springer-Verlag, Berlin, 1963), vol. 15; M. Eto, *Organophosphorus Pesticides: Organic and Biological Chemistry* (Chemical Rubber Company, Cleveland, Ohio, 1974).
18. H. E. Smalley, J. M. Curtis, F. L. Earl, *Toxicol. Appl. Pharmacol.* **13**, 392 (1968).
19. J. F. Robens, *ibid.* **15**, 152 (1969); K. S. Khera and D. J. Clegg, *Can. Med. Assoc. J.* **100**, 167 (1969); E. M. Mrak, chairman, *Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health* (Department of Health, Education, and Welfare, Washington, D.C., 1969), pp. 655–677.
20. T. Tanimura, T. Katsuya, H. Nishimura, *Arch. Environ. Health* **15**, 609 (1967); V. A. Gofmekler and B. B. Khuriyev, *Gig. Sanit.* **1**, 27 (1971); C. H. Budreau and R. P. Singh, *Toxicol. Appl. Pharmacol.* **24**, 324 (1973); J. S. Bus and J. E. Gibson, *Food Cosmet. Toxicol.* **12**, 313 (1974); see also references cited in these four publications.
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## Peripatus: Its Affinities and Its Cuticle

Abstract. *The cuticle of* Peripatoides leuckarti *is arthropodan in structure and in chemical composition. The cuticular protein resembles that of other arthropods; it is not a collagen. This peripatus cuticle has no affinities with the annelid cuticles.*

The Onychophora are of interest because of their assumed relation to both the Annelida and the Arthropoda. Manton (*1*) regards the group, together with the Myriapoda and Hexapoda, as well-defined taxa of the phylum Uniramia which are descended from an early group of land arthropods. The cuticle of the Onychophora is very thin, is arthropodan in structure, and contains protein and chitin [for example, see Robson (*2*)]. The chitin is $\alpha$-chitin (*3*), identified by x-ray diffraction and by histochemical techniques. Beneath the cuticle, but not part of it, is a thick layer of connective tissue which has the properties of a collagen. Since $\alpha$-chitin is not associated with collagen (*4*), the report by Krishnan (*5*) that protein in the cuticle of *Eoperipatus weldoni* is, like that of annelids, of a collagenous type requires reinvestigation. Also, in view of the evolutionary status of the Onychophora, the report is contrary to that expected from our studies on the comparative chemistry of arthropod cuticular proteins. Working with another species of Onychophora, we have been unable to confirm the presence of collagen in the cuticle.

Specimens of *Peripatoides leuckarti* (Sänger) were collected from litter in sclerophyll forest near Canberra. In this species the body cuticle is about 1 $\mu$m thick, increasing to about 10 $\mu$m in the spines, and consists of procuticle and a multilayered epicuticle. Pieces of body cuticle, uncontaminated with noncuticular material, were isolated as follows. The body wall was soaked in 70 percent ethanol and, from places where the cuticle had begun to separate from the underlying layers, pieces were carefully peeled back with the aid of forceps and removed. The pieces of cuticle were extracted with ethanol and ether, dried, hydrolyzed, and the hydrolyzate analyzed to determine its amino acid composition (Beckman amino acid analyzer). The results are given in Table 1, together with those for *Bombyx mori* larval cuticle (*6*), as residues per 1000 total residues. From the glucosamine content (corrected for losses during hydrolysis) the chitin content of *P. leuckarti* cuticle was calculated to be about 8 percent. Extraction of the body wall with hot normal aqueous NaOH left an insoluble residue in the form of pieces of very thin colorless sheets. The residue on hydrolysis gave glucosamine as the only sugar (identified chromatographically) and so the residue has the properties of chitin.

Table 1. Amino acid analyses of cuticular proteins. The *B. mori* data are from (*6*).

| Amino acid | Residues per 1000 total residues | |
|---|---|---|
| | *Peripatoides leuckarti* | *Bombyx mori* (larval) |
| Alanine | 56.5 | 123.7 |
| Arginine | 30.7 | 24.0 |
| Aspartic acid | 41.0 | 88.9 |
| Cysteine | 9.2 | 6.1 |
| Glutamic acid | 125.0 | 94.3 |
| Glycine | 97.2 | 112.3 |
| Histidine | 26.7 | 13.0 |
| Leucine | 47.4 | 35.0 |
| Isoleucine | 37.7 | 43.2 |
| Lysine | 74.4 | 34.6 |
| Methionine | 11.7 | 1.5 |
| Phenylalanine | 27.3 | 24.1 |
| Proline | 84.9 | 98.9 |
| Serine | 77.9 | 74.7 |
| Threonine | 160.2 | 65.2 |
| Tyrosine | 44.1 | 78.9 |
| Valine | 47.9 | 81.6 |

The amino acid composition of the cuticle shows that the protein is not a collagen, it does not have the high glycine content, hydroxyproline, or hydroxylysine, all of which are characteristic of collagens. In this respect it differs from that in the cuticles of annelids. The protein resembles other arthropod cuticular proteins, even though there are differences in the amounts of individual amino acids when compared with that from other species. Hackman (*7*), when considering the amino acid composition of unspecialized (for example, larval) insect cuticles and of cuticular proteins from them, has shown that the sums of the amino acid residues, when grouped according to the second letter of their genetic codes, have similar values. (The same values are given by the proteins from several crustacean cuticles and from *Limulus polyphemus* cuticle, and this subject is being reported on in detail separately.) The values appear characteristic and are markedly different from those for sclerotized cuticles and for unhardened cuticles with specialized functions, such as being elastic or being able to expand rapidly. This result confirms the polymorphic nature of the cuticular proteins. The same groupings of the amino acid residues for *P. leuckarti* cuticular protein give uracil (U) = 172, cytosine (C) = 379, adenine (A) = 311, and guanine (G) = 137, values very similar to those given by the proteins from unspecialized, unhardened arthropod cuticles, as exemplified by *B. mori* larval cuticle, for which U = 185, C = 362, A = 310, and G = 142. The protein in *P. leuckarti* cuticle is of the type commonly found associated with $\alpha$-chitin. The cuticle has the structure and composition of an arthropod cuticle, not the properties of an annelid cuticle.

It is of interest that the proteins from unspecialized cuticles from species of Insecta, Onychophora, Crustacea, and Arachnida have similar amino acid compositions when allowance is made for protein polymorphism. This suggests either that convergent evolution within the arthropod taxa has resulted in the appearance of the same cuticular proteins (together with $\alpha$-chitin) on a number of occasions, or that all the taxa are derived ultimately from a common ancestor, which might not have been annelid [see Tiegs and Manton (*8*)], and that the overall amino acid composition of the cuticular proteins has remained unchanged.

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