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.
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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.
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21. Supported in part by a fellowship to N.H.P. (1 F22 ES00314) and grants from the National Institute of Environmental Health Sciences (5 P01 ES00049) and the Rockefeller Foundation. For advice and assistance, we thank J. Engel and A. D. Moscioni of this laboratory.

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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 α-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 α-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 μm thick, increasing to about 10 μm 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 α-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 α-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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3 July 1975

Detection of an Antigen Related to Mason-Pfizer Virus in Malignant Human Breast Tumors

Abstract. *An antigen related to the major structural protein (p27) of Mason-Pfizer monkey virus has been found in malignant human breast tumors by radioimmunoassays. This antigen was not detected in normal placental tissues or in tumors that were not of breast origin.*

The Mason-Pfizer monkey virus (M-PMV) was originally isolated from a mammary carcinoma of a female rhesus monkey (*1*); it has biochemical and biophysical properties characteristic of the known RNA tumor viruses (*2*). Molecular hybridization experiments demonstrated that ^{3}H-labeled DNA complementary to the RNA of M-PMV hybridizes preferentially to the RNA of human malignant breast tumors (*3*).

The presence of antigens related to the major structural protein (p30) of mammalian type C viruses has been detected in tissues of animals (*4*) and man (*5*) by competition radioimmunoassay (RIA). Antigens related to the p30 protein of primate type C viruses were also found in peripheral white blood cells of patients suffering from acute leukemia (*6*). Recently, our laboratory has developed a specific RIA for the major structural protein (p27) of M-PMV (*7*). We used this assay to look for the presence of proteins related to M-PMV p27 in various types of human tissues. We now report the detection of a protein antigenically related to M-PMV p27 in human malignant breast tumors.

Frozen malignant breast tumors (Hackensack Hospital, New Jersey) were selected after gross and microscopic pathological examination. Frozen human malignant tumors that were not of breast origin were obtained through the Office of Program Resources and Logistics, National Cancer Institute. Normal human placentas were obtained from Hackensack Hospital. Tissues were homogenized and extracted with ether to remove the lipids and fats (*4*). The soluble proteins contained in the aqueous phase were further purified by diethylaminoethyl (DEAE)-cellulose ion exchange column chromatography as described for M-PMV p27 (*7*). The fractions of the column where M-PMV p27 would normally be eluted were collected, concentrated, and used as competing antigens in RIA for M-PMV p27 and simian sarcoma virus (SSV-1) p30 proteins (*8*).

Of 18 breast tumor specimens that were tested, 8 induced the release of > 25 percent of the maximum precipitable ^{125}I-labeled M-PMV p27 (Fig. 1). The range of ^{125}I release was from 27 to 78 percent. Figure 1A shows the competition curves obtained with four of the eight positive specimens. The other four positive specimens showed curves similar to those of the four presented. Each of these tumor specimens showed competition curves of similar slope to the competition curve developed with purified M-PMV p27 as the competing antigen, which suggests that the reacting proteins in the tumor tissues are similar to M-PMV p27. From the RIA curves, the amount of p27-like proteins detected in these eight breast tumors ranged from 0.3 to 5.7 ng per milligram of tissue extract. The remaining ten tumors each induced the release of less than 15 percent of the maximum precipitable ^{125}I-labeled M-PMV p27, and were considered negatives. No correlation between the age of the patient and the presence of reactive antigen was noticed.

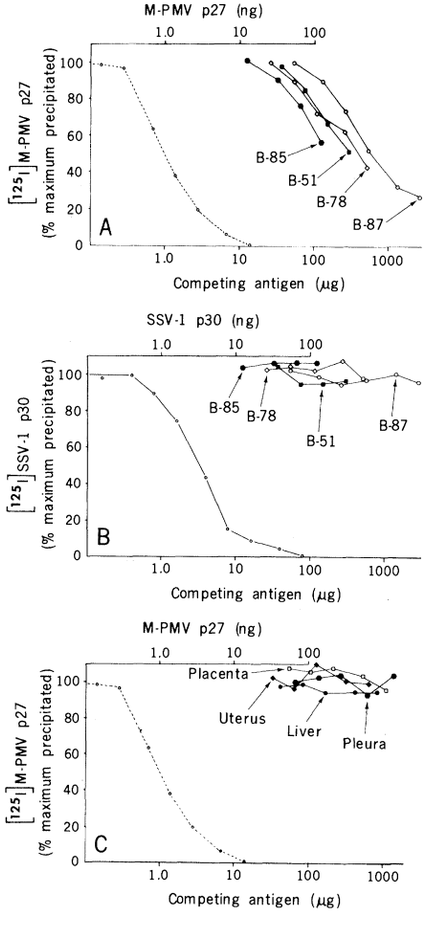

Fig. 1. Radioimmunoassay for M-PMV p27 and SSV p30. The conditions for competition RIA were as follows: A quantity of specific rabbit antiserum sufficient to precipitate 50 percent of the ^{125}I-labeled precipitable antigen was added to varying amounts of competing proteins and incubated for 1 hour at 37°C. Three nanograms of ^{125}I-labeled M-PMV p27 [8.6 × 10^4 count/min, 98 percent precipitable by trichloroacetic acid (TCA) and 85 percent precipitable with specific antiserum] or 5 ng of ^{125}I-labeled SSV p30 (6.2 × 10^4 count/min, 97 percent precipitable by TCA and 90 percent precipitable with specific antiserum) were added and incubated for 1 hour. Appropriate quantities of goat antiserum to rabbit serum was added, incubated for 1 hour at 37°C and 18 hours at 4°C. Precipitates were sedimented, washed with TNE (tris, sodium chloride, EDTA) buffer, and counted. Competition RIA of (A) M-PMV p27; (B) with SSV-1 p30, with four human breast tumors: B-85, B-78, B-51, and B-87; and (C) M-PMV p27, with nonbreast tissues: uterus, pleura, liver, and normal human placenta. Purified M-PMV p27 (o--o) and SSV p30 (o—o) were used as positive controls (top scale).

In order to determine if these M-PMV p27-related proteins found in malignant breast tissues can be found in other human tissues, 12 human tumors not of breast origin and two normal human placentas were extracted in the manner described above and used as competing proteins in RIA's. None of these tissues contained detectable antigen related to M-PMV p27. Four representative samples were plotted (Fig. 1C) for comparison with the results obtained with extracts derived from breast tumor tissues.

The possibility of nonspecific competition by the eight malignant breast tissue extracts was ruled out by allowing these extracts to compete with SSV-1 p30 in an RIA developed for that protein. None of the 18 breast tumors demonstrated competition against SSV-1 p30. The 14 nonbreast tissue extracts tested also showed no competition. Figure 1B illustrates the results obtained in RIA for SSV-1 p30 in assays conducted with four of the breast tumor extracts that were found to be positive for M-PMV p27.

As an additional control, to eliminate the possibility that some proteolytic enzyme from the malignant breast tumor extracts might have caused the release of ^{125}I from ^{125}I-labeled p27 and thus produced a