TITLE: Böcek gelisme düzenleyicilerinin Yesil seftali aphidi`nin morfogenesis`i üzerine etkileri

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Effects of insect growth regulators on morphogenesis of the green peach aphid, Myzus persicae (Sulzer)

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Summary

Relatively high doses of Zoecon insect growth regulators indirectly administered to **Myzus persicae** (Sulzer) resulted in one to three supernumerary larval moults. None of the compounds interfered with embryogenesis at the concentrations used, but abnormal development of the female genitalia, particularly the absence of a gonopore, was observed Parturition could not occur, and daughter larvae sometimes underwent an intramaternal moult.

Supernumerary larvae not only differed from normal larvae and adults in color and body size, but malformations of antennae and hind tibiae were also observed. Alatiform larvae showed reduced wing development and cuticle pigmentation. The cauda underwent predictable morphological changes from the fifth to the seventh larval instar, which allowed identification of specific supernumerary instars. Compounds ZR-512, -515, -619, and -777 evoked qualitatively similar effects, and ZR-512 proved to be particularly effective when administered to young larvae.

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Introduction

Various insect growth regulators (IGR) were reported to adversely affect morphogenesis, fecundity, and survival of different aphid species when applied at the proper phase of larval development (White, 1968; White and Lamb, 1968; Hangartner et al., 1971; Kuhr and Cleere, 1973; Nassar et al., 1973; Elliott and McDonald, 1976; Mittler et al., 1976). These studies indicated that IGR could, in principle, be useful for the control of aphid pests, particularly in synchronously developing populations. Thus, studies were undertaken to evaluate the effects of IGR on the green peach aphid, **Myzus persicae** (Sulzer). This report describes the morphogenetic effects induced by the IGR and the sensitive phases in larval development and supplements the less detailed findings on the same species by Nassar and Staal [cited in Nassar et al., (1973)].

Material and Methods

The aphids were reared in small clip cages (diameter 25 mm) that were mounted to the lower surface of the leaves of the food plant - chinese cabbage (**Brassicae pekinensis**) - at a maximal density of five aphids per cage. They were maintained at 22°C, 60 % RH, and in an 18-hr photoperiod.

The IGR were supplied by Zoecon Corporation (Palo Alto, California) and consisted of ethyl 3,7,11-trimethyl-2-4=dodecadienoate (ZR-512); isopropyl 11-methoxy-3,7,11-trimethyl=2,4-dodecadienoate (ZR-515); ethyl 11-methoxy-3,7,11-trimethyl=2,4-dodecadienethiolate (ZR-619); and prop-2-ynyl 3,7,11=trimethyl-2,4-dodecadienoate (ZR-777).

Leaf discs (22 mm dia.) were punched out of full-grown leaves and dipped into aqueous solutions of the formulated IGR concentrate ranging in concentration from 0.0001 to 0.1 % (w/v). The amount of IGR adhering to the discs after drying was determined to be 0.1 mg per cm^2 of upper and lower leaf surface for the 0.001 % dilution. The discs were mounted upside down on a water surface, and 0- to 24-hr-old larvae were then placed in the center. After 6 days, the larvae were transferred to the clip cages, and the number of surviving and dead larvae was recorded daily. The number of cast larval skins was counted to determine the instar that individuals had reached. Animals that died during a moult were considered to have reached the following instar.

Fifty 0- to 4-hr-old first-instar larvae were caged on treated artificial diet for each experiment at a density of one or 10 larvae per diet. The diet was presented as a droplet of feeding solution enclosed by two parafilm membranes (Dadd, 1967). The surface of the diet had been sprayed uniformly with ZR-512 aerosol for three seconds. The animals were transferred to fresh diet every three days. For microscopic examination of morphogenetic effects, the aphids were placed in 75 % lactic acid for one day, cleared in chloral phenol for an additional day, and then mounted in gum acacia.

Results

Morphogenetic Effects

Supernumerary moults: When larvae were exposed to IGR, many animals underwent a supernumerary larval moult to the fifth larval instar (L_5) instead of developing to an apterous or alate virginopara. Several L_5 larvae moulted to a L_6 or even L_7 larva, but mortality during each supernumerary moult ranged from 50 to 90 percent. These effects were induced by concentrations of ZR-512 and -515 as low as 0.001 %; lower concentrations allowed part of the population to develop into normal females. Generally, L_5 larvae were similar in size to normal females, but L_6 and L_7 larvae were larger. However, as a consequence of large individual variations in size, the different supernumerary instars could not be determined by measurement of body dimensions. Thus counts of the number of cast larval skins were used to indicate the specific supernumerary larval instars.

External abdominal morphology: The abdomen of normal females terminates in a rather long cauda with a broad base and an elongated cylindrical tip. The ventral part of the last abdominal segment bears an anal plate. More anteriorly, there is a large genital plate that covers a gonopore through which larvae pass during parturition from the mother. The genital plates are sclerotized, darkly pigmented structures in alate females and bear a fixed number of setae arranged in a characteristic pattern (Fig. 1a). Supernumerary larvae showed several morphological differences, and the structures mentioned above had a rather juvenile appearance. The cauda was shorter than those in adults and had a conical shape, the anal plate was larger, and the genital plate was nearly absent - marked only by a row of setae (Fig. 1b). In L_6 and L_7 larvae, these structures revealed a more adult appearance, but the genital plate and gonopore were again absent (Figs. 1c, d). This transition from larval to larval -adult intermediate structures was consistent and typical for each supernumerary instar. Thus, it was possible to use these morphological characters to identify specific instars arising from supernumerary moults.

Reproduction: The supernumerary instars did not produce offspring because of the absence of the gonopore. Embryogenesis in treated animals appeared normal. Abdomens that contained as many as 24 fully developed embryos were observed (Fig. 2). In addition, intramaternal moulting of embryos was regularly observed.

Pigmentation: The color of the aphids was different in IGR-treated and untreated individuals. Whereas the abdomens of normal larvae and apterous adults were light green, those of supernumerary larvae were usually dark green. The darker green appearance was caused by the presence of the red-eyed embryos, which made the abdomens less transparent. The green coloration became progressively darker as additional larval moults occurred.

The head, thorax, and appendages of normal alatae are darkly pigmented. Also, there are several pigmented spots and sclerotized plates on the abdomen (Fig. 3). When exposed to leaf discs dipped in 0.001 % of ZR-512, the deposition of black pigment was prevented, and the animals remained pale yellowish-green. Such alatae were otherwise normal and produced normal numbers of offspring.

Development of appendages: In larvae destined to become alatae, the growth of the wing pads normally starts in the third larval instar, proceeds in the fourth instar, and wing expansion is completed in the adult. However, this process could be easily inhibited by IGR, even at concentrations of 0.001 and 0.0001 % for ZR-512 and -777, respectively. Several degrees of retarded wing development were noted in IGR-treated larvae and adultoids, including crumpled wings of a reduced size and fourth-instar wing pads (Figs. 4,5). Supernumerary larvae never showed normal wing expansion, and most had rudimentary wing pads similar in size to those of normal fourth-instar larvae.

The hind leg tibiae of normal animals are straight. The application of IGR often resulted in adults or supernumerary larvae with tibiae that were curved (Figs. 4,5). This phenomenon might have been caused by reduced cuticle hardening whereby the tibiae were unable to support the weight of the freshly moulted individuals. Also, these animals showed reduced locomotory activity. The number of animals that showed curved tibiae was variable, and there appeared to be no correlation with the concentrations of IGR applied. We concluded that this phenomenon most likely represented a secondary IGR effect that resulted from a disturbance of the normal sequence of events involved in moulting.

The length of extremities such as legs, siphunculi, and antennae increases during normal larval development. Measurements were taken to determine whether there was a fixed relationship between the length of the appendages and body size of subsequent larval instars and whether such measurements could be used to identify the specific instar. Apparently the antennae of larvae affected by IGR reached a length that was intermediate to that of L_4 larvae and adults, regardless of the number of supernumerary moults. All six antennal segments were shortened proportionally. Occasionally, the antennae were either crumpled (Fig. 6) or were distal segments lost during ecdysis. The length of the siphunculi in L_5 and L_6 larvae was usually similar to that of normal adults.

The length of hind tibiae increased during subsequent moults although there was considerable variation. Figure 7 shows the range in measurements of 15-30 larvae per series of treatment as compared with control adults. Tibiae of the L_5 larvae were similar to those of adults, but those of L_6 larvae were larger on the average. Moreover, the tibiae of L_5 larvae that were treated with ZR-512 were unusually short, which may have been the result of the repellent action of the IGR, which retarded feeding activity and growth during the early larval instars.

Sensitive Stages During Larval Development

A series of experiments using membrane - lined artificial diet was conducted to determine the variations in susceptibility to IGR at different larval stages. We assumed that the IGR concentration in parafilm remained constant for at least three days (i.e., the maximal time of feeding before replacement of the diet). Since the hormonal effects described in the previous section could be induced by each of the four IGR tested, the sensitive period was established for ZR-512 only.

Groups of larvae were exposed to the IGR for three different periods: (1) during the whole larval period; (2) during the first three days: first- and early second-instars; and (3) during the last three days: late third- and fourth-instars. The results showed that treatment 1 produced the greatest effect with all animals undergoing one or more supernumerary moult (Table 1). Further, the early instars (treatment 2) proved to be much more susceptible than the later instars (treatment 3). According to Hangartner et al. (1971), **Aphis fabae** showed a maximum susceptibility during the third instar, a phase not specifically tested by us. We assume that the strong effects obtained in **M. persicae** after treating first- and second-instar larvae resulted from the persistence of the IGR in the animals until a critical period (possibly the early third larval stage) was reached. There was no fixed relationship between the time of exposure to IGR and the incidence of successive supernumerary moults. In these particular experiments, only one L_7 larva was obtained.

The percentage of larvae that developed into alatae was rather high under these experimental conditions. This is a common phenomenon for aphids reared on artificial diet (Mittler and Kunkel, 1971). Also, the incidence of wing formation in animals kept at a density of 10 per unit of diet was higher than in those that were kept in isolation. The influence of crowding on production of alatae has been well established for **M. persicae** and other aphid species (Wadley, 1923; Bonnemaison, 1951; Johnson and Birks, 1960).

Whereas the treatment of younger larvae resulted in abnormal wing development in all alatae, treatment of the older larvae allowed full wing expansion in 25 percent of the alatae. This might indicate that this morphogenetic effect probably had been induced at about the time the wing pads in the third-instar began actual outgrowth.

Animals with curved hind tibiae were found in all three experimental groups. However, no relationship was found between the occurrence of curved tibiae and supernumerary moults. This indicated that IGR had no direct influence on leg formation.

Discussion

The study of the effects of IGR in aphids is complicated by the fact that the transition from the final larval to the adult instar is not usually marked by major abnormalities in external adult morphology or pigmentation such as is in other hemimetabolous and holometabolous insects (Sláma et al., 1974). Examination of treated larvae of **M. persicae** by low-power microscopy nevertheless revealed that development of the anal plate, genital plate, cauda, antennae, and hind tibiae could be markedly suppressed. These abnormalities did not represent IGR effects induced in the adult but were associated with larval adult intermediates. Developmental abnormalities manifested by adults were incomplete wing expansion and pigmentation of the alatae. Our observations (unpublished) and those of Hangartner et al. (1971) in **Aphis fabae** showed that reproduction in such adults was not impaired.

From the above considerations, to achieve significant pest control by IGR, supernumerary moulting must be induced. We can detect the event of a supernumerary moult most easily by observing the shape of the cauda. However, the most important IGR effect is the suppression of development of the genital plate and the associated gonopore since this kind of effect results in an early death of such creatures due to congestion. Several other aphid species have been shown to react in a comparable way - **Megoura vicia** (Lees, 1966); **Brevicoryne brassicae** (White, 1968); **M. persicae** (cf. Nassar et al., 1973); **A. fabae** (Hangartner et al., 1971); **Acyrthosiphon pisum** and **Amphorophora agathonica** (Kuhr and Cleere, 1973).

The inhibition of wing pad differentiation and wing expansion induced by very low doses of IGR might have special importance for the prevention of aphid migration when habitat conditions favor the induction of alatiform aphids (Tamaki, 1973).

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Özet

Böcek gelişme düzenleyicilerinin Yeşil şeftali aphidi [**Myzus persicae** (Sulzer)]'nin morfogenesis'i üzerine etkileri

Böcek gelişme düzenleyicileri adı da verilen Juvenil Hormon Analogları, **M.persicae'nin** nimflerine dolaylı olarak ve nisbeten yüksek dozlarda uygulandığı zaman, nimflerin 1-3 kez daha fazla gömlek değiştirerek N_5, N_6 ve N_7 dönemlerinin meydana gelmesine neden olmuşlardır.

Kullanılan dozlarda, bileşiklerin hiçbirisi dişide embriyo gelişmesini önlememiş, ancak anormal başkalaşım geçiren nimflerin abdomen ucunda görülen değişiklikler, özellikle gonopor'un yokluğu nedeniyle dişinin nimf bırakmadığı, bazı nimf'erin dişi vücudu içinde gömlek değiştirdikleri saptanmıştır.

Anormal başkalaşım geçirerek ekstra nimf döneminde olan bireyler, normal nimf ve erginlerden yalnızca vücut büyüklüğü ve renk bakımından farklı olmayıp, anten ve arka bacak tibia'larında da bozulmalar olmuştur. Uygulanan juvenil hormon analoglarının etkisiyle anormal başkalaşım geçiren kanatlı bireylerin hepsinde kanatlar küçük ve buruşuk veya kanat tomurcuğu şeklinde olmuş ve vücut renklenmesi azalmıştır.

Uygulanan bileşikler nedeniyle N_5, N_6 ve N_7 dönemlerindeki nimflerin cauda şeklinde görülen morfolojik farklılıklar her dönem için karakteristik olup Şekil 1'de gösterilmiştir.

Bu çalışmada kullanılmış olan bileşikler, ZR-512, -515, -619 ve -777, birbirlerine benzer etkiler göstermişlerse de ZR-512 özellikle genç nimflere uygulandığı zaman, yukarıda sözü edilen özellikler yönünden daha etkili olmuştur.

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Table — 1

Morphogenetic effects induced by rearing larvae for different periods on diet treated with ZR-512 (aerosol)

Treatment			Incidence of supernumerary moults				Number of alatae with		Ap cur
instars exposed	period of exposure (days)	n	% of total	number in L_5	L_6	L_7	normal wings	abnormal wings	
$L_{1,2,3,4}$a/	0–9	27	100	24	3	0	0	23	
$L_{1/2}$b/	0–3	40	85	19	15	0	0	13	
$L_{3/4}$a/	6–9	63	36	5	17	1	9	36	

a/ 10 animals per unit of diet.

b/ 1 animal per unit of diet.

Captions of Figures 1-7

Fig. 1. Drawings showing terminal segments of ventral abdomen of adult alate aphid (a) and supernumerary fifth-instar (b), sixth-instar (c), and seventh-instar larvae (d). Note rows of setae in b-d marking the place where genital plate normally would develop. A, anal plate; C, cauda; G, genital plate; go. gonopore, covered by G.

Fig. 2. Sixth-instar larva with moulted embryo; legs are clearly visible (arrow). (X 25).

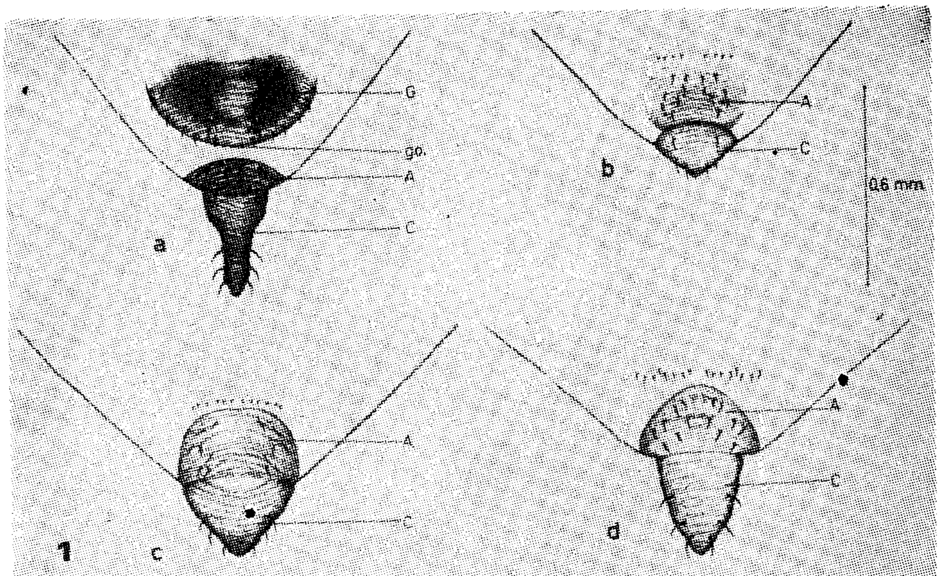
Fig. 3. Alate female showing darkly pigmented head, thorax, abdominal cuticle patches, and extremities. Embryo (asterisk) has been forcet out during preparation. (X 50).

Fig. 4. Alate female without pigmentation and with crumpled wings of roughly normal size and one curved tibia. (X 25).

Fig. 5. Fifth-instar larva with poorly developed wings and curved hind leg tibiae (X 25).

Fig. 6. Crumpled antenna of fifth-instar larva with abnormally broadened segment joints. (X 200).

Fig. 7. Lengths of hind leg tibiae of normal adults and apterous fifth- (L_5) and sixth-instar larvae (L_6), treated with four IGR. Bars indicate ranges of measurements (in mm).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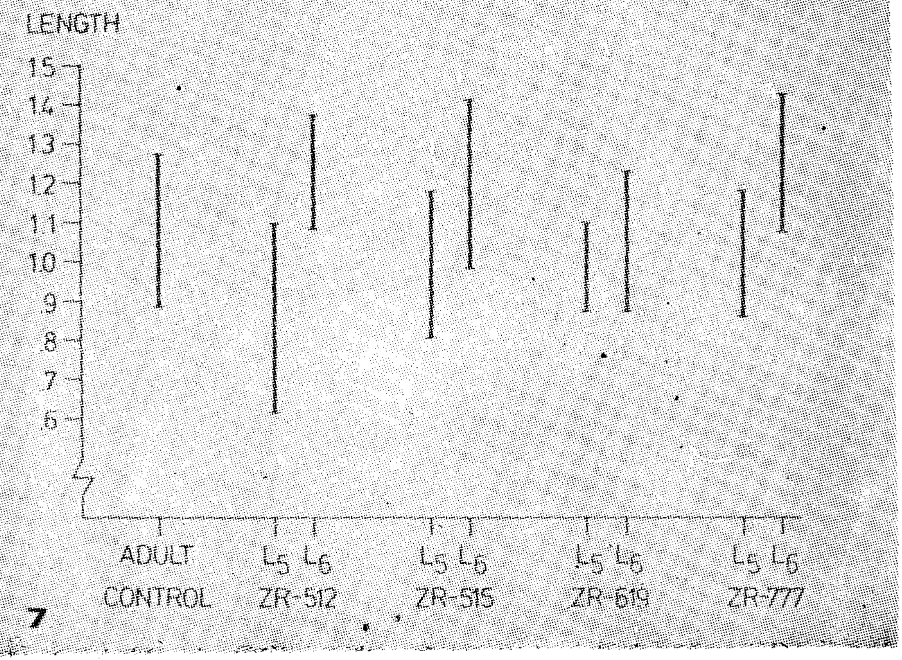
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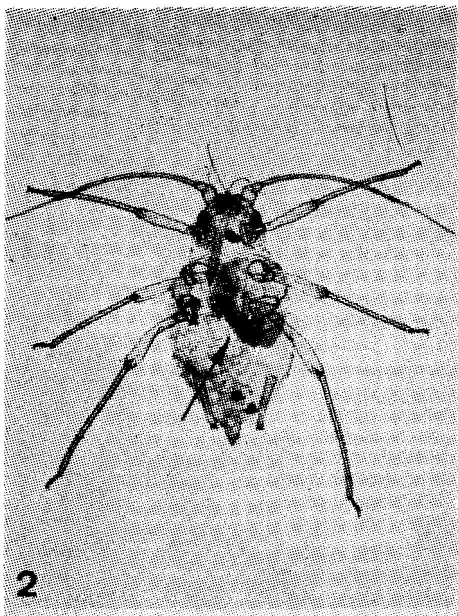
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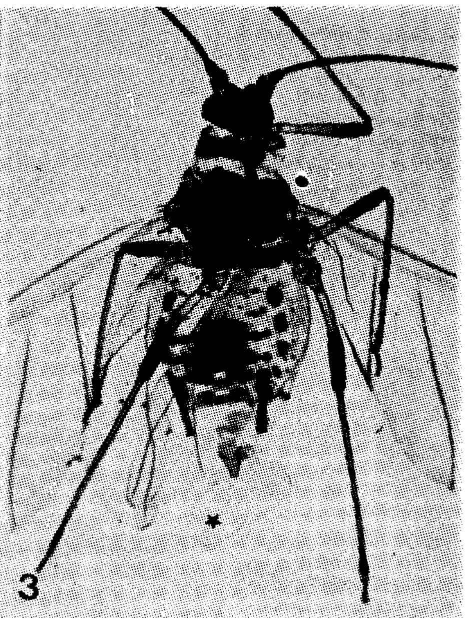
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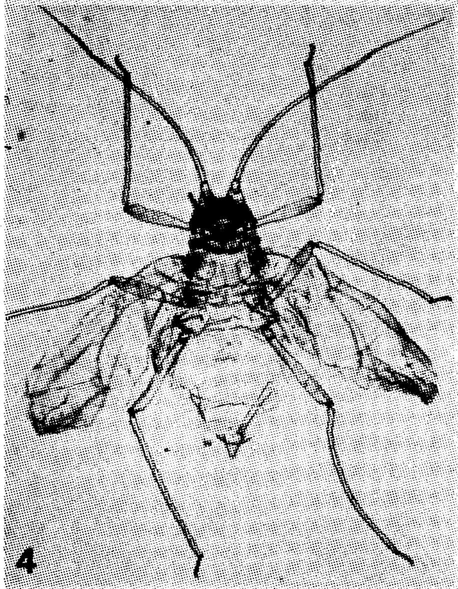
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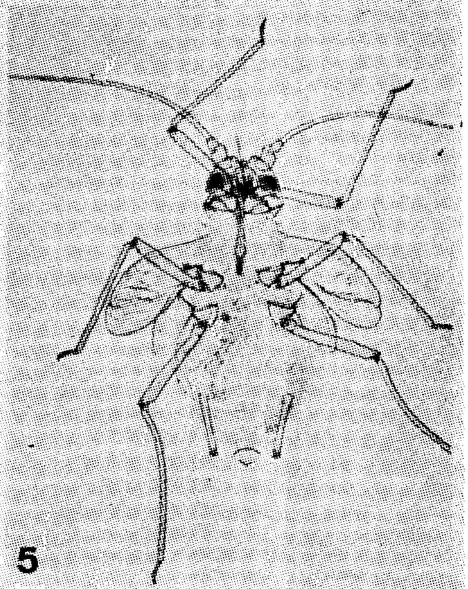
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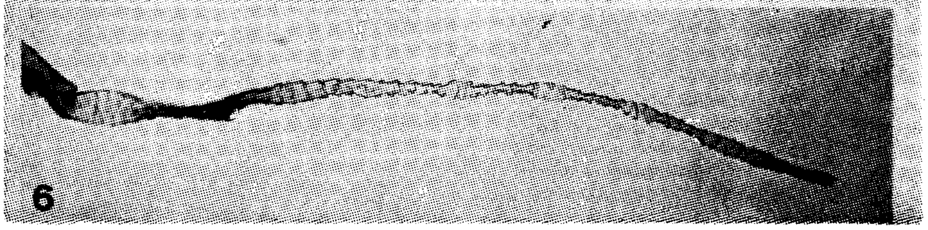
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