Ph. D. Entomology

Edmond Yehuda Lipsitz

ANALYSIS OF LIPID DURING THE LIFE CYCLE OF THE HOUSE CRICKET, ACHETA DOMESTICUS (L.)

ABSTRACT

Changes in lipid content during the life cycle of the house cricket have been investigated. On a wet weight basis, total and neutral lipids decreased in the egg stage, increased considerably, though not in a steady manner, in the larval stage, and decreased gradually, while exhibiting a sexual dimorphism, in the adult stage. Phospholipid remained relatively constant during the life cycle. The pattern of change of triglyceride closely followed that of the neutral and total lipids. Some neutral lipid components remained constant while others showed variations with age. Phosphatidylcholine exhibited a pattern similar to triglyceride; other phospholipid components had different patterns of change. Differences were observed in the fatty acid composition of five neutral lipid fractions at two distinct larval ages, the most striking of which was found in the monoglyceride fraction. Haemolymph lipid from 28-day adult males and females exhibited a sexual dimorphism.

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ANALYSE DES LIPIDES DURANT LE CYCLE VITAL DU GRILLON DOMESTIQUE, ACHETA DOMESTICUS (L.)

RESUME

Les variations du contenu en lipides durant le cycle vital du grillon domestique ont été étudiées. Sur une base de poids frais, les lipides totaux et les lipides neutres diminuent au stade de l'oeuf; augmentent considérablement, mais non d'une façon constante, au stage larvaire; puis diminuent graduellement, tout en offrant dimorphisme sexuel, au stage adulte. Les phospholipides demeurent relativement constants durant le cycle vital. Le mode de variations des triglycérides suit de près celui des lipides neutres et lipides totaux. Certains lipides neutres demeurent constants tandis que d'autres varient avec l'âge. La phosphatidylcholine varie d'une façon similaire à celle des triglycérides. Par contre d'autres phospholipides varient de façons différentes. De toutes les différences qui furent observées dans la composition des acides gras de cinq fractions de lipides neutres entre deux âges larvaires distincts, la plus frappante est celle dans la fraction contenant les monoglycérides. Les lipides de l'hémolymphe des mâles et des femelles adultes âgés de 28 jours montrent un dimorphisme sexuel.

ANALYSIS OF LIPID DURING THE LIFE CYCLE OF THE HOUSE CRICKET, ACHETA DOMESTICUS (L.)

by

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CLAIM OF CONTRIBUTION TO KNOWLEDGE

1. A detailed analysis of the changes in lipid content of the house cricket, Acheta domesticus (L.), during the life cycle of the insect was carried out on two egg, eleven larval, eight female and nine male age groups. The insects were reared under controlled conditions, a precaution not always observed in other studies of cricket lipid, which makes this study of greater use to others. It is also the most complete survey of the lipids of any insect species over the whole life cycle.

2. Total lipid of all the above age groups was fractionated into neutral and phospholipids. Neutral lipid was further separated into seven components by means of column and thin-layer chromatography, and the amounts of the various components were determined. Phospholipid components were obtained by two-dimensional thin-layer chromatography from two egg, five larval and four each of adult male and female ages, and each component was determined quantitatively.

3. A detailed fatty acid analysis was carried out on two distinct ages during larval development by means of gas-liquid chromatography. This included analysis of the total, neutral and phospholipid fatty acids of the two ages, and of the total lipid of the insect food. It also included fatty acid analysis of

five neutral lipid components of the two ages.

4. A new method was developed for rapid collection of insect haemolymph. This involved low speed centrifugation of crickets placed in compartmentalized plexiglass cylinders.

5. Total lipid extracted from 28-day-old adult male and female haemolymph was separated into neutral and phospholipid by means of silicic acid column chromatography. The neutral lipid fraction was separated into its components by column chromatography and the phospholipid fraction by two-dimensional thin-layer chromatography. Qualitative and quantitative analyses were carried out on the lipid components. The fatty acid composition of the haemolymph total lipid was determined.

6. Curves for changes in weight were obtained for the adult male and female stages.

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I. INTRODUCTION

The role that lipid plays in biological systems is only partially understood, and work with lipid has lagged behind that of carbohydrate and protein because of the lack, until recently, of suitable techniques for comprehensive analysis. There has been, however, a growing interest, in recent years, in all aspects of the biochemistry of lipid in insects. This greater interest stems from the realization that lipid plays an important role in metamorphosis, flight, water retention and insecticide resistance, and that it is related in some way to the ability of various species to withstand extremes of temperature. Although the characterization of lipid has been reported for about 170 species of insects, the existing knowledge of lipid composition in insects is insufficient and highly fragmentary and cannot be extrapolated to all insects. Fast, who in 1964 reviewed the literature on lipid in insects, wrote: "It is apparent from this review that really very little is known about the composition of insect lipid and that a great deal more analytical work will be required."

A strong impetus to the investigation reported in this thesis was given by McFarlane and Henneberry (1965), who suggested that fatty acids play a role in the control of growth and development, that is, a physiological role. They showed that certain fatty acids and their methyl esters inhibited the growth of the cricket, Gryllodes sigillatus (Walk.), when these substances were presented in such a way that they

entered the body through the cuticle. Subsequently, McFarlane (1966a, b) showed that certain methyl esters stimulated growth of the house cricket, Acheta domesticus (L.). These results are of great interest, although an interpretation of them in terms of mechanism awaits further work. Such an interpretation would seem to require a more or less precise knowledge of the changes in lipid in growth and development. The purpose of the work presented in this thesis was to initiate studies directed towards this end.

The insect selected for this study, the common house cricket, Acheta domesticus (L.) (Gryllidae, Orthoptera), is a cosmopolitan and easily available insect. Because of the relative ease of maintaining laboratory colonies, it is most suitable for both biologically and chemically oriented studies.

The reported study was designed to examine and analyse lipid changes during the entire life cycle of the insect and to relate possible lipid changes to the developmental processes occurring within the organism. Lipid determinations were made both quantitatively and qualitatively by means of the modern techniques of column, thin-layer and gas-liquid chromatography. The studies have been carried out on eggs, developing larvae, and adult males and females reared under controlled conditions. A detailed study of the lipid composition of two distinct stages in larval development was included. Some comparisons of the lipid content of the haemolymph of adult male and female crickets were made.

II. REVIEW OF LITERATURE

Reports on lipid in insects indicate that there is considerable variation in lipid composition. Most of this variation is due to the many different types of analytical methods that have been used for extraction and quantitative analysis. Many reports do not state the age, developmental state or the diet of the insects that were used, which renders comparisons and generalization difficult. It should be emphasized, however, that reported differences in the composition of insect lipid are frequently due to variations among the different insect taxa. This is not difficult to understand when one considers the large diversity of insects, their modes of living and varying habitats.

Many oils and fats had been fairly well characterized by the end of the nineteenth century, but insect lipid remained virtually unstudied. Physiological studies relating to insect lipid were seriously begun in the nineteen-twenties. In 1926, Rudolfs showed that in the tent caterpillar, the amount of fat decreased during incubation of the eggs and increased considerably during the larval stage. Along with the increase of fat, there was a decrease in water content, especially in the later stages of larval life. In 1937 two workers showed independently that the constituent lipid of two different insects were strongly influenced by the lipid in their diets. Yuill and Craig (1937) demonstrated that the degree of unsaturation of fatty acids from the flesh fly Lucilia sericata (Meig.) (Diptera) closely resembled the degree of

unsaturation of its dietary fatty acids: high when reared on fish heads and low in response to a synthetic diet. In the same year Melampy and Maynard (1937) showed that the lipid of the cockroach Blattella germanica (Linn.) (Orthoptera) also mimicked the degree of unsaturation of its dietary lipid. Following these pioneering studies, many more detailed analyses have allowed insect lipid to be placed in a general framework; even so, the details of insect lipid metabolism are, as yet, not fully understood.

Fast (1964) made a rather extensive survey of the literature on lipid content of insects and found the three-fourths of the species studied contained less than 10% lipid based on their wet weight. On a dry weight basis the data indicated about 20% lipid for adults and 30% lipid for larvae. In two-thirds of the insects Fast examined, 60% to 80% of the fatty acids were unsaturated; in this respect the fatty acids present in insect lipid seem to follow the same pattern as those found in other animals.

A. Total lipid

Yuill and Craig (1937) reported that the total lipid varied from 60% of the dry weight in young larvae to 30% in full grown larvae of Lucilia sericata. Blackith and Howden (1961) showed that adult locusts (Orthoptera) exhibit interspecific variation as to lipid content. Locusta migratoria (R. & F.) ranged from 7.5% to 10.7% lipid; Schistocerca gregaria (Forskål) ranged from 5.3% to 9.3% lipid; and Nomadacris septemfasciata (Audinet-Serville) contained about 13% lipid.

Clark and Chadbourne (1962), working with the pink bollworm Pectinophora gossypiella (Sand.) (Lepidoptera), found that the lipid content tended to decrease in diapausing larvae while in the feeding larvae lipid content tended to increase. The fat content of the alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera), was at a low level (6% to 10% of the body weight) during the winter months when egg deposition was occurring. It rose to about 30% at the beginning of aestivation in May and fell to an intermediate level, 10% to 16%, by early fall (Bennett and Thomas, 1964). Strong (1964) stated that the fresh weight of eggs of Euceraphis punctipennis (Zett.) (Homoptera) contained 13.9% lipid. The total extractable lipid decreased from 39.5% to 23.2% of the dry weight of the ootheca during embryogenesis of Periplaneta americana (L.) (Orthoptera): of the major lipid classes, only the triglyceride fraction decreased, and this by 75% (Kinsella and Smyth, 1966).

Gilbert and Schneiderman (1961), while studying the juvenile hormone at representative stages during the life cycle of Hyalophora cecropia (L.) (Lepidoptera), noted a sexual dimorphism in lipid content in the adult stage. By preparing lipid extracts at various stages in the life cycle they observed that lipid content per individual larva increased with age and size, but the rate of increase varied in the various instars. The newly hatched larva contained 4.53% lipid, but this decreased to a more or less constant value in the fourth and fifth instars (1.3% - 1.9% fresh weight), although the lipid content increased as the larva grew. At pupation the lipid content decreased and lipids constituted

5% and 7% of the wet weight of the insect. At this time a sexual dimorphism in lipid content was evident; the male pupa contained 40% more lipid than the female per gram wet weight. During the pupal-adult transformation this dimorphism increased markedly until in the adult moth the tissues of males contained about 5 times as much lipid per gram fresh weight as the tissues of the female. Further results extended this dimorphism to a total of seven families of Lepidoptera

Niemierko et al. (1956), examining the lipid content of Bombyx mori L. (Lepidoptera), found that the females used 50% of their stored lipid between spinning and adult emergence while the males utilized only 30%. The lipid content of the newly emerged adult male was 48% dry weight while that of the female was only 25% (17.5% in males and 6% in females on a fresh weight basis). In Antheraea pernyi G. (Lepidoptera), Demyanovsky and Zubova (1957) found sexual dimorphism in lipid content evident as early as the fifth instar larva and it continued until adult emergence. In the 5th stage there were sex differences in the level of fat storage, the male caterpillar being richer in fat than the female.

B. Glycerides and free fatty acid

It is generally accepted that triglycerides form the major part of insect lipid, indicating their importance as the major storage form of lipid (Gilmour, 1965). The lipid of various aphids contained 80% triglyceride and less than 3% unesterified fatty acid

(Strong, 1963a). In the greater wax moth larva, Galleria mellonella (L.) (Lepidoptera), triglyceride made up 85% of the total lipid (Young, 1964); this held true despite major changes in the lipid content of the diet. In a study of the lipid of adult Anthonomus grandis Boheman (Coleoptera), Lambremont et al. (1964) showed that newly emerged adult boll weevils had 2% - 6% body lipid of which only 2% was triglyceride. After 2-3 weeks of feeding, non-diapausing adults contained 6% to 10% lipid (40% to 60% of which was triglyceride), whereas diapausing adults had 18% to 25% body lipid containing 75% to 85% triglyceride. In Hyalophora cecropia, triglyceride constituted about 90% of the pupal and adult fat bodies (Chino and Gilbert, 1965a).

Van Handel (1965) reported that when starved adult mosquitoes were fed sucrose or protein, triglyceride began to accumulate before the storage of glycogen reached a maximum. In seven-day-old mosquitoes triglyceride may be fifty times higher in the female than in the male (Van Handel and Lum, 1961). Investigating changes in the amount, composition, and distribution of neutral lipid and phospholipid during the metamorphosis of the blowfly of the genus Lucilia, D'Costa and Brit (1966) found that neutral lipid decreased immediately after pupation, then rose to a value maintained for a period in mid-pupal life, followed by a steady decline during adult development. Most of these variations were found to occur in the triglyceride, the major fraction. Smaller amounts of free fatty acid and diglyceride were found to be present, but the levels remained constant throughout the life cycle. In Formica polyctena

Förster (Hymenoptera) the total lipid decreased during metamorphosis with a concomitant increase in percentage of free fatty acid (Schmidt, 1966).

Fawzi, Osman and Schmidt (1961) reported that triglyceride accounted for the bulk of neutral fat in Locusta migratoria. The absolute content of the female is higher than that of the male, but the reverse is true when this is considered as percentage of the body weight since the female is twice as heavy as the male. Allais et al. (1964), studying the nature and evolution of different lipids in the eggs of Locusta migratoria, found that the lipid, which at laying comprises 26% of the egg dry weight, showed a decrease of 31.2% to form only 20.7% at the end of the embryonic development. The study proved that this fall was due only to the catabolism of triglyceride; the mono- and diglycerides showed no variation.

Kinsella (1966a) found that triglyceride accounted for most of the lipid of the ova, nymph and adult of Oncopeltus fasciatus (Dallas) (Homoptera). The ova were found to contain a higher percentage of triglyceride than any other stage of the life cycle. Monoglyceride and diglyceride and free fatty acid varied during the life cycle, indicating a dynamic state of these metabolites. In Periplaneta americana triglyceride was found to decrease by 75% during embryogenesis, but mono- and di-glycerides remained constant until the time of nymphal emergence when they increased sharply, indicating intense triglyceride hydrolysis (Kinsella and Smyth, 1966).

The eggs of Euceraphis punctipennis contained 85.9% triglyceride (Strong, 1964). Bumgarner and Lambremont (1966) reported that triglyceride accounted for more than 70% of the neutral lipid of boll weevil eggs. Free fatty acid occupied a secondary position (6.2%) while mono- and diglycerides constituted less than 3% of the total lipid. Svoboda, Pepper and Baker (1966) showed that the eggs of the grasshopper, Aulocara elliotti (Thomas) (Orthoptera), contained some 60% triglyceride with only trace amounts of free fatty acid and monoglyceride. No change in the total lipid was detected during diapause, but triglyceride declined during postembryonic development.

Several reports in the literature show that free fatty acid was usually the second largest fraction of neutral lipid with smaller amounts of monoglyceride and diglyceride. However, Giral, Giral and Giral (1946) found that 74% of the neutral fat of Melanoplus atlanis Riley [= M. sanguinipes (Fabr.)] (Orthoptera) was free fatty acid, and Giral (1946) reported that most of the lipid of Sphenarium purpurascens Charpentier (Orthoptera) was also free fatty acid. It should be noted that these two papers have been disputed on the basis of the extraction method utilized (Kilby, 1963).

C. Phospholipids

While glycerides are an important energy reserve, phospholipids have been well established as structural components of cellular membranes in many organisms. Most insect orders which have

been analyzed for phospholipid exhibit a predominance of phosphatidylcholine, similar to the vertebrates. Most dipterous insects, however, seemed to have a preponderance of phosphatidylethanolamine.

The total lipid of Bombyx mori was found to contain 18.1% phospholipid which on analysis proved to be 62.5% phosphatidylcholine, 16.1% sphingosine, 8.1% phosphatidylserine and 3.7% phosphatidylethanolamine (Sridhara and Bhat, 1962). In a study of the boll weevil lipid in relation to diet and diapause, Lambremont (1964) found 9% of the total lipid as phospholipid in diapausing weevils and 20% as phospholipid in non-diapausing weevils. In adult Tenebrio molitor L. (Coleoptera), Kamienski et al. (1965) found that of the total phospholipid 43% was phosphatidylcholine and 41% was phosphatidylethanolamine. They also detected small amounts of phosphatidylinositol and trace amounts of sphingolipid. No phosphatidylserine or lysolecithin was detected.

Phospholipid was found to account for 16% of the total lipid in adults of Drosophila melanogaster Meig. (Diptera) (Keith, 1966). D'Costa and Brit (1966) have shown that in Lucilia, lipid phosphorus increased to a maximum during larval-pupal transformation, but the exact time at which this maximum was reached varied with different batches of flies. An analysis of this phospholipid revealed 55% phosphatidylethanolamine and 26% phosphatidylcholine. These two fractions always made up the majority of the phospholipid,

and no sphingomyelin was detected. Bieber et al. (1961) reported 60% phosphatidylethanolamine and less than 25% phosphatidylcholine in Phormia regina (Meig.) (Diptera). In Musca domestica L. (Diptera), 65% phosphatidylethanolamine, 17% phosphatidylcholine, 3.5% phosphatidylserine, 3% phosphatidlylinositol and polyglycerophosphatides were found in the phospholipid; no sphingosine was detected (Crone and Bridges, 1963). Crone and Bridges also reported that Periplaneta americana and Blatella germanica (Orthoptera) contained 44% and 53% phosphatidylcholine respectively. The phospholipid of Oncopeltus fasciatus was found to consist primarily of lecithin and cephalin (mainly phosphatidyl-ethanolamine), with lecithin as the major fraction (Kinsella, 1966a).

Fast (1966) examined the phospholipid of 27 species of insects representing 6 orders and 20 families to determine the choline/ethanolamine ratios. He found that ethanolamine accounted for approximately 50% of the total phospholipid in aphids (Homoptera) and in all but one family (Cecidomyiidae) of Diptera while choline accounted for only about 25%. Ethanolamine and choline were present in approximately equal proportions in one family of Diptera and in the Coleoptera examined. In the other insects examined phosphatidylcholine predominated, phosphatidylethanolamine comprising only 25% to 30% of total lipid phosphorus as in most mammalian tissues. Analysis of the lipid of the cricket Gryllus bimaculatus (DeGeer) (Orthoptera) by Fast (1967) showed that phosphatidylcholine formed most (54.2%) of the phospholipid.

Phosphatidylethanolamine comprised 35.4% of the total. The unidentified acid phospholipid (probably phosphatidic acid or cardiolipin) and sphingomyelin together made up about 10% of the total.

There are at least two reported cases where no nitrogen was found in the phospholipid fraction. Locusta migratoria and Phalera bucephala L. (Lepidoptera) are both reported to have no nitrogen in their phospholipid (Fawzi et al., 1961; Schmidt and Osman, 1962). This finding rules out the well known bases, ethanolamine and choline, as phospholipid constituents in these two organisms.

In addition to their role in the post-embryonic development of insects, phospholipid plays an important role in insect embryogenesis. The egg lipid of Locusta migratoria was found to be about 20% phospholipid which actually constituted about 5% of the total dry weight of the egg (Allais et al., 1964). Phosphatidylethanolamine predominated but phosphatidylserine, phosphatidylcholine and sphingomyelin-like lipid were also identified. There was little variation in phospholipid during embryogenesis although blastokinesis occurred simultaneously with an 11% decrease in phosphatidylcholine. Allais and his colleagues also demonstrated synthesis of phospholipid at the termination of blastokinesis. This phenomenon of phospholipid synthesis during embryonic development has also been reported for Bombyx mori (Niemierko et al., 1956). Total lipid amounted to 12% of the fresh mass of the diapause eggs, and 5.5% of newly hatched larvae. This meant that a considerable amount of lipid was consumed during embryonic development. However,

the content of phospholipid in eggs amounted to 3%, and in newly hatched larvae 5.5% of the dry substance. Thus a synthesis of phospholipid took place in the course of development of the embryo.

Pearincott (1960) showed a 30% increase in the lecithin fraction during embryonic development of the housefly; Bieber et al. (1961) found a threefold increase in both the phosphatidylcholine and phosphatidylethanolamine fractions of the developing eggs of Phormia regina. Kinsella (1966b) reported that phospholipid increased fourfold during embryonic development (from 6% to 24% of the total extractable lipid) of Periplaneta americana. The phospholipid was composed of three major fractions, namely, lecithin 60%, cephalin 24% and sphingomyelin 6%, while the minor constituents identified were lysolecithin, phosphatidylinositol, phosphatidic acid and cerebrosides.

The function and metabolism of phospholipid may be better understood if their distribution in the subcellular fractions is known, since phospholipids occur primarily as components of biological membranes. Several workers have studied the phospholipid of subcellular particles in Diptera.

Crone (1964) studied the sarcosomes of Musca domestica flight muscles and reported 69% phosphatidylethanolamine, 9% phosphatidylcholine, 6% polyglycerophosphatides and 3% unidentified, alkali-stable phospholipid. The phospholipid profiles of mitochondria, microsomes, soluble fraction and residue from Phormia regina do not differ appreciably from the phospholipid profiles of the whole animal (Taylor and Hodgson, 1964). Khan

and Hodgson (1967) studied the phospholipid of subcellular fractions from the housefly Musca domestica. Their analyses showed that there are variations in the distribution of some phospholipid among these fractions. The most unusual was the microsomal fraction which contained only 36% phosphatidylethanolamine whereas the other fractions contained 50% to 59% of this phosphatide. The microsomal fraction also differed from other fractions in its higher content of phosphatidylcholine, lysophosphatidylethanolamine, and sphingolipid. Mitochondria contained 5% cardiolipin, whereas the microsome and residue fraction contained 4% and 5% respectively. The supernatant fraction contained only 7% of the total phospholipid, most of this being in the form of phosphatidylethanolamine.

Chaudhary and Lemonde (1963) have made a study of phospholipid changes during metamorphosis in Tribolium confusum Duval (Coleoptera). They reported that phospholipid changed as a linear function of larval fresh weight. At the prepupal stage, a slight loss of phospholipid occurred which was attributed to excretion. They also found evidence of synthesis of phospholipid in the newly emerged adult. Taylor and Hodgson (1964) have studied the synthesis of phosphatidylethanolamine in Phormia regina. Their findings indicated that the metabolic origin of phosphatidylethanolamine in the blowfly was similar to that in mammals, involving the formation of serine from glycine and the decarboxylation of serine to yield ethanolamine. The beta carbon of serine, as in mammals, originates from formate.

D. Sterols

One of the basic tenets in insect biochemistry is the inability of insects to convert simple precursors to sterol. This was first demonstrated by Hobson (1935), who showed that the fleshfly, Lucilia sericata, required an exogenous source of sterol for normal growth.

In a review of the literature dealing with the utilization of sterols by insects, Clayton (1964) found that a sterol is a dietary essential for at least 22 insects and concluded that sterols play a structural role that is similar to that of cholesterol in mammalian tissue. No insect has been shown not to need a dietary sterol but there is no rule as to when a sterol deficiency will manifest itself; this varies in different insects. However, it is interesting to note that Clayton (1962) found that ingestion of sodium-1-C^{14}-acetate by the silverfish (Ctenolepisma sp.) led to the appearance of labelled cholesterol in the tissues of the insect. He concluded that it was therefore evident that the total absence of sterol biosynthesis in insects in general is far from rigidly established. Clayton suggested that the most primitive insect may retain the capacity to synthesize sterols, in contrast to the higher forms in which this capacity has been lost in the course of evolution.

Gilbert (1967) lists four main categories of experiments dealing with the sterol requirement in the diet of insects. First, a survey of sterols and sterol precursors that can replace

cholesterol, the main insect sterol. Second, investigations aimed at proving that insect can or cannot synthesize sterol from simple precursors. Third, experiments showing how insects can modify the dietary sterols. Finally, investigations which attempt to define a physiological role for these sterols.

Strong (1964) reported that sterol ester made up 4.8%, and free sterol together with hydrocarbon accounted for 7.8%, of the neutral lipid in an aphid, Euceraphis. Sterol was found to make up 15% of the total lipid of wax moth larval hemolymph (Wlodawer and Wisniewoska, 1965).

In a study of the sterol and sterol ester of female houseflies and eggs, it was found that eggs of the fly have 41% of their sterol esterified and that this figure drops to 8.4% in adult females (Dutky et al., 1963). In the eggs of Hyalophora cecropia about 36% of the cholesterol is esterified (Goodfellow and Gilbert, 1963). Drosophila melanogaster was reported to have about 35% of its sterol esterified, and this percentage seemed to be independent of diet and the absolute amount of sterol either free or esterified (Keith, 1966).

A considerable amount of work has been done on sterol metabolism in Musca. Silverman and Levinson (1954) reported that steroids appear to act as both growth and pupation factors in Musca vicina (Macq.). Levinson and Bergman (1957) found that M. domestica required cholesterol for normal growth, and while some molecular modifications inhibited its use, others did not. Pearincott (1960) reported that cholesterol levels in all tissues

remained relatively constant during metamorphosis of the housefly, and that it was probably a tissue constituent. Monroe et al. (1967) have found that there was an accelerated accumulation during larval development in M. domestica, and that there was a quantitative carry-over through pupal and adult stages. This confirmed a sterol storage mechanism.

E. Fatty acids

Prior to the late 1950's the reports in the literature on insect fatty acid composition were meager, but have become more numerous since the classic efforts of Martin and Synge (1941) who first suggested the concept of gas chromatography in which gas is the transient phase rather than a liquid (as in paper or column chromatography).

The most complete survey by one individual of the fatty acid constitution of insects is that of Barlow (1964), who, by gas chromatography, analysed 30 species of insects. Due to lack of material only one analysis was conducted on most of the species. Barlow's findings indicate that the fatty acid composition is characteristic of the species. For the family Aphididae (Homoptera) more than 80% of the fatty acid was myristic acid, whereas in most species outside of this group, this fatty acid constituted only about 15% of the total. All the Diptera had a high proportion of palmitoleic acid (19% to 60%) while most other species yielded no more than 2% to 6% of this acid. Barlow's work suggested that the stage of the insect had little effect on the

fatty acid composition.

Herodek and Farkas (1960), examining the unsaturated fatty acids during the embryonic and post-embryonic development of Bombyx mori, found that the percentage of total unsaturated fatty acids declined only slightly from the embryo to last instar larva but fell from 80% to 70% in the pre-pupa. During adult development, the percentage of unsaturated fatty acids increased in the female where they may play a role in egg development, but fell in the male. The percentage of oleic, linoleic and linolenic acids remained rather constant during embryogenesis. Once the first-instar larva hatched, however, linoleic acid increased from about 10% of the total to more than 50% by the third instar, presumably at the expense of oleic and linolenic which decreased during this period. After the third instar, the concentration of linoleic acid decreased to reach a level of about 15% in the prepupa after which the concentration remained constant. With the decrease in percentage of linoleic acid, increases occurred in both linolenic and oleic acids, indicating that there was an interconversion of linoleic to and from oleic and linolenic by hydrogenation and dehydrogenation, or a utilization of linoleic acid during the larval pupal molt.

Giral (1946) reported a high proportion of oleic acid in Sphenarium purpurascens with 11.4% stearic acid and only 9.6% palmitoleic acid. Giral, Giral and Giral (1946) found a preponderance of stearic acid over palmitic acid and a high proportion of

arachidonic acid in the unsaturated fraction of Melanoplus. Saha et al. (1966) have studied the fatty acids of grasshoppers, but did not find the predominance of stearic acid as reported by Giral, Giral and Giral (1946). Instead, they found that palmitic and oleic acids predominated in all five species studied.

Svoboda et al. (1966) found that although linoleic acid was the major fatty acid in the eggs of the grasshopper, Aulocara elliotti, lauric, myristic, palmitic, stearic, oleic, and linoleic acids were also detected. Kinsella (1966c) reported that 30 fatty acids could be detected during the life cycle of Periplaneta americana, but that palmitic, stearic, oleic, and linoleic acids together accounted for 95% of the total. Fawzi, Osman and Schmidt (1961) have evidence that palmitic and oleic are the most abundant fatty acids in Locusta migratoria. Myristic, stearic, linoleic, linolenic and arachidonic acids were also detected.

Several workers have studied the effect of diet on the fatty acid composition of insects. Tombes (1966) has reported that the alfalfa weevil, Hypera postica, has a fatty acid composition which reflects the fatty acids of its diet. Lambremont (1964) has shown that the fatty acids of the boll weevil are diet-dependent. Bumgarner and Lambremont (1966) found that the boll weevil, on a low fat diet, had 26.8% palmitic, 13.1% palmitoleic, 49.4% oleic and 3.4% linoleic acids. On a high fat diet the boll weevil was found to have 26.8% palmitic, 2.4% palmitoleic, 18.7% oleic and 42.1% linoleic acids. Hutchins and Martin (1968) reported that the fatty acids of Acheta domesticus (L.) (Orthoptera) did not

change significantly from the third through the eleventh week of the crickets' postembryonic life. The major fatty acids were linoleic, oleic, palmitic and stearic acids. The fatty acid composition of the cricket lipid reflected the fatty acids of the dietary lipid. However, Strong (1963a) has shown that the fatty acids of 21 species of aphids were species specific and did not reflect the composition of the diet. All 21 species of aphids had a high myristic acid content and a low stearic acid content.

Kato (1965) reported that genetic strains of Drosophila melanogaster could be grouped according to variations in palmitoleic, oleic, linoleic, and linolenic acids. Kato (1965) also found that the fruit fly adults contained 38.6% saturated fatty acids, composed of butyric, caproic, caprylic, lauric, myristic, palmitic and stearic acids. Keith (1966), analysing the same insect, reported 19.2% palmitic, 25.3% oleic, 18.5% palmitoleic, 12.5% linoleic, and only 4.1% stearic acid. This relatively high amount of linoleic acid was attributed to the cornmeal molasses diet. Kinsella (1966a) found that linoleic acid accounted for as much as 58% of the fatty acids of Oncopeltus fasciatus. He stated that this was due to the large amount of linoleic acid found in the diet, the milkweed seed.

A study of phospholipid composition and of fatty acid spectra within the neutral lipid and the main phosphlipid classes of a broad group of insects was carried out by Fast

(1966) in order to determine if any relationships existed. Of the 27 species of insects representing 6 orders and 20 families, all Diptera except the Cecidomyiidae contained very high proportions of palmitoleic acid and more than 50% of the fatty acids were less than 18 carbons long in these Diptera. In the Cecidomyiidae, as in the Lepidoptera, Hymenoptera, Coleoptera and Orthoptera, only about 30% or less of the fatty acids were less than 18 carbons long. In the Aphididae (Homoptera) more than 18% and in the related Cercopidae only 12% of the fatty acids were less than 18 carbons long. Thus in those species in which more phosphatidylethanolamine than phosphatidylcholine was present, and only in those species, more than 50% of the fatty acids in the neutral lipid were less than 18 carbons long.

The high level of palmitoleic acid found in the neutral lipid of most Diptera was found also in the phosphatidylcholine fractions of these insects with generally lower but still significant levels in phosphatidylethanolamine. However, in the Aphididae, the phospholipid fatty acid composition did not reflect the neutral pattern at all but resembled those of Lepidoptera or Hymenoptera. Palmitic acid levels are, in general, higher in the neutral lipid than in any phospholipid fraction, and linoleic acid was always higher in the phospholipid than in the neutral lipid fraction. Fast (1966) postulated that, as the fatty acid composition observed in insects which contained large amounts of phosphatidylcholine was similar to that

found in vertebrates, the fatty acids may play a greater role in phospholipid function than has heretofore been demonstrated.

Much of the work on the synthesis of fatty acids and lipid moieties in insects has been done using C^{14}-acetate or C^{14}-glucose, and it has been shown by numerous workers that insects can synthesize long chain saturated fatty acids from simple precursors.

Robbins et al. (1960) investigated the role of acetate in lipid synthesis in the adult house fly, Musca domestica, and found that female flies incorporated at least 2.5 times as much acetate into the fatty acids as into the unsaponifiable fraction. The rate of fatty acid synthesis was 3.7 to 8 times greater in females than in males. By contrast, both sexes utilized about the same amount of acetate in the synthesis of unsaponifiable material. Strong (1963b) fed the green peach aphid, Myzus persicae (Sulzer), through an artificial membrane on a diet containing 18% sucrose solution with the addition of C^{14}-glucose, and the insect was found to incorporate 75% of the label into stearic, palmitoleic and oleic acids. Small amounts were incorporated into myristic, linoleic and linolenic acids although there was no significant incorporation of the precursor into short chain fatty acids.

Zebe and McShan (1959) demonstrated that the fat body of Prodenia eridania Cram. (Lepidoptera) contained a system in

which saturated fatty acids were synthesized at a faster rate than the unsaturated series. The fatty acids formed were predominantly palmitic with smaller amounts of stearic, myristic and lauric acids. They also found a considerable amount of radioactive oleic acid and postulated that the presence of oleic acid was due to desaturation of stearic acid.

Lambremont (1965) showed that adult Anthonomus grandis was incapable of synthesizing linoleic acid from acetate, but readily synthesized palmitic, palmitoleic, stearic and oleic acids from C^{14}-1- and C^{14}-2-acetate. The results were consistent with the hypothesis that biosynthesis of even numbered fatty acids takes place through the successive condensation of a two-carbon unit. Lambremont et al. (1965) found that of the principal fatty acids from newly emerged unfed adults reared on a standard larval diet containing C^{14}-acetate, oleic acid had 60% of the label whereas stearic, palmitic, palmitoleic acids were of a secondary order and contained equal radioactivity.

Lambremont et al. (1966) reported that newly emerged adult boll weevils incorporated C^{14}-1-acetate-synthesized fatty acids primarily into phospholipid; after several days of feeding the new fatty acids were distributed into the glycerides. Larvae fed on C^{14}-1-acetate had most of the radioactivity in the free fatty acid fraction.

Van Handel (1965) fed starved mosquitoes sugar and protein meals and found that newly synthesized triglyceride contained 16 and 18 carbon fatty acids, with 33% palmitoleic acid, but only a trace of linoleic acid. The fatty acid composition was similar, regardless of whether the meal was sugar or protein. Sridhara and Bhat (1965) showed that palmitic acid-1-C^{14} was not incorporated into glycogen in Bombyx mori, but Chino and Gilbert (1965b) found long chain fatty acids to be synthesized from C^{14}-glucose in Hyalophora cecropia. Thus the synthesis of lipid from carbohydrate has been demonstrated but not the synthesis of carbohydrate from a lipid source.

III. MATERIALS AND METHODS

A. Maintenance of the insect colony

The procedure for maintaining the insect colony followed the methods of Ghouri and McFarlane (1958) and McFarlane (1962).

Adult male and female Acheta domesticus were kept in one gallon jars with 20-25 adults per jar in incubators at 28 ± 1.5°C. Humidity was maintained at 50 ± 5% R.H. using saturated $Ca(NO_3)_2 \cdot 4H_2O$ solutions. A shell vial of 32 ml capacity, tightly plugged with cotton wool, served as a satisfactory source of water for about 10 days. "Baby rabbit pellets" sold by Ogilvie Flour Mills Co. Ltd. Montreal were placed in the bottom of the jar and served as food for the crickets. The surface area of the jar was increased with strips of folded paper towelling.

Eggs were obtained by supplying jam cups filled with wet sand to a batch of gravid females for a 24 hour period. The water vials were removed from the jars during oviposition to attract females to the dishes. Egg dishes containing eggs were emptied on a paper towel and sand and towel were placed in an airtight jar containing a small pad of moist cotton to ensure 100% atmospheric humidity. Excess moisture had to be avoided, by wiping the jar with a dry towel at the seventh day of incubation, since it condensed on the sides of the jar and

trapped the newly emerged larvae.

B. Test insects and rearing

Larvae emerged after 9 or 10 days of incubation, and for one set of experiments several thousand larvae were divided into groups of 10 and each group reared in a 16 oz ointment jar containing food, a water vial and a 1½x6 inch strip of folded filter paper. The cop of the jar was covered by a piece of muslin secured by a rubber band. The food was ground to at least 20 mesh and placed in a small filter-paper boat in the bottom of the ointment jar. The temperature in the incubator was 31 ± 1°C, the R.H. 50 ± 5%. The larvae were from one brood and less than 24 hrs old at the time of being placed in the jars.

Larvae required for analysis were removed at random at 0 days and thereafter at 4 day intervals in suitable numbers depending on age, then weighed and the lipid extracted. After 42 days of the larval stage the insects began to become adults, so the collection of larvae for analytical purposes was terminated. From 43 days on collection of newly emerged adults began. These new adults were collected and removed daily and placed in separate jars each day (20 - 30 insects in a jar) for lipid determination in the adult stage. Not more than 10 adult males or females were removed at random from the jars at seven day intervals (exact age of the insects was always

known), weighed and the lipid extracted.

C. Extraction of lipid

Groups of insects (not less than 1.5 g in the larval stage; not more than 10 crickets in adult stage) were homogenized in 10 volumes of chloroform : methanol (2:1, v/v) in a high-speed Virtis 45 homogenizer and the homogenate was incubated at 50°C for 30 min. Eggs (1-2 g) were homogenized in a glass tissue grinder provided with a Teflon pestle.

The solid matter was separated out in a sintered glass funnel, with slight suction, and re-extracted once more with 10 volumes of chloroform : methanol (2:1). The combined extracts were washed with 0.02% $CaCl_2$ by the Folch procedure (Folch et al., 1957). When the phases separated clearly, the upper phase was discarded and the washed lipid extract was transferred to a round-bottomed flask and the solvent evaporated to near dryness using a rotary evaporator at a temperature not exceeding 40°C. The lipid residue was taken up in small portions of chloroform : methanol (2:1) and filtered through a Whatman No 1 filter-paper disk in a Buchner funnel. The extract was made up to 25 ml and stored at -15°C until used for analysis.

D. Separation of neutral and phospholipid

The extracted lipid was separated into neutral lipid and

phospholipid by means of columns of silicic acid (Mallinkrodt, 100 mesh). The columns were packed in 20 mm I.D. tubes and were charged with not more than 10 mg of lipid per gram of adsorbent.

Silicic acid for chromatography was prepared according to the method of Wlodaver (personal communication). Two or three volumes of methanol were thoroughly mixed with 1 volume of silicic acid and the slurry was allowed to stand for 30 min. The small particles which remained in suspension were decanted and discarded. This washing procedure was repeated once more with methanol and then twice with diethyl ether. The washed silicic acid was air-dried and was activated before use by heating to 110°C for 18 hrs in an oven. The removal of the silicic acid fines gave a product that was superior to unwashed silicic acid with respect to flow characteristics and separation of lipid.

The activated silicic acid was mixed with chloroform : methanol (2:1) and the slurry poured into the glass tubes. The column was packed and settled by flushing with 50 ml of petroleum ether and chloroform. The lipid was dissolved in chloroform and added to the column. The neutral lipid was eluted first by eluting the column with chloroform (150 ml)

and the phospholipid fraction was recovered next by eluting with methanol (100 ml).

E. Fractionation of neutral lipid and thin-layer chromatography

A number of methods have been developed recently for the separation of neutral lipid into its components. The three materials most commonly used for column separation of lipid classes are silicic acid, alumina, and florisil. The author has found that the florisil column method (as described by Carroll, 1961) was most suitable for the recovery of neutral lipid.

Florisil (60 to 100 mesh) consists of hard, porous, white granules with the following composition: magnesium oxide 15.5 ± 0.5%, silicone dioxide 84.0 ± 0.5% and sodium sulfate 0.5%. Carroll (1961) noted that different samples of florisil exhibited different efficiencies in the separation of sterol and diglyceride fractions. This variation was attributed to differences in the adsorption strength of the florisil. The adsorption strength of the florisil packing was dependent on the degree to which it had been activated. The best separation of sterol and diglyceride was achieved when florisil was de-activated by addition of 7% by weight of water.

Florisil columns were prepared in glass tubes (1.2 cm I.D., 25 cm long). The tubes were partly filled with hexane then 12 g of dry florisil was added in small portions. The tube was tapped between the additions of florisil to ensure uniform packing. The excess of hexane was allowed to drain off from the column until the surface of the liquid reached the top of the packed column. Lipid material, dissolved in hexane, was then applied to the top of the column and eluted with solvents as described by Carroll (Table 1). The solvent was evaporated from the collected fractions which were then weighed on a Mettler semimicro balance. The lipid in some of these fractions was identified by means of thin-layer chromatography.

Classes of neutral lipid was separated on Silica Gel G chromatoplates by the uni-dimensional method of Kelley (1966). To prepare the chromatoplates 30 g of Silica Gel G was dispersed in 65 ml of distilled water and applied to the plates as described by Stahl (1956). The prepared plates were allowed to dry on the template for 20-25 min at room temperature, then were stored in a desiccator over a drying agent ($CaCl_2$). The plates were activated by heating to 110°C for 30 min just before use. The samples of lipid in chloroform : methanol (2:1) solvent were applied at points 2 cm from the bottom of the plates by means of micropipettes.

Table 1. Elution pattern of neutral lipid components on a florisil column as described by Carroll (1961).

Class of eluent	Eluting solvent	ml
Hydrocarbon	Hexane	20
Sterol ester	5% ether in hexane	50
Triglyceride	15% ether in hexane	75
Cholesterol	25% ether in hexane	60
Diglyceride	50% ether in hexane	60
Monoglyceride	2% methanol in ether	75
Free fatty acid	4% acetic acid in ether	75

The chromatographic chambers were lined on three sides with Whatman 1 M paper which was wetted with the developing solvent 45 - 60 min prior to the insertion of chromatoplates. Plates were first developed in a solvent system comprised of petroleum ether : ether : acetic acid (70 : 30 : 1, v/v). When the solvent front reached a height of 16 cm, the plates were removed and air-dried. Redevelopment in the same direction in a solvent system comprised of ether : petroleum ether : acetic acid (70 : 30 : 1, v/v) was then carried out. In the second development the plates were removed when the solvent front rose 11 cm (a position just below the triglyceride spot). The first development separated the hydrocarbon, sterol ester and triglyceride in positions near the top of the plate. The second solvent separated free fatty acid, cholesterol, monoglyceride and diglyceride.

The lipid spots were located by placing the developed plate in an iodine atmosphere in an enclosed glass jar. The lipid appeared as dark yellow spots on a white background. The plate was removed from the iodine atmosphere and set aside until the adsorped iodine had evaporated, which was noted by the disappearance of the yellow spots. The plate was then sprayed with 50% H_2SO_4 and heated for about half an hour in a preheated Fisher Junior drying oven at a temperature of 175°C. A permanent record of the chromatograms was obtained by

photographing them with a 35 mm camera using Kodak Panatomic-X film.

F. Analysis of phospholipid

One-dimensional separation of phospholipid was performed by thin-layer chromatography with chloroform : methanol : 7N ammonium hydroxide (65 : 30 : 5, v/v) according to the methods of Skidmore and Entenman (1962). The components were detected with iodine vapor and identified by means of standard mixtures and characteristic color reactions.

A modified method of two-dimensional thin-layer chromatography was used for quantitative analysis (Rouser et al., 1966). Silica Gel G chromatography plates were heat-activated for 20 min at 120°C just before use. After heating, the plates were cooled for 30 minutes, spotted with sample from a microsyringe, and placed immediately in the developing chamber. Plates were first developed in a solvent mixture of chloroform : methanol : 7N ammonium hydroxide (65 : 30 : 5, v/v). When the solvent front had migrated about 16 cm, development was halted and the plates were air-dried for 5 - 10 min. They were developed in the second dimension (90 degree rotation clockwise) with chloroform : acetone : methanol : acetic acid : water (50 : 20 : 10 : 10 : 5, v/v); the solvent front was again allowed to move about 16 cm. Fresh solvent and chamber linings were used for each run.

Detection of the spots was carried out by means of iodine vapor and the area of each spot was marked using a glass marker-pen. The plate was left at room temperature until residual iodine had disappeared and the zone corresponding to each phospholipid was scraped off the plate, collected separately on glazed paper and carefully transferred to centrifuge tubes.

Elution of the spots were performed according to the method of De Bohner et al. (1965). Four ml of 1N HCl in methanol was added to each centrifuge tube. The tubes were placed in a water bath at 50 - 60°C for 15 min; each was stirred periodically with a glass rod. The tubes were then centrifuged for 5 - 10 min and the supernatant was carefully decanted, to remove the silica gel. The whole procedure was repeated once more and the combined supernatants were evaporated to near dryness under nitrogen.

G. Phosphorus determination

Lipid phosphorus was determined colorimetrically by a modification of the method of Martin and Doty (1949). Digestion of the phospholipid was carried out with 60% perchloric acid. The heating was done on an electrically heated Kjeldhal rack with water-pump suction to remove any escaping fumes.

The temperature was adjusted to give gentle refluxing and digestion was completed in about 30 min.

After digestion, the sides of the cooled tubes were rinsed with 5 ml of distilled water after which 5 ml of isobutanol : benzene (1 : 1, v/v) and 0.5 ml of 10% ammonium molybdate was added to the tubes. The tubes were shaken for about 30 seconds then allowed to stand until the phases separated. Three ml of the upper phase was transferred to a clean 10 ml volumetric flask followed by 3 ml of acid alcohol and 0.5 ml of stannous chloride. After color development samples and blanks were transferred to cuvettes and the optical density determined at 660 mμ (using a red filter) with a Beckman Model B spectrophotometer.

H. Collection of haemolymph and lipid extraction

Plexiglass was used to construct small containers to hold crickets while collecting haemolymph. Small cylinders (24 mm O.D.) were cut from plastic 20 mm thick. The cylinder ends were marked off in quadrants and in each was drilled a 7 mm I.D. hole for a distance of 17 mm. The remaining 3 mm was pierced by a 3 mm I.D. hole. Each cylinder then contained four compartments. A small opening was cut between the eyes and the ice-cooled insect was placed head first into one of the compartments of the plexiglass cylinder which was inserted

in a pre-cooled Pyrex centrifuge tube (I.D. 25 mm). The smaller hole at the base of the cylinder did not permit the movement of the insect body into the centrifuge tube during centrifugation, but permitted free flow of the haemolymph. Details of tube and plexiglass cylinder are shown in Fig. 1.

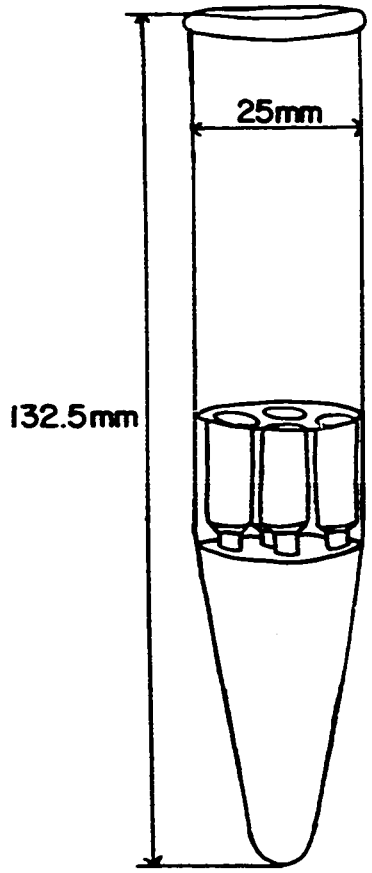
The cooled tubes were placed in an International Clinical model centrifuge having a four-place angled head and spun for 5 to 10 min at low speed. Groups of 40 crickets were processed thus in batches of sixteen or eight (4 insects per tube). A group of 40 adult insects yielded from 1.1 - 1.3 ml of haemolymph; females gave the greater yield.

One ml of haemolymph was used for the determination of lipid, which was extracted and purified according to the method of Sperry and Brand (1955), Procedure no. 2.

I. Transesterification and gas-liquid chromatography

Free fatty acids and fatty acids in esterified lipids were converted to methyl esters for gas chromatographic analysis by transesterification using the method of Metcalfe, Schmitz and Pelka (1966). Five ml of 0.5N methanolic sodium hydroxide was added to a sample in a 50 ml volumetric flask and heated on a steam bath until the fat globules went into solution (about 2 - 3 min). Five ml of 10% boron trifluoride-methanol reagent was added to the flask and the resultant mixture was

FIGURE 1



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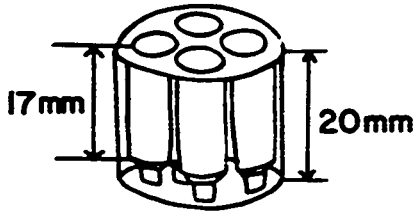
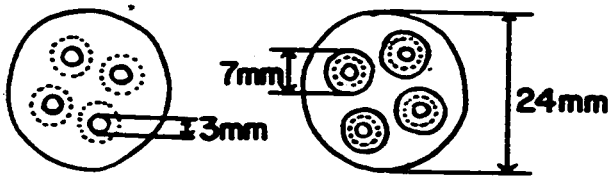


B



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boiled for 2 min. After cooling, sufficient saturated sodium chloride solution was added to the flask to float the methyl esters into the narrow neck of the flask.

The entire mixture was transferred to a separatory funnel to which 20 ml of distilled petroleum ether was added. The funnel was shaken for 1 min and the layers were allowed to separate. The lower aqueous layer was drained off and discarded and the upper layer was drained through a filter paper into a beaker. About 1 g of anhydrous sodium sulfate was added to remove any residual water. The solvent was evaporated on a steam bath and the methyl esters stored until used.

Gas-liquid chromatography was carried out on an F and M dual-column chromatograph (Model 5750), equipped with a flame ionization detector and a Honeywell recorder. The stainless steel columns (6 ft x 1/8 inch) were packed with acid washed Chromosorb W which was coated with 6% diethylene glycol succinate (DEGS). The operating conditions were as follows: carrier gas (helium) flow rate, 30 cc/min; injection port temperature, 260°C; detector temperature, 280°C; isothermal operation, 180°C; programmed operation range, 60°C - 190°C; temperature programming rate, 8°C/min; upper limit interval, hold until all fatty acids were removed by the carrier gas (about 15 min); range setting, 10^2 (approximately); attenuation, 16 - 32 (approximately); and sample size, 1.5 - 3.5μl.

Individual peaks were identified by reference to qualitative mixtures of fatty acid methyl esters (Applied Science Laboratories, Inc., State college, Pa., U.S.A.) which were subjected to gas chromatography under the same operating conditions as were the unknown samples. Peak areas were determined by the triangular approximation method.

IV. RESULTS

The results presented here were obtained from two entirely different populations reared during the course of the experiments. Analysis of Batch A, a population of about 6000 insects, was performed during 1966 - 1967; Batch B, a population of 7500, was analysed during 1967 - 1968. A third batch (Batch C) was used only for the determination of the growth curve for adult males. This batch was set up because of the great variation in weight exhibited by the male population.

A. Growth curve

The changes in wet weight of Acheta domesticus during the entire life cycle are shown in Tables 2, 3, and 4. Figures 2 and 3 represent the combined results obtained from all batches in the determination of the growth curves.

Figure 2 shows that the wet weight during the larval stage more or less doubled every 4 days until 32 days; thereafter, until adult emergence, the rate of growth slowed down. Newly emerged male and female adults had nearly identical weights. The wet weight of males remained more or less constant until 21 days after emergence, followed by a substantial decline at 28 days. This decline was evident in all three batches (Table 3) and no adequate explanation was found for

this phenomenon. The adult males exhibited a slow and gradual increase in weight and reached their maximum mean weight (359 mg) in 49 days. Adult females showed a considerable increase in their weight between 0 and 21 days after which (at 28 days) a decline in weight was evident. The mean wet weight of the female reached its maximum (485 mg) 35 days after adult emergence, and then declined to 444 mg at 49 days.

B. Total lipid

The results of the analyses for total lipid content, neutral lipid and phospholipid were obtained from two entirely different populations. Batch A and Batch B results are presented separately in order to show that the pattern of changes were similar in both cases. Batch B was reared and the lipid analyses were performed about twelve months after Batch A. Values were not necessarily always identical but they were closely correlated even when differences were quite apparent.

The total larval lipid, on a wet weight basis, decreased at 4 days after emergence followed by an increase for the next 12 days (Figure 4) to reach a plateau between the 16th and 24th days. From the 24th days until adult emergence, the total lipid content showed a sizable increase.

Newly emerged adults (Figs. 5 and 6) contained between 8.5% and 11.5% total lipid depending upon batch and sex,

females being richer in fat than males. The lipid content of females showed a decline during the first 3 weeks after adult emergence and total lipid diminished to 6.6% of the wet weight. This meant that a considerable amount of lipid was consumed during this period, which corresponds to the early egg laying period of the female. During the next 7 days (at 28 days) the percentage of female total lipid increased to 9.2% of the wet weight. This rapid increase was followed by a gradual decrease in the total lipid content to the end of the observation period. Figure 5 shows that the percentage of total lipid in adult males gradually declined with age, but in an erratic manner.

C. Neutral lipid

The pattern of neutral lipid content closely followed the one obtained for total lipid in all stages. The neutral lipid in the larval stage, as percentage of wet weight, is shown in Table 5 and Figure 7. The results show that there was a small decrease at 4 days and a rapid increase between 4 days and 16 days. The amount of neutral lipid remained more or less constant for the next 8 days, and then increased during the rest of the larval period.

The percentage of neutral lipid in the adult female (Table 6 and Figure 8) decreased during the first 21 days followed by an increase for the next two weeks and a slight

decrease between 35 and 49 days. In adult males neutral lipid declined from 7.3% at adult emergence to 5.1% in Batch A and 3.8% in Batch B 56 days later (Table 7 and Figure 8).

Neutral lipid was separated into the following components by means of florisil columns: hydrocarbon, sterol ester, triglyceride, monoglyceride, diglyceride and free fatty acid. Figure 9, a photograph of a Silica Gel G thin-layer chromatograph, shows a typical separation pattern of neutral lipid. This particular sample was the neutral lipid fraction from a 35-day-old adult male. The lipid recovery in individual experiments ranged between 95% and 104%.

The distributions of the neutral lipid components for 3 different stages are shown in Tables 8, 9 and 10. Changes in the triglyceride component closely resembled the changes in the percentage of neutral lipid in larval and adult male stages. Adult females demonstrated a somewhat different pattern in later stages of life.

Triglyceride constituted 1.2% of the wet weight at 0-day for larva (Figure 10), the value increased to 6.2% at adult male emergence, and 7.6% - 8.5% for 0-day adult females. At the end of the life cycle triglyceride declined in the male (Figure 11) to 2.6% in Batch A and 1.3% in Batch B and to 1.4% and 1.8% respectively in the female cricket (Figure 12).

Noteworthy is the intense catabolism of triglyceride between 35 and 49 days of female life.

Free fatty acid comprised the second major component in the early period of larval life. Figure 13 illustrates that at larval hatching free fatty acid constituted 0.26% of the wet weight but at 24 days had declined to about 0.15% and remained more or less constant throughout the larval stage and into the adult stage (up to 35 days of age in the adult male and 28 days in the adult female). Beyond this age a gradual increase in free fatty acid was observed in male insects and a very sharp increase in the case of females (Figures 14 and 15). This augmentation in the value of free fatty acid closely corresponded to the sharp decrease in the triglyceride value and indicated an intense triglyceride hydrolysis.

Hydrocarbon content remained relatively constant during the larval stage, increased moderately in females and to a much larger measure in the males (Figures 16 and 17).

Sterol ester and free cholesterol remained more or less constant during the larval stage but were generally higher in the adults. No appreciable changes were observed in the monoglyceride and diglyceride concentrations during the entire life cycle.

D. Phospholipid

Tables 5, 6 and 7 and Figures 7 and 8 summarize the results observed for phospholipid during the entire life cycle of the cricket. It can be seen that the percentage wet weight of the phospholipid remained nearly constant in all three stages. The values ranged between 1.38% and 2.20% including both Batch A and Batch B.

E. Egg lipid

To determine the extent of lipid metabolism associated with egg development, eggs at 0 day and 7 days of incubation were extracted and lipid content determined (incubation period at 31°C is 9 days). It should be noted that cricket eggs absorb an amount of water equal to their new laid weight during the early part (2 - 4 days) of their development (See Discussion).

In Table 11 is summarized some of the changes that occurred during embryogenesis in the egg. The total lipid content dropped from 18.2% (based on wet weight) for new laid eggs to 4.1% at time of hatching. Neutral lipid comprised 15.9% of the wet weight of the 0-day egg and decreased to 1.95% of the 0-day larva; phospholipid decreased from 2.25% of the wet weight to 1.81%.

Separation on a florisil column was employed to detect variations in the distribution of the neutral lipid fraction during the course of egg development. The results of the analysis (Table 12) clearly show that triglyceride metabolism accounted exclusively for the lipid diminution during embryogenesis. Expressed as percentage of wet weight all neutral lipid components decreased during the incubation period of 9 days from 0-day egg to 0-day larva. However, expressed as percentage of neutral lipid, six fractions showed a more than 50% increase while the triglyceride fraction exhibited a decrease of 30% from the 85.4% of the 0-day egg to the 60.0% of the newly hatched larva.

F. Analysis of phospholipid

Qualitative and quantitative analyses of the composition of the phospholipid during the 3 stages of the life cycle (egg, larva, male and female adults) revealed that it consisted predominantly of three major fractions, namely, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. Other phospholipid constituents identified but present in very small quantities were lysolecithin and phosphatidylinositol; solvent line components which were not identified probably contained phosphatidic acid and some cerebrosides.

Two dimensional thin-layer chromatographic separation and subsequent chemical analyses were performed on the

following stages and ages. Egg stage: 0 and 7 days; larval stage: 0, 4, 16, and 40 days; adult male stage: 0, 21, 42 and 56 days; adult female stage: 0, 21, 42 and 49 days.

In Table 13 and Figure 18 are shown the changes in the distribution of the major fractions during the life cycle. Noteworthy is (a) the dramatic decrease of the phosphatidylcholine fraction from 64.3% of the total phospholipid of the new laid egg to 38.7% at larval hatching, and (b) a further decrease in the 4-day larva (to 30.4%). From this lowest value a continuous increase was evidenced until adult emergence. Adult males showed a slow decrease in their phosphatidylcholine content with age. However, a decrease in the adult female was observed only after an initial increase.

The second major fraction, phosphatidylethanolamine, did not change during the first 7 days of embryogenesis, but increased to 30.4% of the total phospholipid at larval hatching and remained more or less constant during the entire life cycle.

The sphingomyelin fraction exhibited a cyclical pattern of change in the egg and larval stages. It increased during the first 7 days of the egg stage and dropped slightly at larval hatching. After an increase in the first 4 days of the larval stage (to 16.2%) it gradually diminished to 8.2% at adult female and 10.8% at adult male emergence. For 42 days thereafter it remained constant and increased only slightly at the end of the observation period.

G. Fatty acids

Separate experiments were performed to ascertain the changes in the distribution of fatty acids associated with lipid extracts at two different developmental stages during larval life.

The lipids of 12-day-old and 30-day-old larvae (about 15 g of insects in each) were separated into neutral and phospholipids and the neutral lipid was fractionated on a florisil column. All lipid extracts and fractions were methylated as described in the section on Methods. The columns for gas-liquid chromatography were maintained at 180°C for isothermal operation and between 60°C - 190°C for programmed determination; the column temperature was regulated to rise 8°C per min. The upper limit interval was set at hold until all fatty acids had been removed from the column by the carrier gas.

The results of the analyses of the fatty acid content of the total, neutral and phospholipid fractions from the two different age groups and their food are presented in Table 14. All groups contained four major fatty acid components: palmitic acid, stearic acid, oleic acid, and linoleic acid, which together accounted for about 90% of the fatty acids. The fatty acid composition reflected, but was not identical with, the fatty acid composition of the dietary

lipid. The most striking difference was exhibited by linoleic acid which comprised 40.3% of the food but 54.6% of the phospholipid, 25% - 28% of the neutral lipid, and from 30% to 34% of the total lipid depending on the age of the larvae. Stearic acid comprised 5% of the fatty acid of the dietary lipid but appeared in more than double this quantity in all other fractions. On the other hand, linoleic acid, which comprised 7.9% of the fatty acid of the food, did not reach higher than 4.5% in the total, neutral or phospholipids. A close correlation did exist between the palmitic and oleic acid content of food and that of the total lipid; both acids were slightly higher in neutral lipid and considerably lower in phospholipid.

No appreciable changes were observed in the distribution of fatty acids between 12-day- and 30-day-old larvae, with the possible exception of linoleic acid, which was higher in the total and neutral lipids at 30 days, and stearic acid, which was lower at 30 days than at 12 days.

The analysis of the fatty acid content of the neutral lipid components was initially performed isothermally at 180°C. However, repeated trials indicated that a considerable amount of short-chain fatty acids were present in some components and therefore a programmed analysis was initiated.

The results obtained from the programmed analysis of five fractions in the two different age groups are summarized in Table 15.

Distinct differences were observed between individual components and also between the two developmental ages. Short-chain fatty acids (caproic and caprylic) and two unidentified components were observed in sterol ester, triglyceride and 12-day-old monoglyceride fractions. None of the above (with the exception of X-1 component) appeared in the 30-day-old monoglyceride or free fatty acid fractions. These four short-chain components increased, in some cases considerably, in the sterol ester and triglyceride fractions of 30-day-old larvae, but decreased in the di- and monoglyceride and free fatty acid fractions. Undecanoic acid (C 11:0) was observed in measurable amounts in the 30-day diglyceride and free fatty acid fractions. A small component, provisionally identified as an isomer of myristic acid, appeared only in the monoglyceride fraction. One of the most interesting differences was the large percentage of myristic acid (34.5%) in the 12-day-old monoglyceride fraction and its subsequent decrease to 10.9% in the 30-day-old larvae.

Palmitic, stearic, oleic and linoleic acids formed the bulk of the fatty acids in all five fractions but differences between the components did exist, especially in the

monoglycerides. All of these fatty acids were present in much lower concentrations in the monoglyceride fraction of the 12-day-old larvae, and low values were found also for stearic and linoleic acids in the 30-day-old larvae. One unidentified component, which was not C 19:0 or C 20:0, was found only in the monoglyceride and free fatty acid fractions.

H. Haemolymph lipid

The composition of lipid in the haemolymph of 28-day-adult male and female crickets was investigated and the results are listed in Table 16.

Total lipid comprised 3.4% of the male and 2.3% of the female haemolymph. Male haemolymph contained 2.1% neutral lipid and 1.3% phospholipid whereas female haemolymph contained 1.6% neutral and 0.7% phospholipid.

The neutral lipid components were also examined and the results are summarized in Table 17. It was found that hydrocarbon made up the largest fraction in males (37.2%) and free fatty acid in females (23.9%). Free fatty acid was the second largest fraction in males and hydrocarbon in females. Both sexes exhibited the same concentration of diglyceride (5.0%) but a sexual dimorphism existed in the monoglyceride fraction, the female value being double the male. In contrast to the

large amount of triglyceride found in the cricket body lipid, haemolymph had a low concentration of triglyceride in both sexes.

Qualitative and quantitative two-dimensional thin-layer chromatographic analysis revealed that the major components of the phospholipid fraction were phosphatidylcholine and phosphatidylethanolamine. These two fractions comprised more than 96% of the cricket haemolymph.

The composition of the phospholipid components in the insect haemolymph, expressed as percentage of phospholipid, was as follows:

	Males	Females
Phosphatidylcholine	61.9	71.6
Phosphatidylethanolamine	34.7	24.4
R_f similar to sphingomyelin	-	4.0
Solvent front	3.4	-

The fatty acids of the total lipid of the haemolymph of 28-day-adult males and females were analysed by gas-liquid chromatography.

The data presented in Table 18 reveals that no great sex differences existed in the distribution of fatty acids associated with haemolymph total lipid. The fatty acid composi-

tion of the haemolymph total lipid was very similar to that of total lipid extracted from the whole insects in two ages (12 and 30 days) of the larval life. Palmitic, stearic, oleic and linoleic acids accounted for about 94% of the fatty acids of the haemolymph total lipid.

V. DISCUSSION

The growth curve for Acheta domesticus (Figure 2) shows that a 0-day larva with a weight of 0.8 mg increased its weight by a factor of 400 during larval growth, an increase which is roughly approximating a geometrical progression. This growth measurement at 31°C closely resembles the results obtained by Patton (1963) at 35°C. Patton fed the insects with several kinds of food and found that growth and development were dependent upon the quality of the food and yielded sigmoid curves with the majority of the food tested. His experiment showed that the most rapid growth took place between the third and fourth week. The largest larvae and adults were produced when baby rabbit pellets were used as the food, but the rate of development up to the third week was significantly lower than it was with other food. Ghouri and McFarlane (1958) found that the development and duration of larval life and stadia were also temperature dependent; however, there is no difference in the shape of the growth curve in this work and in the work of Patton (1963). In the present study it was found that adult females continued to increase in weight (Figure 3) probably because of intensive feeding during the early period of egg laying. There is no apparent explanation for the sharp decrease in the adult male weight (between 21 and 28 days) but it may be related to the male sexual life, of which little is known.

An examination of Table 5 reveals that the growth process is not accompanied by a steady rate of accumulation of lipid material. The accumulation of total lipid, on the basis of wet weight, took place in four stages during larval development: an initial decrease followed by a moderate increase, then a plateau followed by a sharp increase. The most intensive period of lipid accumulation took place during the last 18 days of larval development.

A pattern of change similar to that observed with the house cricket was recently reported by Agarwal and Rao (1969) for larvae of Dysdercus koenigii F. (Hemiptera). The newly hatched larvae contained 10.7% total lipid, which declined to 8.3% in the second instar followed by an increase in the third instar. The percentage remained constant in the fourth instar followed by a small decline in the fifth instar, then by a rapid increase to the adult stage.

Other roughly similar patterns of lipid change are exhibited by various insects. In the silkworm, B. mori, Niemierko et al. (1956) reported that the percentage of total lipid amounted to 5.5% of the wet weight of the newly hatched larvae. In the first and second instar the percentage of lipid decreased (to 2.8% and 2.1% respectively). In the third and fourth instars the content of lipid remained almost constant

(2.0% - 2.2% wet weight). Only in the last instar did the percentage of lipid in the fresh substance increase to reach a value of 3.8%. Gilbert and Schneiderman (1961) reported similar observations with H. cecropia. The percentage of lipid in the newly hatched larvae was 4.5%, but this percentage decreased to a more or less constant level of 1.3% to 1.9% in the fourth and fifth instars. The lipid amounted to 5% and 7% of the wet weight of the female and male respectively at pupation.

Hutchins and Martin (1968) have recently reported the characterization and analysis of the lipid of Acheta domesticus. Their crickets were obtained from a commercial source and no information was given on the conditions under which the crickets were reared. The analyses began at the second week of postembryonic life and continued weekly until the 8th week, followed by an analysis of 10½ weeks-old crickets. The report did not state whether the crickets were in the larval or adult stages or whether both stages were present at this age. The analyses indicated that there was a build up of depot fat during the first seven or eight weeks of the postembryonic life of the cricket. The increase in depot fat was followed by a loss of lipid between the 8th and 11th week. The progress of lipid change as reported by Hutchins and Martin is not in agreement with the experimental results

obtained by the present author. Hutchins and Martin (1968) found that, on a percentage basis, the total lipid increased from 4.8% wet weight to 7.7% between the second and sixth weeks. This increase was followed by a plateau between the sixth and eighth weeks, after which a decline began, from 7.7% at 8 weeks to 4.9% at 10½ weeks. No meaningful comparison can be made between the present results and those of Hutchins and Martin because of the lack of information on their experimental conditions and rearing data.

A considerable amount of information is available on lipid changes occurring in holometablous insects during pupal-adult transformation. However, there is very little published information on the changes in the lipid content during the aging of male and female adult insects.

An examination of Figures 5 and 6 reveal that the total lipid of both the adult male and female crickets gradually declined with age, with the exception of a small period (21 - 28 days) when a sharp increase in the female lipid was observed (this increase will be discussed later). A total lipid decline with age was found also by Munson and Gottlieb (1953) for adult P. americana where females contain more lipid than males. The highest lipid content was found in 0-day-old adults.

It fell rapidly in the first week of adult life and thereafter, with fluctuations, declined slowly in both the adult male and female.

Neutral lipid, the major component of the total lipid, followed very closely the pattern of total lipid (Figures 7 and 8). Agarwal and Rao (1969) obtained similar results in their analyses of the total and neutral lipid in the egg, larval and adult stages of D. koenigii. A steady decline in the neutral lipid during adult development was found by D'Costa and Birt (1966) in the blowfly Lucilia.

In Tables 8 - 10 and Figures 10 - 17 are presented the detailed composition of the neutral lipid, viz. hydrocarbon, sterol ester, triglyceride, cholesterol, monoglyceride, diglyceride and free fatty acid of crickets at various stages and ages. It is immediately apparent that the triglyceride fraction forms the greater part of the lipid content of the insect in all developmental stages, and that triglyceride is mainly responsible for the changes in total amount of lipid. In all insect species investigated so far, triglyceride has been found to comprise the major fraction of neutral lipid (Fast, 1964: Gilbert, 1967).

During the first half of larval development, the synthesis and storage of triglyceride occurred at a rather low

rate. Subsequently, the triglyceride formation and storage increased, probably as a reserve for egg development. The triglyceride was depleted during the early life of the adult female (Figure 11), when the ovaries are being developed and eggs laid. The renewed accumulation and synthesis of triglyceride in the female during the 4th week might correspond to a new cycle of egg formation. There is, however, no corroboratory evidence for this hypothesis from the little known reproductive physiology of the female.

The free fatty acid fraction (Figures 14 and 15) increased during the last period of the life cycle, and corresponded closely to the moderate reduction of triglyceride in the male and the extreme depletion of triglyceride in the female. These changes probably reflect the catabolism of triglyceride to free fatty acid. Gilbert and Schneiderman (1961) found that the lipid of both males and females rapidly decreased during the adult life of H. cecropia and postulated that this was perhaps the reason why these moths died in about 10 days. In the case of the house cricket, it is not so much triglyceride depletion but the build-up of free fatty acid that might contribute to death.

The percentage of free fatty acid decreased continuously during larval development between 0 and 24 days (from 0.26% to

about 0.15%); thereafter, it remained more or less constant until the late adult stage. There was a considerable increase in the percentage of free fatty acid during the latter part of embryogenesis. It is possible that this high percentage of fatty acid in the early larval period was a carry-over from the egg stage and it may be important in the early development of the larvae.

The monoglyceride and diglyceride fraction showed only minor fluctuations throughout all stages, and were not influenced by the large fluctuations exhibited by the triglyceride fraction.

Sterol ester remained relatively constant during larval development but showed a slow increase during the early adult stages and a more pronounced increase with age. During this later period, a large pool of fatty acids was available due to intense triglyceride hydrolysis and possibly the cricket was able to convert a larger portion of free sterol to sterol ester. The percentage of sterol ester was higher in the female than in the male, and corresponds to the higher free fatty acid in the later period of the female life cycle.

The increase in the hydrocarbon fraction in the adult male is interesting. Hydrocarbon is known to be a major component of insect cuticular lipid (Gilby, 1965). However, the fact that no hydrocarbon increase in females was observed

indicated that no cuticular function was responsible for this increase.

The number of fatty acids found in insects is varied but usually large. Louloudes et al. (1961) detected 17 fatty acids in the saponifiable fractions of the American cockroach, P. americana. Lambremont and Blum (1963) reported 23 fatty acids in the saponifiable fraction of the boll weevil. Most of these fatty acids occurred as minor components and amounted to less than 2% of the total fatty acids. Qualitative analyses of P. americana, at successive stages during embryogenesis, revealed the presence of at least 30 fatty acids (Kinsella, 1966c). Barlow (1964), who analysed the fatty acid constitution of 30 species of insects, suggested the stage of development of the insect had little effect on fatty acid composition. On the other hand, Herodek and Farkas (1960) and Nakasone and Ito (1967), examining the changes in the composition of fatty acids in B. mori in the course of ontogeny, found significant alterations during the larval period. Young larvae contained much more stearic and linoleic acids and much less palmitic and oleic acids than older larvae.

Hutchins and Martin (1968) reported that fatty acids associated with total lipid extracts did not change significantly from the third through the eleventh week of the postembryonic life of the house cricket. Their results were obtained from

the fatty acids associated with each neutral lipid component (after florisil column separation) of 8-week-old crickets, and indicated that fatty acids derived from mono-, di- and triglyceride fractions were similar. Only the sterol ester fraction was distinctive from the others in that it contained a higher proportion of stearic acid and differed in some of the minor components. No attempt was made to determine the fatty acids of the phospholipid fraction.

In the present work a thorough study was made of the lipid of two distinct larval stages. The data presented in Table 14 show that the differences in the fatty acid composition of total, neutral and phospholipids of 12-day and 30-day old larvae were not dramatic. However, considerable differences were observed in the fatty acid distribution between these three lipid extracts. Less than half the percentage of palmitic and oleic acids was found in the phospholipid than in the neutral lipid, and exactly the opposite was observed for linoleic acid. Palmitic, stearic, oleic and linoleic acids were the four major fatty acids found in this experiment and this observation substantiated the findings of Hutchins and Martin (1968) for the house cricket, and in general were applicable to the Orthoptera (Fast, 1964). The difference between the fatty acid composition of the larvae and that of the food suggests some metabolism of ingested fatty acids.

Much more dramatic differences were observed when the fatty acids of the separated lipid components were analysed. One of the most striking findings was the uniqueness of the composition of and the changes in the monoglyceride fraction. Other interesting aspects were the changes observed in the short-chain fatty acids.

No definite conclusions can be drawn as yet from these changes in the fatty acid composition of lipid at different stages of the insect's development. Little is known regarding the role of individual fatty acids other than their use as a source of energy. If similar changes are observed in other insect species, it would appear, as postulated by McFarlane (1968), that fatty acids (and possibly also their methyl esters) play a crucial role in the growth and development especially in the early larval period.

The water content of the egg must be considered when the results of lipid analyses during embryogenesis are examined. McFarlane et al. (1959) found that the weight of the house cricket egg began to increase at 36 hrs due to water absorption. In terms of the fresh-laid weight a maximum value of 196% was reached after 96 hrs but just before hatching, the increase had declined to 165%. Clearly, it would be preferable to express the quantity of lipid in terms of dry weight, but the usual dehydration procedure applied to insects require

elevated temperatures which may cause to loss of lipid by oxidation. Since it is readily established from inspection of Table 11, that, even on a wet weight basis, the total lipid decreased during development, the precise extent of the decrease is of less significance than the relative changes in the composition of the lipid.

Examination of Table 11 shows that the neutral lipid fraction accounted for the loss of lipid material during egg development. The 46% loss of neutral lipid is in agreement with the results of Allais et al. (1964), who observed a 52% loss in Locusta migratoria, and Kinsella and Smyth (1966), who reported a 54.7% loss of neutral lipid at eclosion in the ootheca of P. americana. The phospholipid content of the cricket represented 12.3% of the total lipid at laying, that is, midway between the 19.5% found in L. migratoria by Allais et al. (1964) and the 6% found by Kinsella (1966b) in the cockroach ootheca. Kinsella postulated that there might be a correlation between duration of embryogenesis and phospholipid content of the ova.

Although the total lipid of the cricket egg diminished considerably, the phospholipid, as percentage of total lipid, increased almost fourfold. Kinsella (1966b) reported an almost threefold increase in phospholipid during embryogenesis, and he attributed the increase to the accumulation and incorporation of low molecular weight fatty acids. The synthesis of

phospholipid in the cricket egg coincided with, and was to a certain extent explained by, a decrease in the total lipid fraction; hence it is suggested that the new phospholipid was partially derived from the neutral lipid moiety. It has been shown by Reiser et al. (1960) that triglyceride serves as a precursor of lecithin in the rat.

Analysis of neutral lipid revealed that the decrease in the lipid fraction during embryogenesis was due to the decrease in triglyceride, which initially had accounted for 85.4% of the neutral lipid. About 30% of the triglyceride was metabolized during the 9 days of incubation time but the rate of metabolism varied. The triglyceride had fallen to 80.1% in 7 days, but in newly hatched larvae the level was 60%, which indicated intensive catabolism of triglyceride during the period immediately preceding hatching. The loss in triglyceride probably also provided for the increase of free fatty acid and other components.

The results presented in Tables 5, 6 and 7 and Figures 7 and 8 demonstrate that phospholipid, expressed as percentage of weight, remained nearly constant during the entire postembryonic life of the cricket. The same holds true for the phospholipid during embryogenesis (Table 11). The composition of the phospholipid in all stages of development consisted pre-

dominantly of three major fractions, namely, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin plus some other minor components.

The qualitative composition of the phospholipid of the house cricket was quite similar to that of the developing eggs of the migratory locust as reported by Allais et al. (1964), and of the developing ootheca of the cockroach according to Kinsella (1966b). Similar qualitative phospholipid values have been reported on the adult cockroach by Siakotos (1960) and on the cricket Gryllus bimaculatus by Fast (1967). In all cases, as in the present report, phosphatidylcholine was the predominant constituent, followed by phosphatidylethanolamine. Similar results were obtained by Fast (1966) for 3 species of Homoptera, 5 species of Lepidoptera, 4 each of Hymenoptera and Coleoptera and one species of Orthoptera. The phospholipid of the house cricket has approximately the same composition as that of vertebrate and most invertebrate animals, and is clearly different from those of Diptera (Fast, 1966).

Considerable changes in the phospholipid components occurred during embryonic and larval development (Table 13 and Figure 18). Phosphatidylcholine was catabolised intensively during egg development and the first 4 days of larval life, after which a vigorous synthesis took place during the rest

of the larval life. A gradual decline was observed in the adult stage of the male but in the female adult this slow decline was interrupted by a slight increase. The pattern of change in this fraction was very similar to that of the triglyceride fraction. Catabolism occurred in the egg stage and first 4 days of larval stage, followed by intensified synthesis during larval development and a gradual decline during the adult stages. The author postulates, that, on the basis of the observations, a close metabolic relationship may exist between the phosphatidylcholine and triglyceride fractions. Reiser et al. (1960) found that lecithins are metabolically very active and rank next to triglyceride as a source of respiratory energy in the rat.

Allais et al. (1964) showed that phosphatidylcholine was significantly catabolized during blastokinesis of the locust embryo (9th day of the embryonic period) and then was rapidly synthesized, whereas the ethanolamine moiety increased gradually throughout embryonic development. Kinsella (1966b) also noted a gradual decrease in the phosphatidylcholine fraction and an increase in the phosphatidylethanolamine and sphingomyelin fractions of the cockroach embryo. He postulated that the accumulating ethanolamine in the embryo of P. americana may have been related to the increasing rate of metabolism as reflected in increased triglyceride metabolism. The data from the present study indicate that a similar situation develops in

A. domesticus. As far as this author is aware no detailed comparative data exist on the quantitative changes in phospholipid components during larval and adult development.

There have been few detailed studies on the lipid content and fatty acid composition of the haemolymph, but those that have been reported point to wide variations in the chemical composition of lipid in various insects (Sridhara and Bhat, 1965). In Table 16 is presented the amount of total, neutral and phospholipids found in the haemolymph of 28-day-old adult male and female crickets. The results indicate a sexual dimorphism, which was also found by Nowosielski and Patton (1965). The amount of lipid reported in the present study is higher than that reported by these investigators; however, the methods of lipid extraction and chemical analysis were considerably different. Nowosielski and Patton found that the lipid content of the haemolymph of the adult cricket ranged between 1.5% - 2.7%. During the last two larval stages, the lipid levels were low, about 1.5%, then increased to a maximum in 5 - 10 day old adults and dropped again after the 15th day of adult life. The drop in the lipid content of the female appeared to be greater and more rapid than that in the male.

The lipid content in the haemolymph of the wax moth larvae was found to be 2.4% by Wlodawer and Wisniewska (1965), who indicated that their results far exceeded the values found by other

authors in the 1920's and 1940's. There are considerable differences in the percentage of phospholipid in the haemolymph; in the wax moth it is 22%, in B. mori (Sridhara and Bhat, 1965) 51%, in the 28-day-old male cricket 38.2% and in the female 30.4%.

Quantitative analysis of insect haemolymph phospholipid also revealed a sex difference and a high concentration of phosphatidylcholine. The amount of phosphatidylcholine in 28-day adult was higher in the haemolymph than in the body lipid. Wlodawer and Wisniewska (1965) found that in the wax moth phosphatidylcholine made up 60% of the haemolymph phospholipid whereas phosphatidylethanolamine constituted only 20%. There appears, however, to be a discrepancy regarding the phospholipid constitution of the wax moth larval haemolymph; Lenartowicz and Niemierko (1964) reported more phosphatidylethanolamine than phosphatidylcholine in the haemolymph. This discrepancy has not yet been resolved.

The fractionation of the various neutral components of the haemolymph showed an entirely different pattern from the one observed with the total larval or adult cricket lipid. The data presented in Table 17 indicate a high percentage of hydrocarbon, free fatty acid, sterol ester and cholesterol in both male and female and double the amount of monoglyceride in the female than in the male.

The following comparison of the percentage of neutral lipid components of the haemolymph during different stages of development shows that a wide variation exists, and consequently, no extrapolation can be made from the content of one insect species to another.

Table 1a. Neutral lipid and phospholipid content in the haemolymph of insects in different species.

Insect	*B. mori*	*G. melonella*	*Hyalophora cecropia*		
Stage	larvae	larvae	pupa	adult	male pupa
Investigator	(1)	(2)	(3)	(3)	(4)
	%	%	%	%	%
Hydrocarbon	5.0	(			
Sterol ester	1.0	(16.0			
Triglyceride	20.0	18.2	10.0	7.9	17.9
Cholesterol	1.9	6.0			7.7
Diglyceride	7.5	28.0	87.5	91.0	66.6
Monoglyceride	13.2	3.8	1.6	0.5	5.1
Free fatty acid	3.0	8.0	0.9	0.6	
Phospholipid	45.0	20.1			

(1) = Sridhara and Bhat (1965). (2) = Wlodawer *et al.* (1966). (3) = Beenakkers and Gilbert (1968). (4) = Chino and Gilbert (1965a).

Nelson et al. (1967) found that the relative amounts of the lipid fractions of the haemolymph of P. americana fluctuated with age and sex whereas those of the fat body remained fairly constant. Haemolymph triglyceride accounted for 54% of the haemolymph lipid at 2 months (4th instar nymph), increased to 83% and 86% at 4 months (8th instar nymph) in males and females respectively, and decreased to 36% and 26% at 6 months (young adults). In contrast, the haemolymph diglyceride decreased from 38% at two months of age to 10% and 12% at 4 months for males and females respectively; then at 6 months it increased to 60% and 70%.

Investigation by Nelson et al. (1967) of the fatty acid composition of haemolymph neutral lipid components revealed that, in the 4 lipid fractions of P. americana which were analysed, the major fatty acids were palmitic, stearic, oleic and linoleic acids, with oleic acid predominant. Palmitic and oleic acid concentrations changed with age and sex. In Table 18 is presented the results of the fatty acid composition obtained from the total lipid of 28-day-old male and female cricket haemolymph. No analysis was made on the neutral lipid components of the haemolymph due to lack of material. From the results presented it can be seen that there was a slight sexual dimorphism in the fatty acid composition at this age and that linoleic

acid predominated. The other 3 major fatty acids were palmitic, stearic and oleic acids. The total haemolymph fatty acid composition was very similar to that of total lipid extracted from the whole insects in two ages (12 and 30 days) of the larval life.

Oleic acid predominated in the total lipid of the haemolymph of B. mori larvae and other fatty acids in high concentrations were, in decreasing order, linoleic and stearic, then linolenic, and palmitic acids (Sridhara and Bhat, 1965). Oleic acid was also the most abundant fatty acid present in H. cecropia according to Beenakkers and Gilbert (1968). Linolenic acid took a second position. Comparing the haemolymph lipid in two stages they found a significant increase in the concentration of palmitic acid during pharate adult development. During this same period, the concentration of linolenic acid decreased in the diglyceride and free fatty acid fractions. The monoglyceride fraction had the highest concentration of oleic acid (64.9%) in the adult whereas in the other fractions, oleic acid ranged between 42% and 46%. Every fraction showed a different pattern of fatty acid composition.

From the information compiled above and the results presented in this report, it can be seen that the amount of lipid in the haemolymph varies considerably among the various species, both during the life cycle of the same insect and between the sexes. More detailed information is necessary in order to arrive at any definite conclusions and to be able to extrapolate from

one insect species to another.

VI. SUMMARY

1. The body weight of Acheta domesticus (L.) increased, in roughly approximating a geometrical progression, during larval growth and the larvae accumulated large quantities of lipid, though not in a steady manner. The gain in body weight extended into the early period of adult life when a sexual dimorphism became apparent: females gradually and continuously lost weight whereas the males experienced a sharp weight loss followed by a slight recovery and then a gradual decline.

2. Total lipid analyses were carried out on eggs of two different ages; at 4-day intervals during larval development; and at weekly intervals on males and females during the adult stage. The total lipid was fractionated into neutral and phospholipids by column chromatography. Neutral lipid was separated into its components by column and thin-layer chromatography and phospholipid by two-dimensional thin-layer chromatography. Haemolymph lipid from 28-day-old adult males and females was analysed following the same procedure as for the body lipid. Fatty acid analysis by gas-liquid chromatography was carried out on two distinct stages of larval development. Determinations were made on total, neutral and phospholipids; insect food; neutral lipid components, and the total lipid of haemolymph.

3. The progress of total lipid change in terms of wet weight, was marked by fluctuations during the life cycle. Total lipid decreased sharply during egg development and continued through the first 4 days of the larval stage. This decrease was followed by a moderate increase for a short period, followed by a plateau during which no increase in total lipid was evident. Finally, a considerable increase in total lipid occurred between the 24th day of larval life and adult emergence. Total lipid change in the adult stage exhibited a sexual dimorphism. Females, after a sharp decline during the early egg laying period, regenerated their lipid content at 28 days and thereafter showed a slow decline. Total lipid of adult males declined in an erratic way with age.

4. Triglyceride formed the predominant fraction of neutral lipid and the pattern of change during the entire life cycle closely followed the pattern of total and neutral lipid, with the exception of the later part of the adult female stage. At this age a sharp decrease in the triglyceride fraction coincided with a sharp increase in free fatty acid content. Free fatty acid, the second largest component in the newly laid egg, gradually declined until about mid way through larval development, and remained more or less constant until near the end of the adult stage where it increased moderately in the male and sharply in the female.

6. Cholesterol, monoglyceride and diglyceride were found to be relatively stable fractions with only minor variations throughout the life cycle. Hydrocarbon and sterol ester were more or less constant until the adult stage when increases occurred. Hydrocarbon showed a greater increase in the male than in the female, and sterol ester exhibited the opposite trend.

7. Palmitic, stearic, oleic and linoleic acids were found to be the major fatty acids in the developing larvae, and together accounted for about 90% of the fatty acids. The fatty acid composition of the larvae reflected but was not identical with the fatty acid composition of the food.

8. Distinct differences were found in the fatty acid composition of the neutral lipid components at two larval ages (12-day-old- and 30-day-old-larvae). Differences in fatty acid content were also observed among the neutral lipid components themselves. Unique among the five fractions examined was a monoglyceride, which had a very high myristic acid concentration in the 12-day-old-larvae but which had decreased considerably in the 30-day-old-larvae. High concentrations of short-chain fatty acids were found in at least three fractions and considerable alterations in these concentrations were exhibited during development.

9. Changes in the components of phospholipid during the life cycle were also evident. Among the three major fractions, phosphatidylcholine was predominant, and showed a pattern of change which was very similar to the change observed with triglyceride, which suggests a close relationship between the two moieties. Phosphatidylethanolamine, the second largest component, was more stable: after an initial increase in the early period of egg development, it remained relatively constant during the entire life cycle. Sphingomyelin increased in the egg and early stages of larval life followed by a decrease midway through larval development until adult emergence, after which it remained nearly constant in both adult males and females.

10. Haemolymph lipid from 28-day-old adult male and female crickets exhibited a sexual dimorphism in total, neutral and phospholipid. Differences between the sexes were observed in the neutral and phospholipid components but the components differed considerably in concentration from the components found in the body lipid. A high concentration of hydrocarbon, free fatty acid and sterol ester, and a very low concentration of triglyceride, was found in the haemolymph. More phosphatidylcholine was found in the haemolymph than in the phospholipid of body lipid. The fatty acid content of the total lipid in the haemolymph was similar to the fatty acid of the total lipid in the developing larvae.

Table 2. Changes in wet weight during larval development.

Age (days)	Number	Mean weight (mg) Batch A	Batch B	Average of Batch A+B
0	3000	0.8	0.7	0.8
4	1100	1.9	1.9	1.9
8	450	5.0	4.7	4.8
12	230	9.0	10.4	9.7
16	150	17.8	18.5	18.2
20	100	18.6	32.9	25.8
24	85	40.8	52.0	46.4
28	60	56.0	83.0	69.5
32	30	126.0	121.0	123.5
36	25	189.0	206.0	198.0
40	20	254.0	221.0	238.0

FIGURE 2

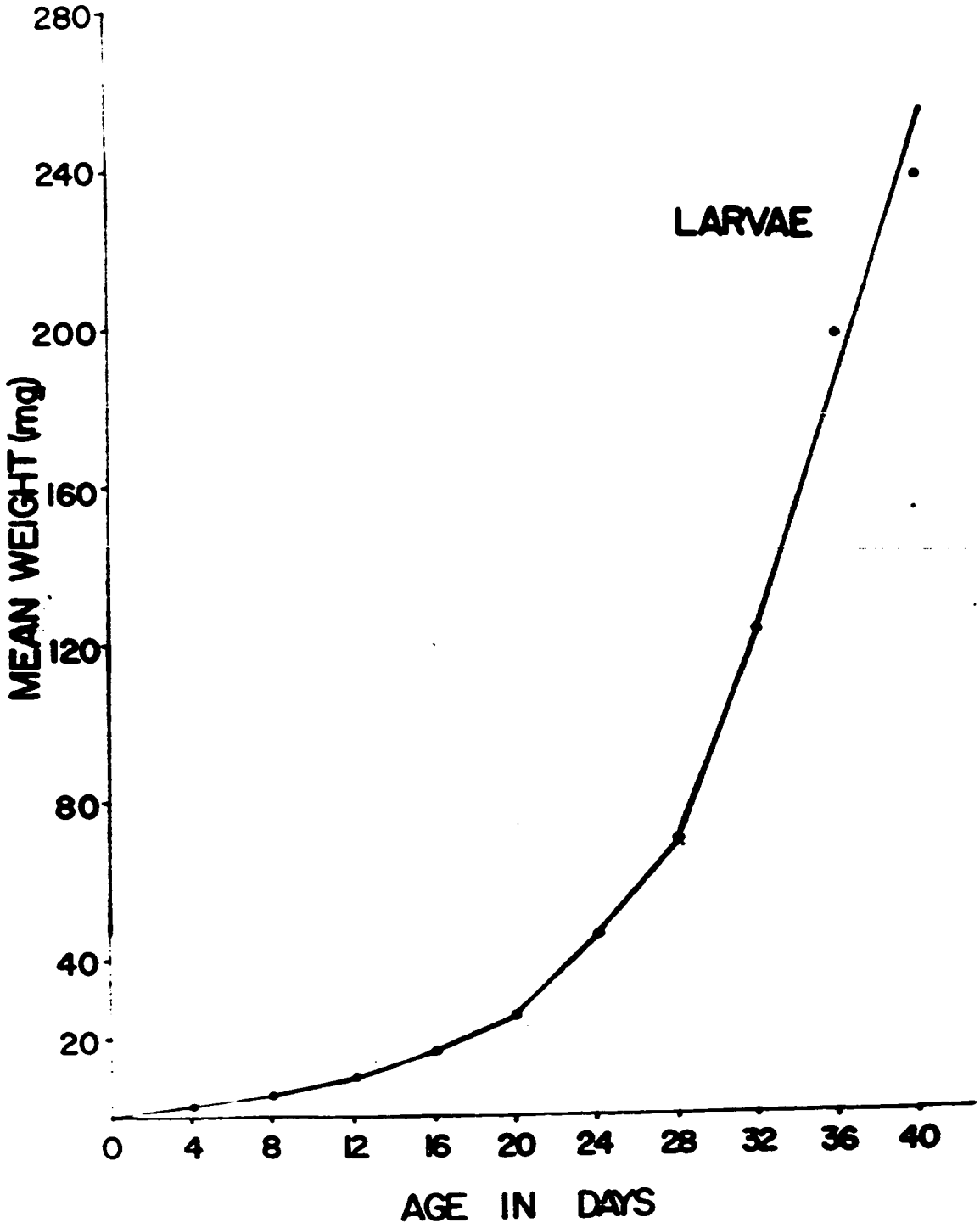

280
240
200
160
120
80
40
20
MEAN WEIGHT (mg)
LARVAE
0
4
8
12
16
20
24
28
32
36
40
AGE IN DAYS

Table 3. Changes with age in wet weight of adult male.

A Age (days)	Number	Mean weight (mg) Batch A	Batch B	Batch C	Average of Batch A+B+C
0	10	345	280	345	323
7	10	299	310	381	330
14	10	337	332	379	349
21	10	345	316	412	357
28	10	281	276	339	298
35	10	335	276	359	320
42	10	291	296	389	325
49	10	376	304	397	359
56	10	316	265	373	318

Table 4. Changes with age in wet weight of adult female.

Age (days)	Number	Mean weight (mg) Batch A	Batch B	Average of Batch A+B
0	10	335	334	335
7	10	385	419	402
14	10	429	424	427
21	10	472	460	466
28	10	515	384	449
35	10	518	452	485
42	10	505	425	465
49	10	479	408	444

FIGURE 3

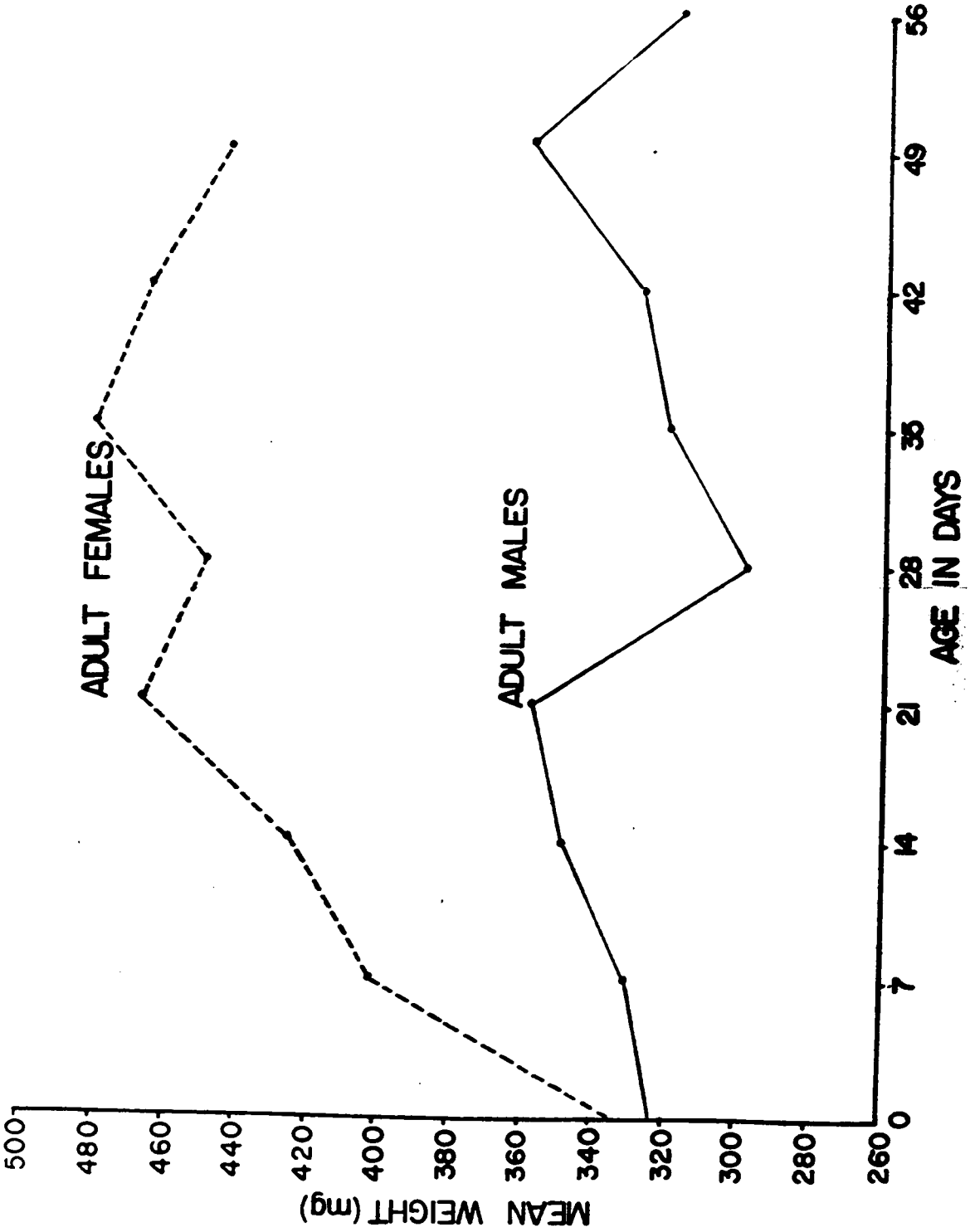

ADULT FEMALES
ADULT MALES
MEAN WEIGHT (mg)
AGE IN DAYS
500
480
460
440
420
400
380
360
340
320
300
280
260
0
7
14
21
28
35
42
49
56

Table 5. Wet weight, total lipid, neutral lipid and phospholipid during larval development.

	Age (days)	0	4	8	12	16	20	24	28	32	36	40
	Batch											
Number	A	3000	1100	450	230	150	100	70	40	20	15	10
	B	3000	1000	430	230	120	100	85	60	30	25	20
Wet weight (g)	A	1.88	1.97	1.98	2.06	3.58	4.15	5.43	4.76	4.66	3.99	4.84
	B	2.16	1.94	2.02	2.42	2.32	3.29	4.43	4.98	3.63	4.31	4.24
Weight total lipid (mg)	A	70.0	64.0	78.0	89.0	203.5	220.0	294.5	298.0	343.0	264.5	508.0
	B	97.5	76.5	96.0	152.0	127.0	176.5	238.0	325.0	239.0	301.0	328.0
Total lipid as % wet weight	A	3.71	3.25	3.93	4.32	5.68	5.30	5.42	6.26	7.36	6.62	10.49
	B	4.50	3.94	4.75	6.28	5.47	5.36	5.37	6.52	6.58	6.99	7.73
Weight neutral lipid (mg)	A	35.3	29.5	44.0	51.5	136.3	139.3	188.8	221.8	257.5	196.5	421.5
	B	45.3	36.3	52.3	95.5	75.5	112.8	163.3	238.0	185.5	235.0	265.5
Neutral lipid as % wet weight	A	1.87	1.50	2.22	2.50	3.80	3.35	3.47	4.65	5.52	4.92	8.70
	B	2.09	1.87	2.58	3.94	3.25	3.42	3.68	4.77	5.11	5.45	6.25
Weight phospho-lipid (mg)	A	32.5	32.5	32.8	34.8	62.0	70.5	99.3	72.8	74.3	62.8	70.8
	B	41.3	30.5	34.0	43.5	41.3	53.8	68.0	81.8	53.3	63.8	64.3
Phospholipid as % wet weight	A	1.72	1.65	1.65	1.68	1.73	1.69	1.82	1.52	1.59	1.57	1.50
	B	1.90	1.57	1.68	1.79	1.77	1.63	1.53	1.64	1.46	1.47	1.51

Table 6. Wet weight, total lipid, neutral lipid and phospholipid of the adult female.

	Age (days)	0	7	14	21	28	35	42	49
	Batch								
Number	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
Wet weight (g)	A	3.35	3.86	4.29	4.72	5.15	5.18	5.05	4.08
	B	3.34	4.12	4.24	4.72	3.84	4.52	4.25	4.79
Weight total lipid (mg)	A	386.5	331.0	293.5	345.0	477.5	470.0	422.5	323.0
	B	340.0	440.0	325.5	309.0	349.0	425.0	350.0	373.0
Total lipid as % wet weight	A	11.50	8.30	6.84	6.61	9.26	9.10	8.37	7.92
	B	10.20	10.50	7.67	6.63	9.08	9.40	8.23	7.78
Weight neutral lipid (mg)	A	328.8	252.0	217.5	253.8	363.8	365.5	323.3	290.0
	B	290.5	362.0	241.0	212.0	270.8	332.5	260.8	297.5
Neutral lipid as % wet weight	A	9.82	6.52	5.06	5.37	7.06	7.05	6.40	7.10
	B	8.71	8.64	5.68	4.54	7.05	7.35	6.14	6.20
Weight phospho-lipid (mg)	A	55.0	68.5	75.0	92.3	113.5	108.5	97.0	63.0
	B	47.0	90.8	74.5	87.3	78.0	94.3	88.0	66.5
Phospholipid as % wet weight	A	1.64	1.77	1.74	1.95	2.20	2.09	1.92	1.54
	B	1.40	2.16	1.75	1.87	2.03	2.08	2.07	1.38

Table 7. Wet weight, total lipid, neutral lipid and phospholipid of the adult male.

	Age (days)	0	7	14	21	28	35	42	49	56
	Batch									
Number	A	10	10	9	10	10	10	9	10	10
	B	10	10	10	10	10	10	10	10	10
Wet weight (g)	A	3.45	2.99	3.05	3.45	2.81	3.35	2.62	3.76	3.16
	B	2.80	3.09	3.92	3.16	2.76	2.76	2.96	3.04	2.65
Weight total lipid (mg)	A	332.5	275.5	257.5	277.5	175.0	196.0	175.0	272.5	220.0
	B	247.0	228.5	198.0	176.5	196.5	152.0	176.0	142.5	140.0
Total lipid as % wet weight	A	9.65	9.23	8.45	8.05	6.22	5.85	6.67	7.26	6.96
	B	8.83	7.39	5.96	5.58	7.11	5.50	5.94	4.68	5.29
Weight neutral lipid (mg)	A	225.3	210.0	193.0	209.0	116.0	131.3	126.5	176.0	162.0
	B	204.8	174.0	147.5	127.0	140.8	108.5	127.0	96.8	100.0
Neutral lipid as % wet weight	A	7.39	7.02	6.34	5.84	4.14	3.91	4.83	4.68	5.12
	B	7.32	5.63	4.43	4.01	5.09	3.93	4.29	3.18	3.78
Weight phospho-lipid (mg)	A	65.8	45.3	56.3	65.3	69.5	54.5	42.5	91.5	56.3
	B	48.5	56.8	57.5	53.0	52.8	52.5	51.5	44.0	38.0
Phospholipid as % wet weight	A	1.91	1.50	1.83	1.79	1.95	1.82	1.62	2.43	1.77
	B	1.73	1.83	1.73	1.67	1.91	1.90	1.73	1.44	1.43

FIGURE 4

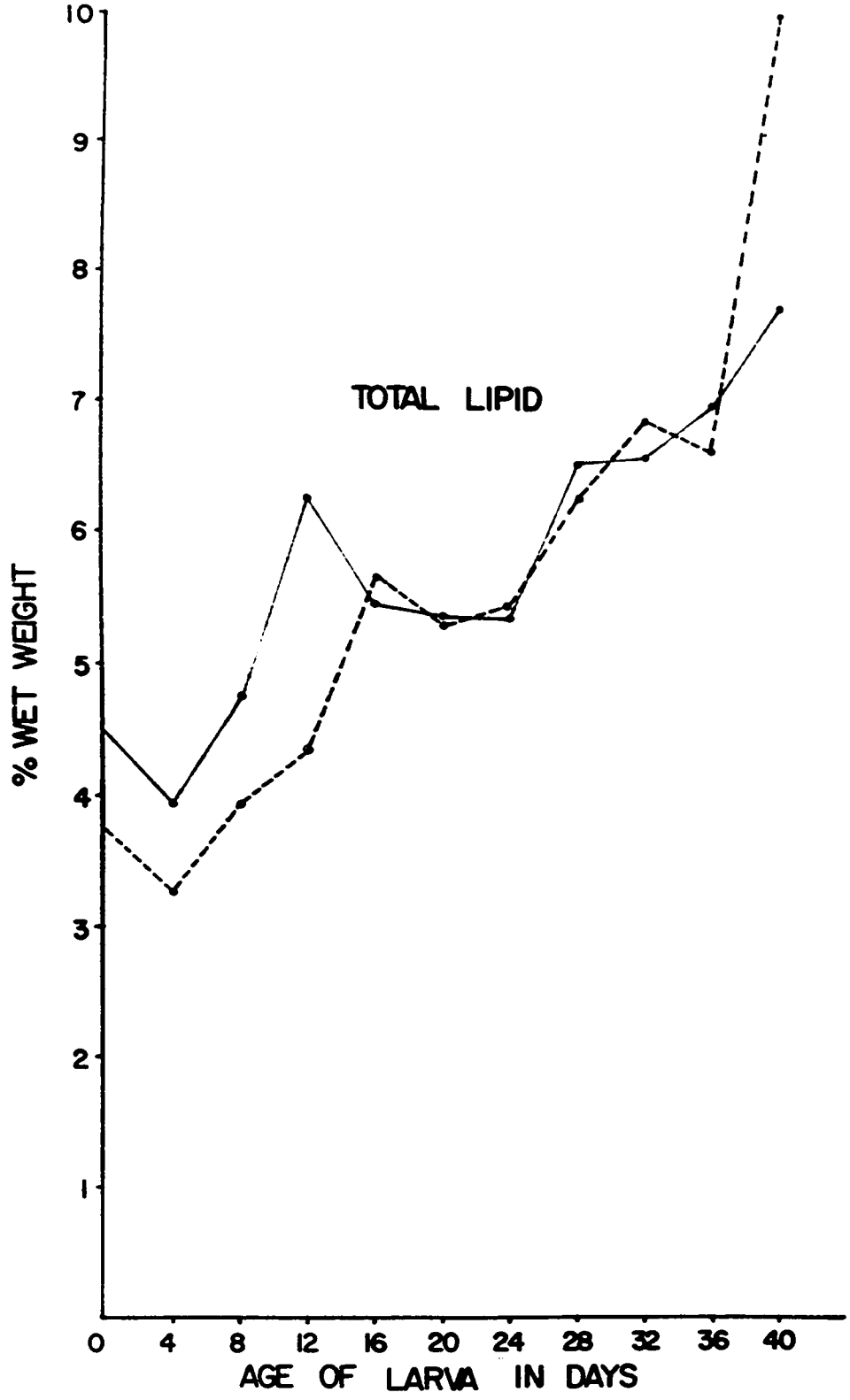

TOTAL LIPID
% WET WEIGHT
10
9
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
28
32
36
40
AGE OF LARVA IN DAYS

FIGURE 5

FIGURE 5.

Changes in total lipid content in adult males.

Broken line - Batch A

Solid line - Batch B

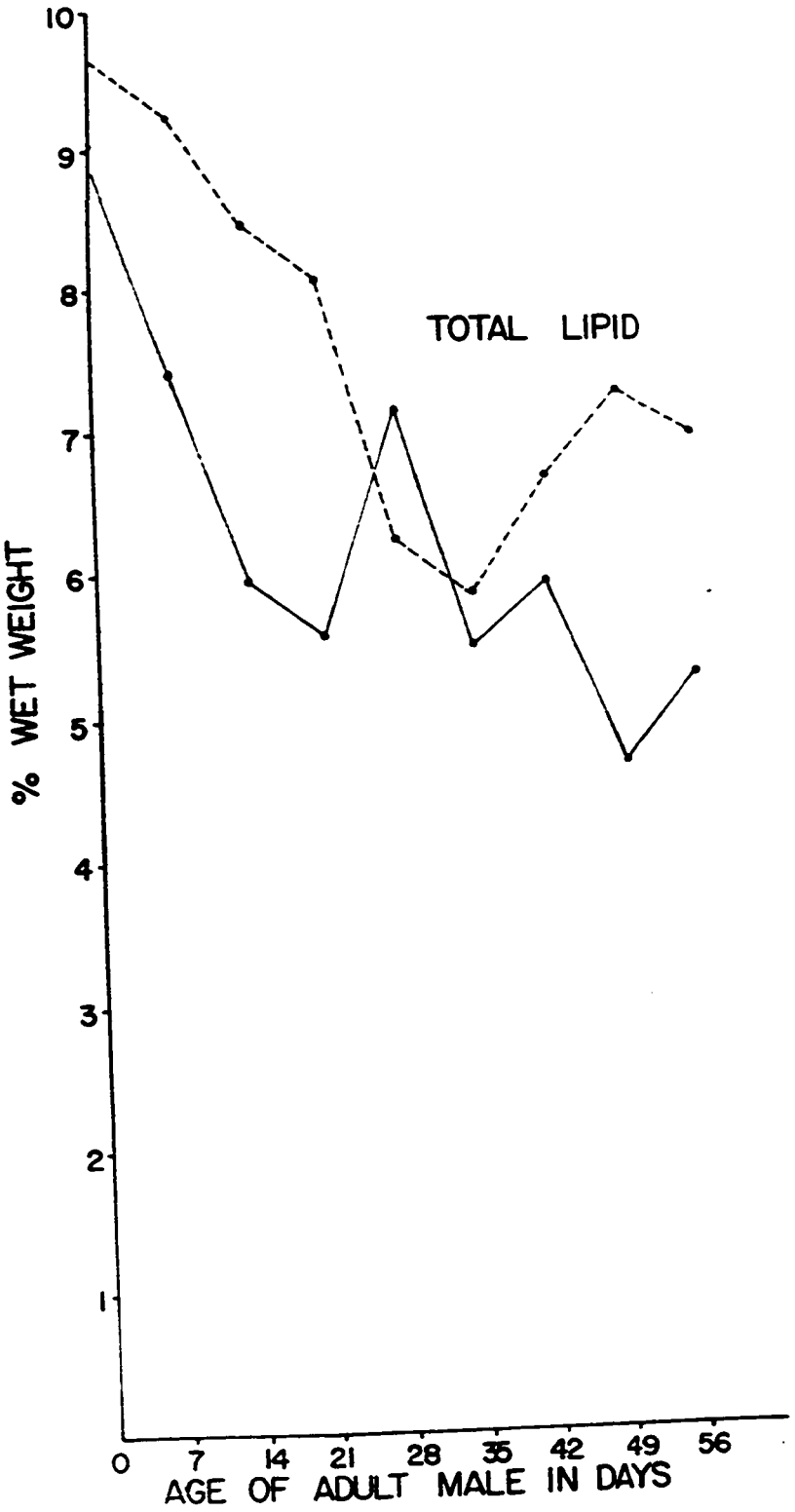

TOTAL LIPID
% WET WEIGHT
10
9
8
7
6
5
4
3
2
1
0
7
14
21
28
35
42
49
56
AGE OF ADULT MALE IN DAYS

FIGURE 6

FIGURE 6.

Changes in total lipid content in adult females.

Broken line - Batch A

Solid line - Batch B

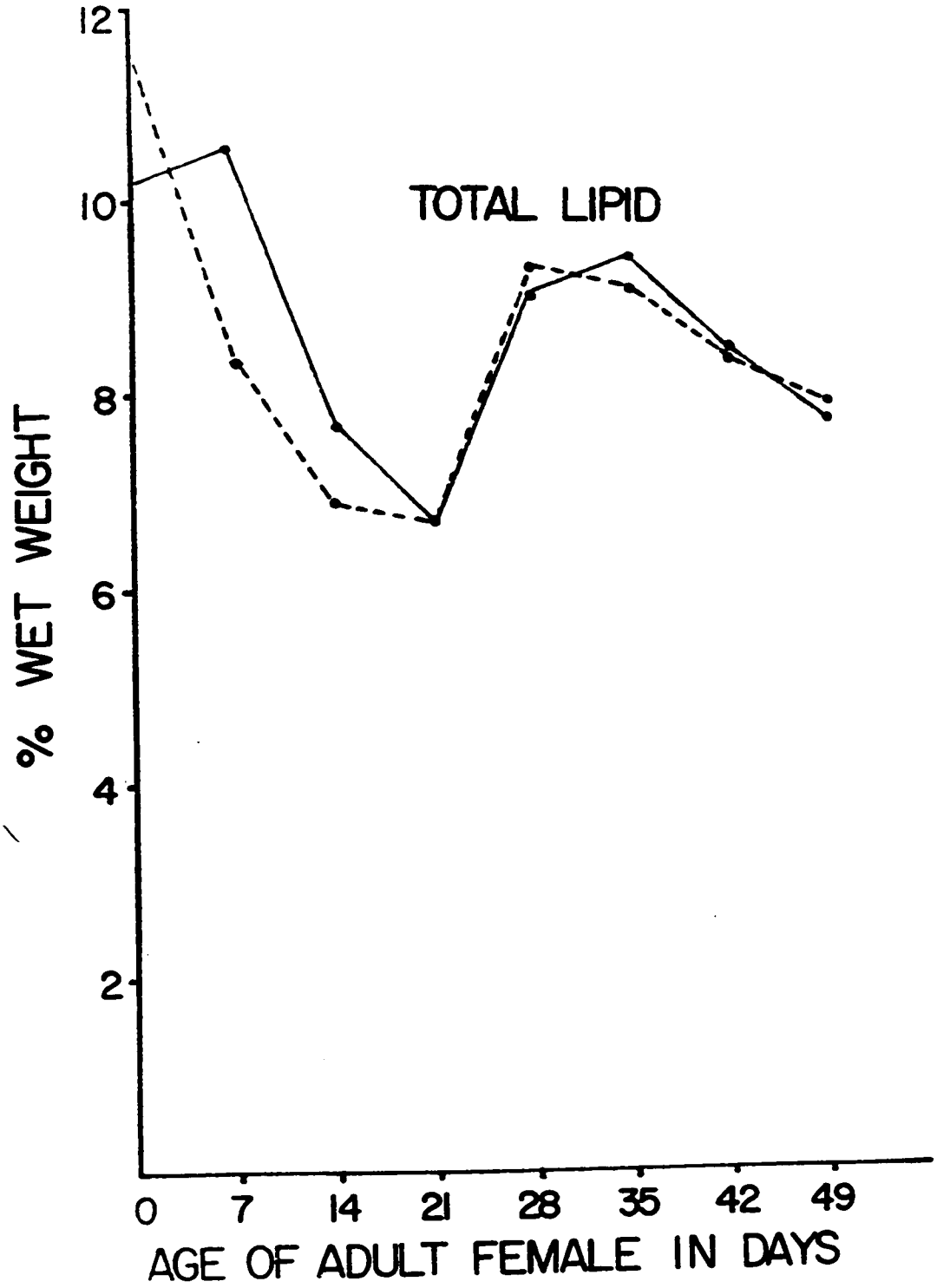

TOTAL LIPID
% WET WEIGHT
12
10
8
6
4
2
0
7
14
21
28
35
42
49
AGE OF ADULT FEMALE IN DAYS

FIGURE 7

FIGURE 7.

Changes in neutral lipid and phospholipid during larval development.

Broken line - Batch A

Solid line - Batch B

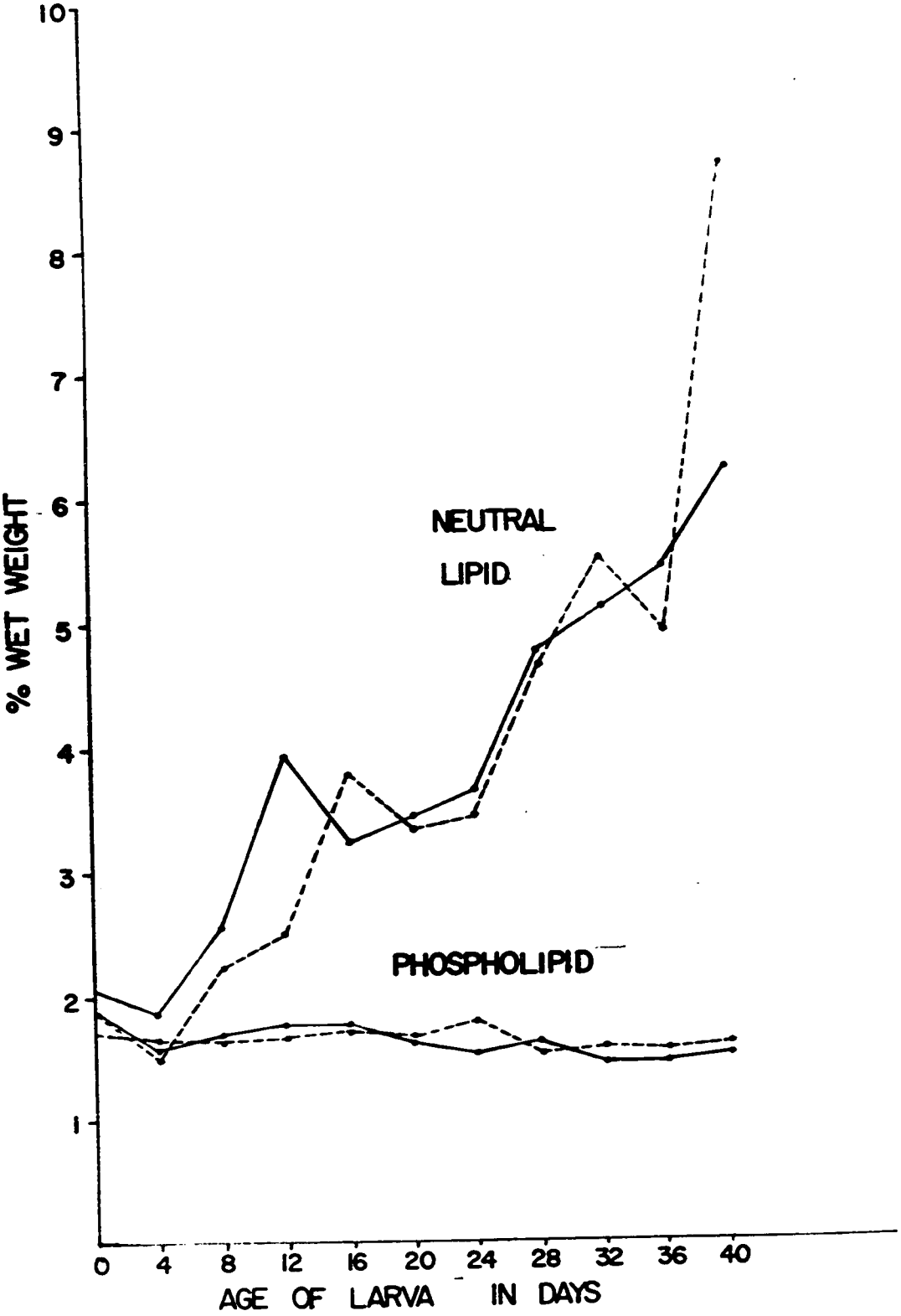

10
9
8
7
6
5
4
3
2
1
% WET WEIGHT
NEUTRAL
LIPID
PHOSPHOLIPID
0
4
8
12
16
20
24
28
32
36
40
AGE OF LARVA IN DAYS

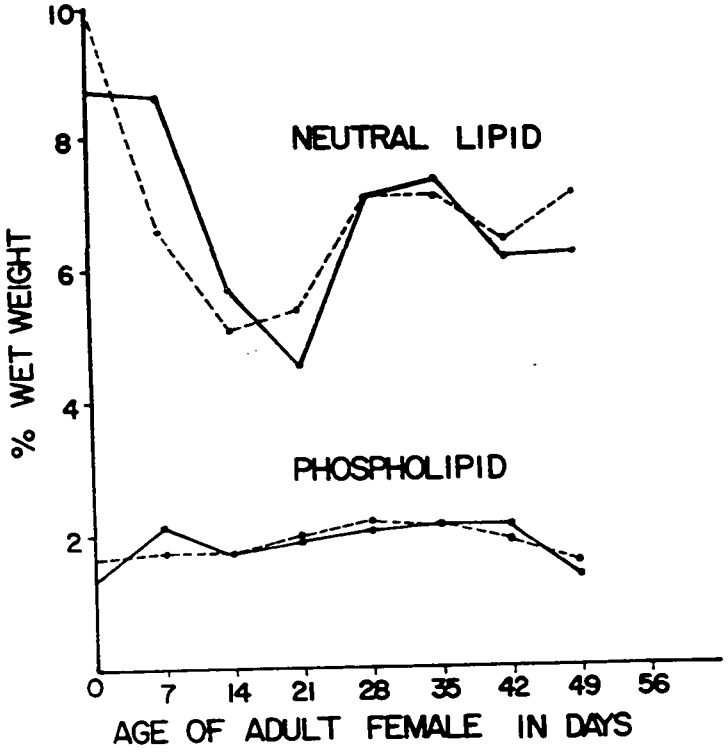
FIGURE 8

FIGURE 8.

Changes in neutral lipid and phospholipid in adult females and males.

Broken line - Batch A

Solid line - Batch B


10
8
6
4
2
% WET WEIGHT
NEUTRAL LIPID
PHOSPHOLIPID
0
7
14
21
28
35
42
49
56
AGE OF ADULT FEMALE IN DAYS

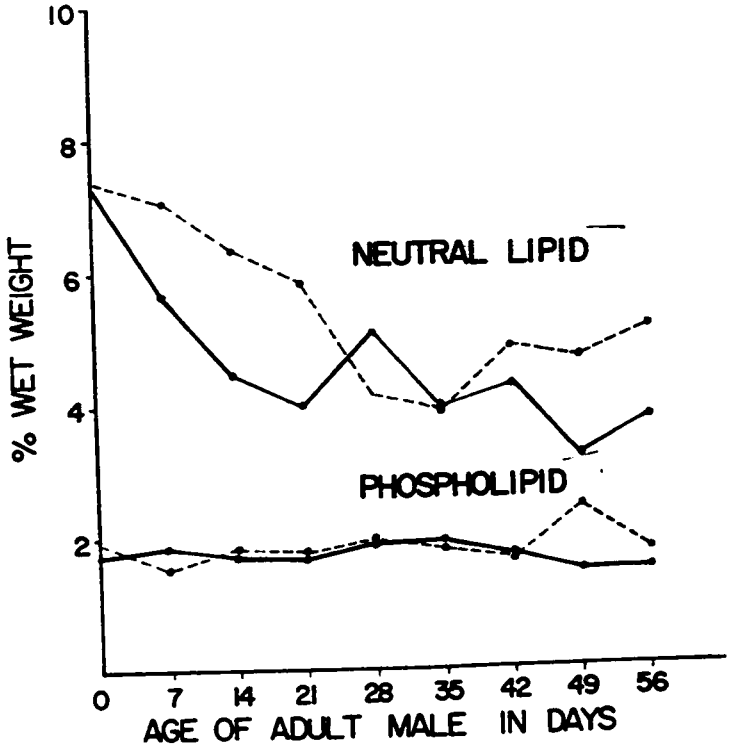

10
8
6
4
2
% WET WEIGHT
NEUTRAL LIPID
PHOSPHOLIPID
0
7
14
21
28
35
42
49
56
AGE OF ADULT MALE IN DAYS

FIGURE 8.

Changes in neutral lipid and phospholipid in adult females and males.

Broken line - Batch A

Solid line - Batch B

Table 8. Distribution of neutral lipid components during larval development.

	Age (days)	0	4	8	12	16	20	24	28	32	36	40
		(% wet weight)										
	Batch											
Hydrocarbon	A	0.10	0.10	0.15	0.16	0.21	0.14	0.16	0.24	0.19	0.19	0.26
	B	0.20	0.14	0.29	0.30	0.22	0.19	0.25	0.21	0.31	0.27	0.29
Sterol ester	A	0.12	0.10	0.11	0.11	0.08	0.11	0.08	0.10	0.26	0.11	0.36
	B	0.06	0.07	0.09	0.12	0.13	0.12	0.13	0.11	0.15	0.12	0.15
Triglyceride	A	1.12	0.82	1.43	1.66	2.93	2.66	2.81	3.82	4.50	4.06	7.39
	B	1.26	1.10	1.60	2.97	2.33	2.55	2.77	3.88	4.03	4.52	5.23
Cholesterol	A	0.16	0.12	0.15	0.16	0.19	0.18	0.17	0.19	0.24	0.22	0.35
	B	0.15	0.12	0.15	0.18	0.16	0.18	0.18	0.20	0.24	0.22	0.25
Diglyceride	A	0.08	0.06	0.09	0.11	0.11	0.07	0.09	0.10	0.10	0.14	0.14
	B	0.11	0.07	0.14	0.09	0.08	0.10	0.13	0.14	0.14	0.13	0.15
Monoglyceride	A	0.03	0.07	0.04	0.08	0.07	0.04	0.04	0.05	0.05	0.05	0.06
	B	0.06	0.07	0.08	0.07	0.09	0.06	0.07	0.07	0.07	0.08	0.05
Free fatty acid	A	0.26	0.25	0.27	0.26	0.21	0.15	0.13	0.16	0.18	0.18	0.14
	B	0.26	0.30	0.24	0.23	0.24	0.24	0.17	0.15	0.17	0.13	0.15

Table 9. Distribution of neutral lipid components in the adult male.

	Age (days)	0	7	14	21	28	35	42	49	56
		(% wet weight)								
	Batch									
Hydrocarbon	A	0.40	0.43	0.45	0.63	0.58	0.59	0.75	0.67	0.97
	B	0.27	0.35	0.35	0.51	0.61	0.64	0.63	0.51	0.93
Sterol ester	A	0.22	0.25	0.29	0.19	0.44	0.28	0.26	0.40	0.48
	B	0.13	0.23	0.23	0.20	0.33	0.31	0.23	0.25	0.41
Triglyceride	A	6.17	4.58	4.25	3.25	1.55	2.50	2.50	2.57	2.61
	B	6.23	4.39	3.15	2.60	3.38	2.20	2.72	1.40	1.30
Cholesterol	A	0.36	0.31	0.39	0.30	0.25	0.29	0.33	0.33	0.44
	B	0.23	0.24	0.27	0.25	0.24	0.20	0.23	0.19	0.28
Diglyceride	A	0.07	0.05	0.07	0.08	0.06	0.07	0.11	0.13	0.11
	B	0.18	0.15	0.21	0.18	0.17	0.16	0.14	0.09	0.11
Monoglyceride	A	0.07	0.09	0.10	0.07	0.07	0.07	0.09	0.10	0.10
	B	0.10	0.09	0.09	0.11	0.12	0.09	0.05	0.05	0.05
Free fatty acid	A	0.16	0.18	0.17	0.15	0.32	0.13	0.22	0.25	0.39
	B	0.19	0.20	0.19	0.19	0.22	0.23	0.31	0.47	0.69

Table 10. Distribution of neutral lipid components in the adult female.

Age (days)		0	7	14	21	28	35	42	49
		(% wet weight)							
	Batch								
Hydrocarbon	A	0.35	0.39	0.32	0.44	0.38	0.37	0.44	0.51
	B	0.33	0.30	0.29	0.31	0.33	0.43	0.31	0.28
Sterol ester	A	0.32	0.19	0.19	0.22	0.43	0.44	0.41	0.67
	B	0.33	0.40	0.27	0.14	0.32	0.43	0.37	0.43
Triglyceride	A	8.48	5.41	3.94	4.05	5.56	5.01	4.44	1.37
	B	7.58	7.12	4.39	3.38	5.69	5.40	2.38	1.88
Cholesterol	A	0.33	0.26	0.27	0.21	0.29	0.43	0.42	0.38
	B	0.18	0.37	0.33	0.31	0.32	0.39	0.56	0.32
Diglyceride	A	0.12	0.16	0.12	0.20	0.18	0.21	0.24	0.10
	B	0.07	0.13	0.11	0.11	0.13	0.12	0.24	0.08
Monoglyceride	A	0.14	0.05	0.06	0.08	0.09	0.08	0.06	0.06
	B	0.05	0.08	0.05	0.07	0.07	0.03	0.05	0.05
Free fatty acid	A	0.16	0.12	0.15	0.19	0.15	0.70	0.41	4.05
	B	0.15	0.19	0.24	0.24	0.22	0.55	2.19	3.13

FIGURE 9

FIGURE 9.

Thin-layer chromatogram of neutral lipid components of 35-day-old adult male house cricket.

HC = hydrocarbon; SE = sterolester; TG = triglyceride; FFA = free fatty acid; DG = diglyceride; C = cholesterol; MG = monoglyceride; O = origin.

The plate was coated with Silica Gel G and developed to a height of 16 cm in petroleum ether : ether : acetic acid (70 : 30: 1, v/v), air dried and developed in the same direction to a height of 11 cm in ether : petroleum ether : acetic acid (70 : 30 : 1, v/v). Spots were located in an iodine atmosphere and by spraying with 50% H_2SO_4 and heating at 175°C for 25-30 min.

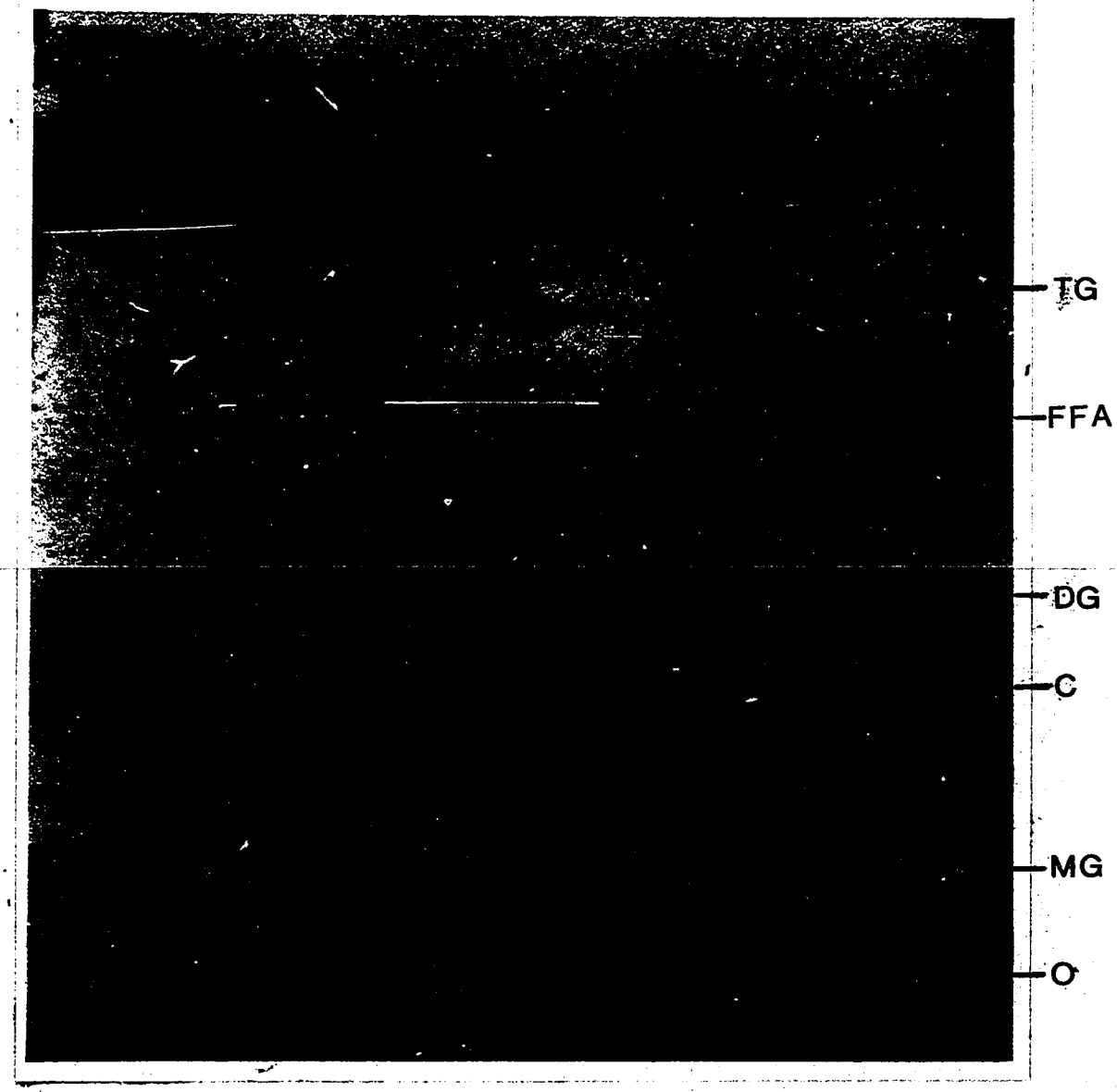

TG
FFA
DG
C
MG
O

FIGURE 10.

Changes in triglyceride content during larval development.

Broken line - Batch A

Solid line - Batch B

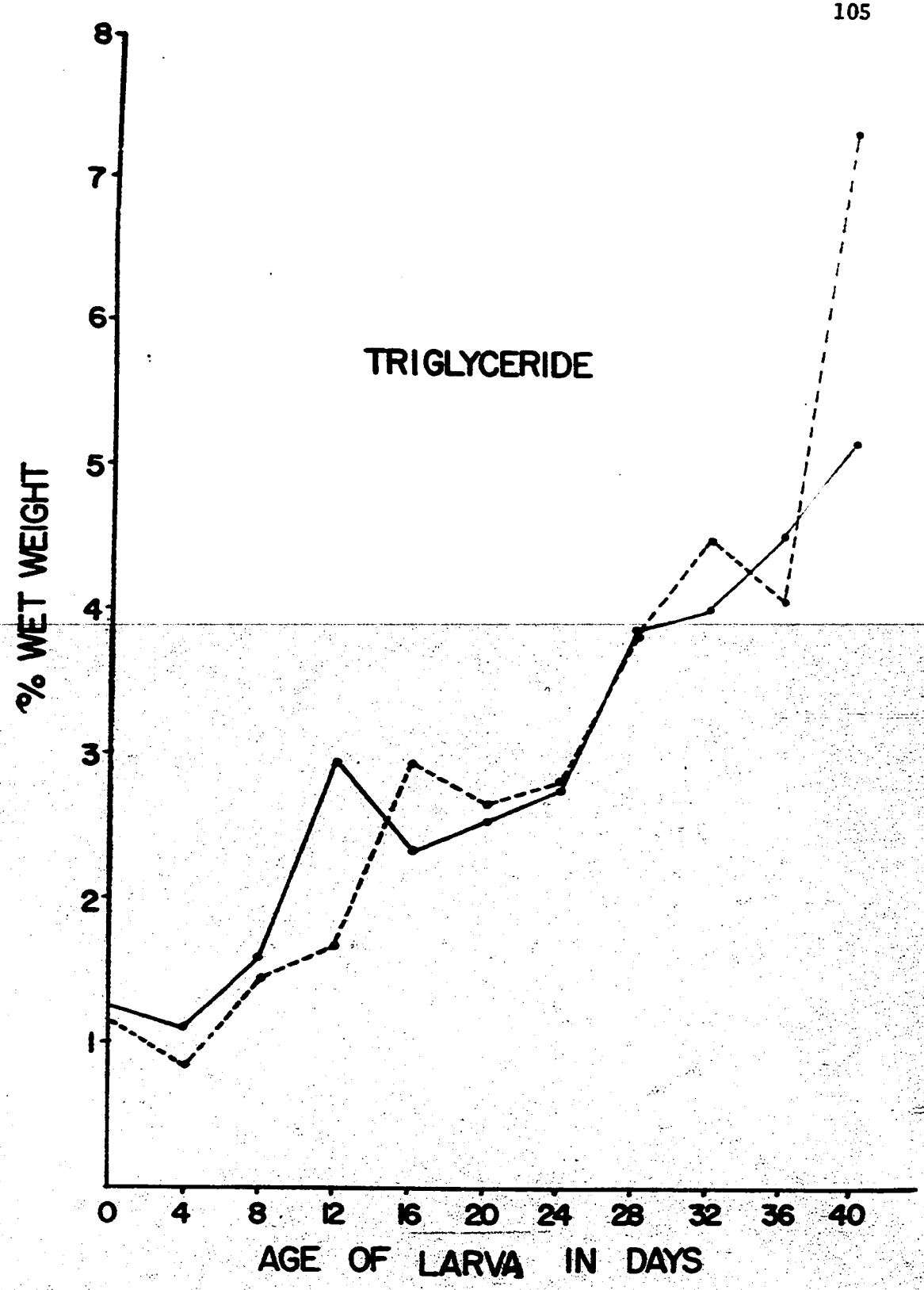

TRIGLYCERIDE
% WET WEIGHT
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
28
32
36
40
AGE OF LARVA IN DAYS

FIGURE 11.

Changes in triglyceride content in adult males.

Broken line - Batch A

Solid line - Batch B

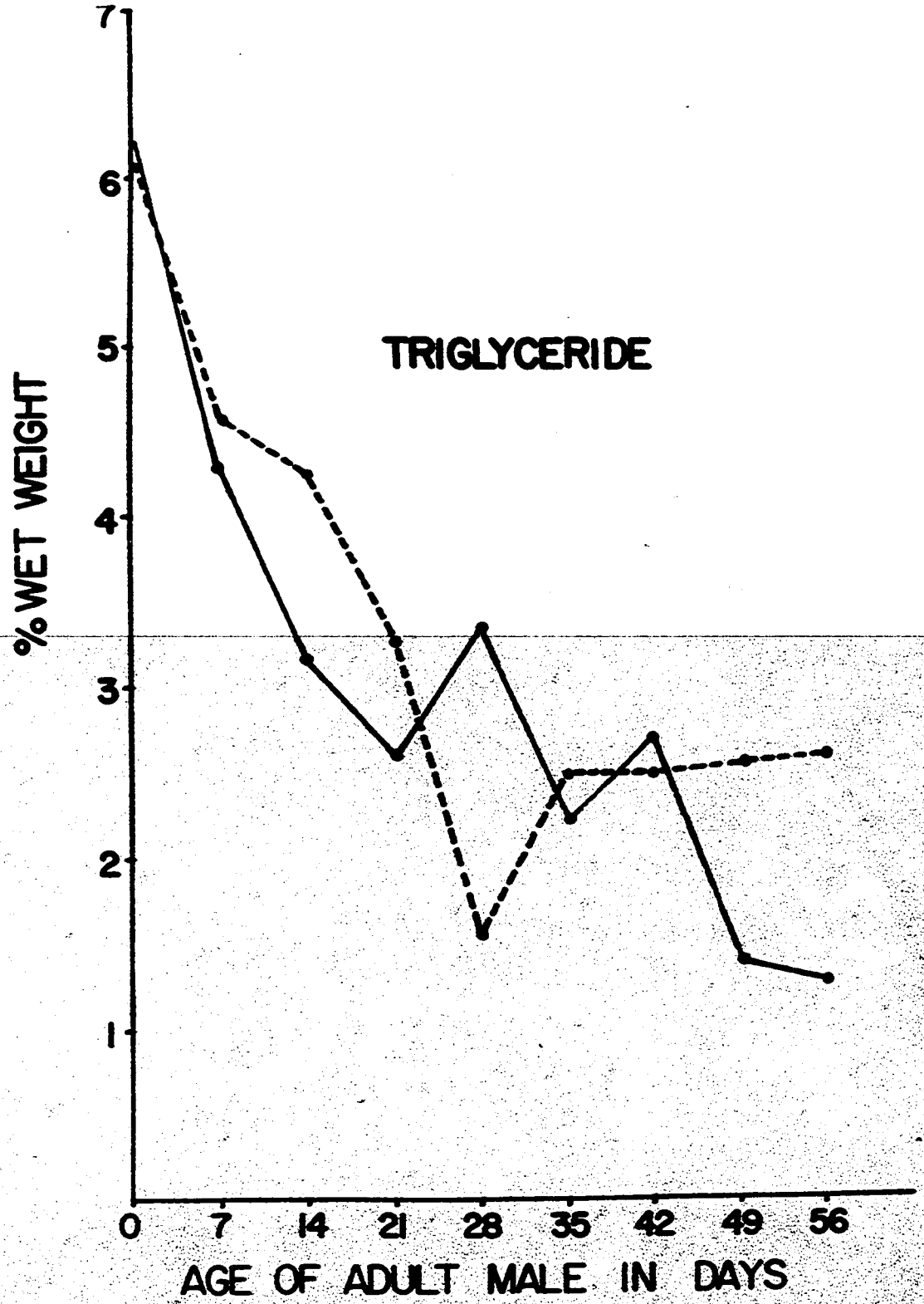

TRIGLYCERIDE
% WET WEIGHT
7
6
5
4
3
2
1
0
7
14
21
28
35
42
49
56
AGE OF ADULT MALE IN DAYS

FIGURE 12

FIGURE 12.

Changes in triglyceride content in adult females.

Broken line - Batch A

Solid line - Batch B

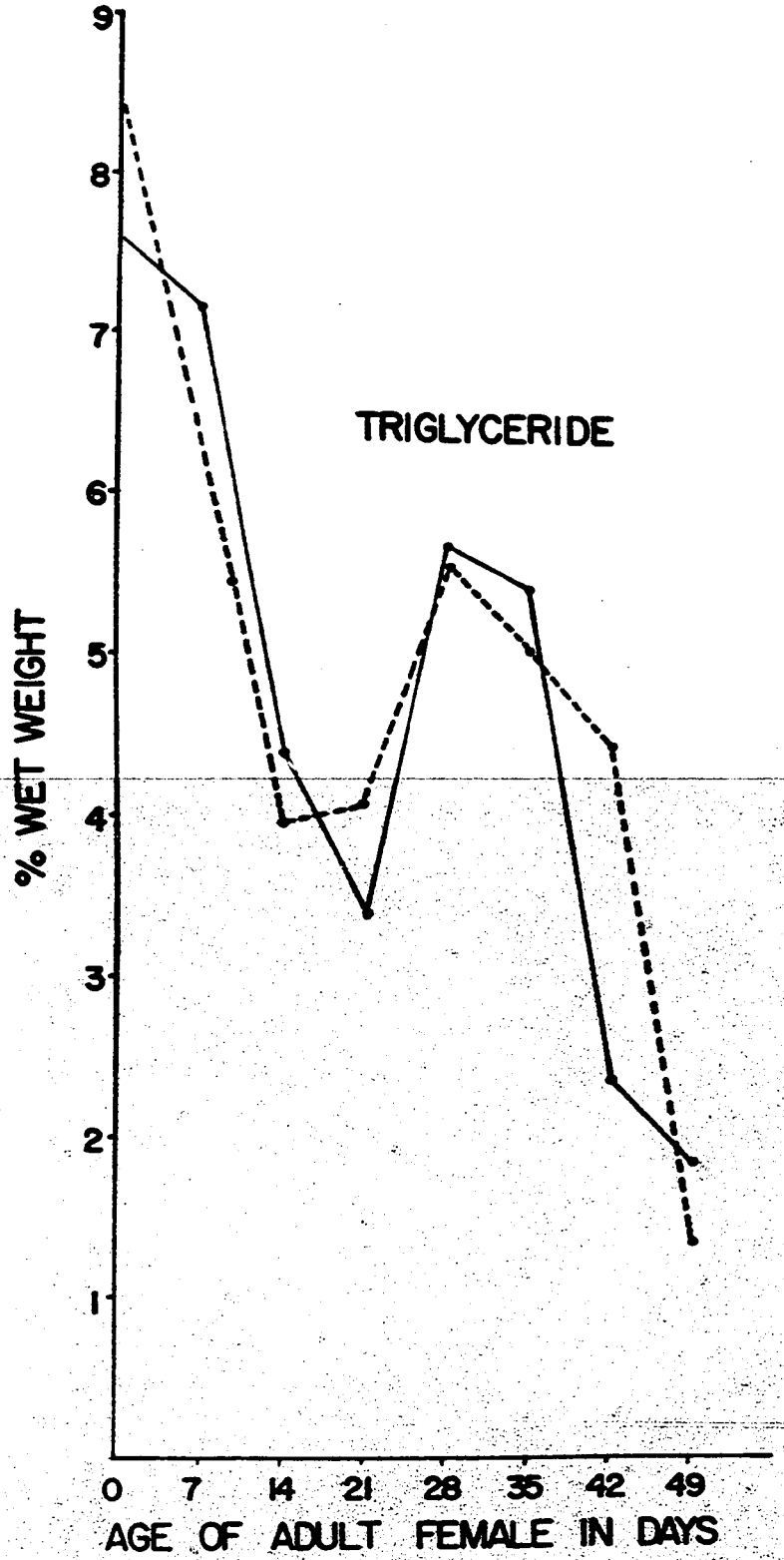

TRIGLYCERIDE
% WET WEIGHT
9
8
7
6
5
4
3
2
1
0
7
14
21
28
35
42
49
AGE OF ADULT FEMALE IN DAYS

FIGURE 13

FIGURE 13.

Changes in neutral lipid components during larval development.

Broken line - Batch A

Solid line - Batch B

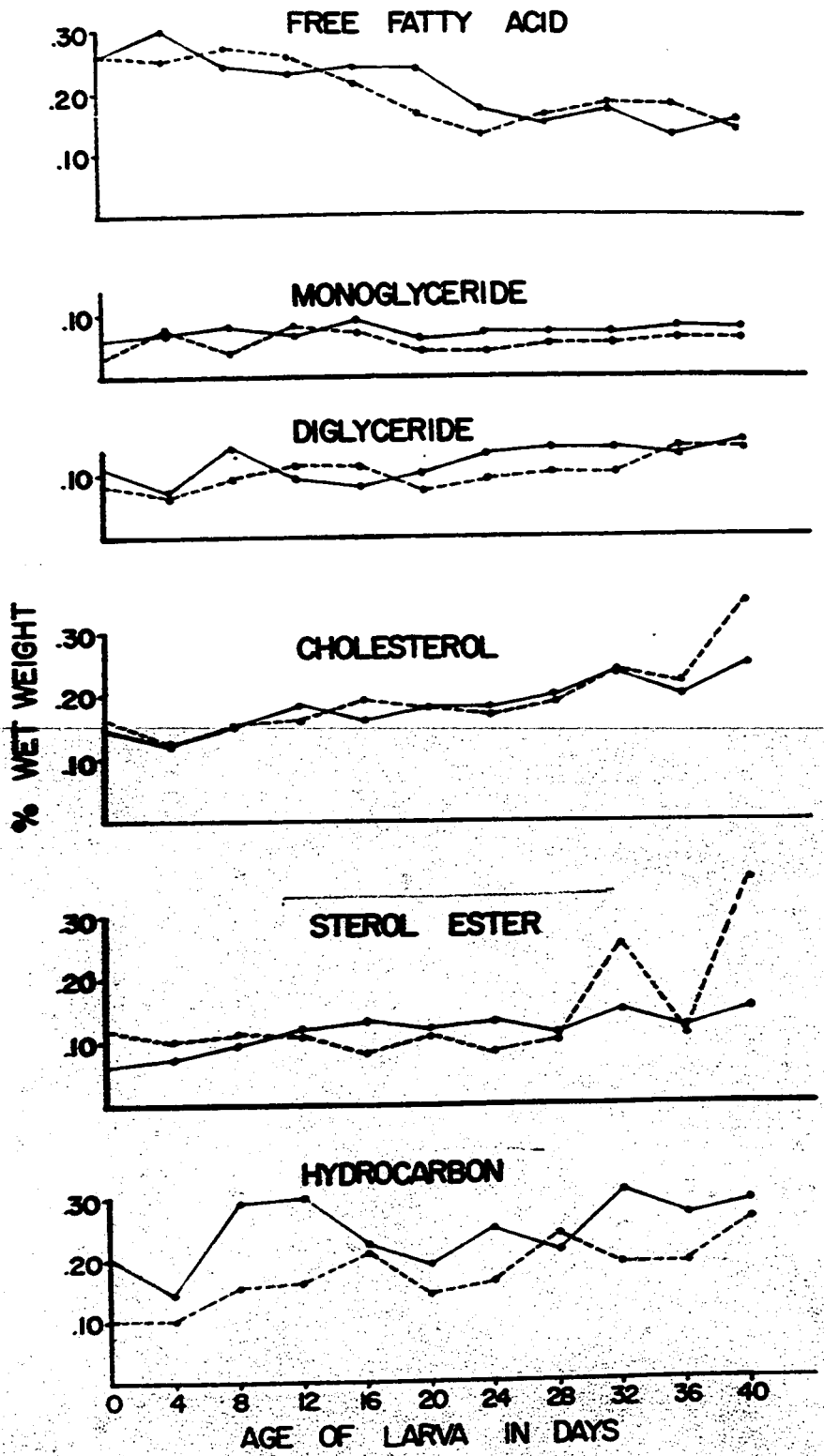

FREE FATTY ACID
.30
.20
.10
MONOGLYCERIDE
.10
DIGLYCERIDE
.10
% WET WEIGHT
CHOLESTEROL
.30
.20
.10
STEROL ESTER
30
20
10
HYDROCARBON
30
.20
.10
0 4 8 12 16 20 24 28 32 36 40
AGE OF LARVA IN DAYS

FIGURE 14

FIGURE 14.

Changes in free fatty acid, monoglyceride and diglyceride fractions in adult males.

Broken line - Batch A

Solid line - Batch B

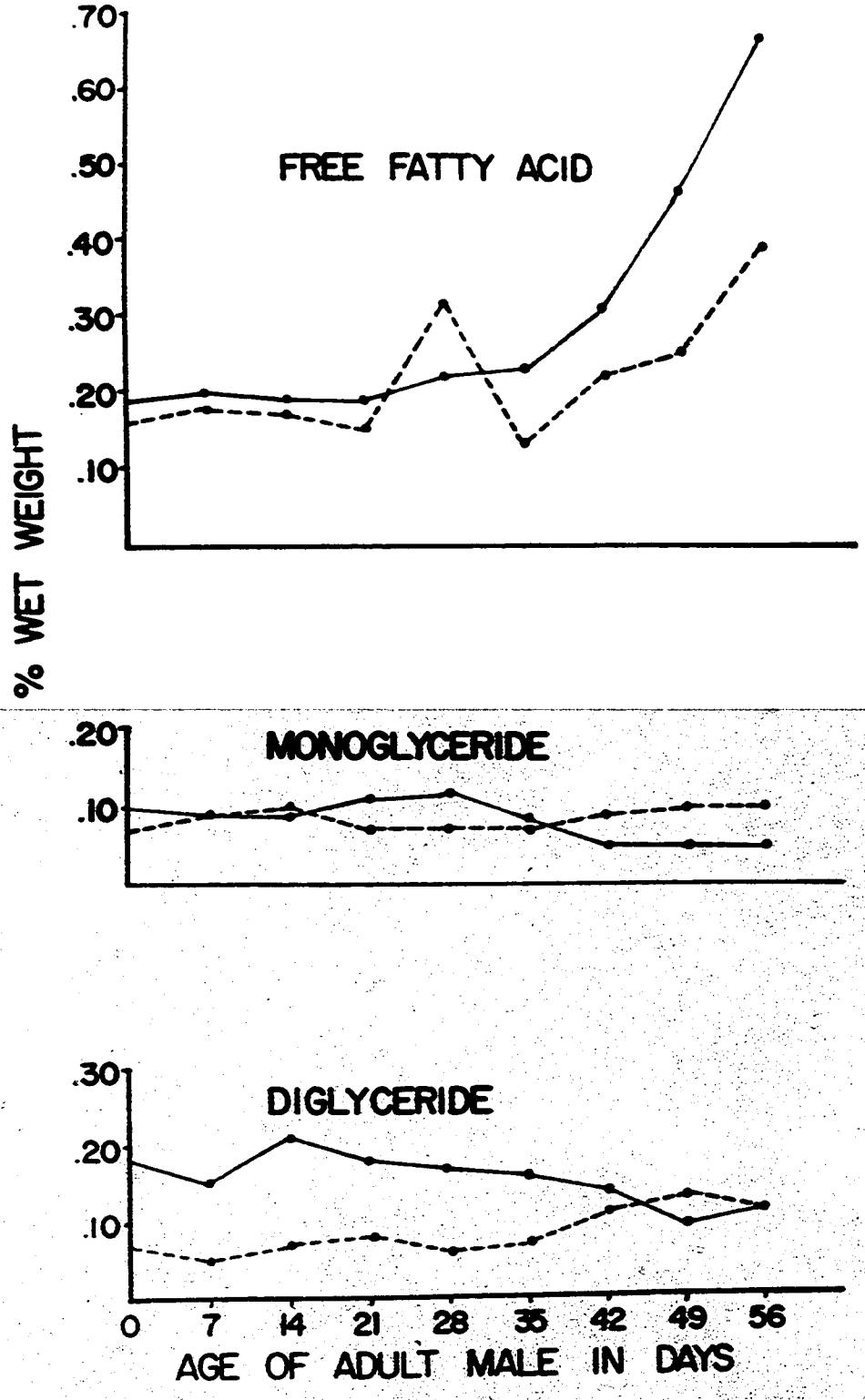

.70
.60
.50
.40
.30
.20
.10
FREE FATTY ACID
% WET WEIGHT
.20
.10
MONOGLYCERIDE
.30
.20
.10
DIGLYCERIDE
0
7
14
21
28
35
42
49
56
AGE OF ADULT MALE IN DAYS

FIGURE 15

FIGURE 15.

Changes in free fatty acid, monoglyceride and diglyceride fractions in adult females.

Broken line - Batch A

Solid line - Batch B

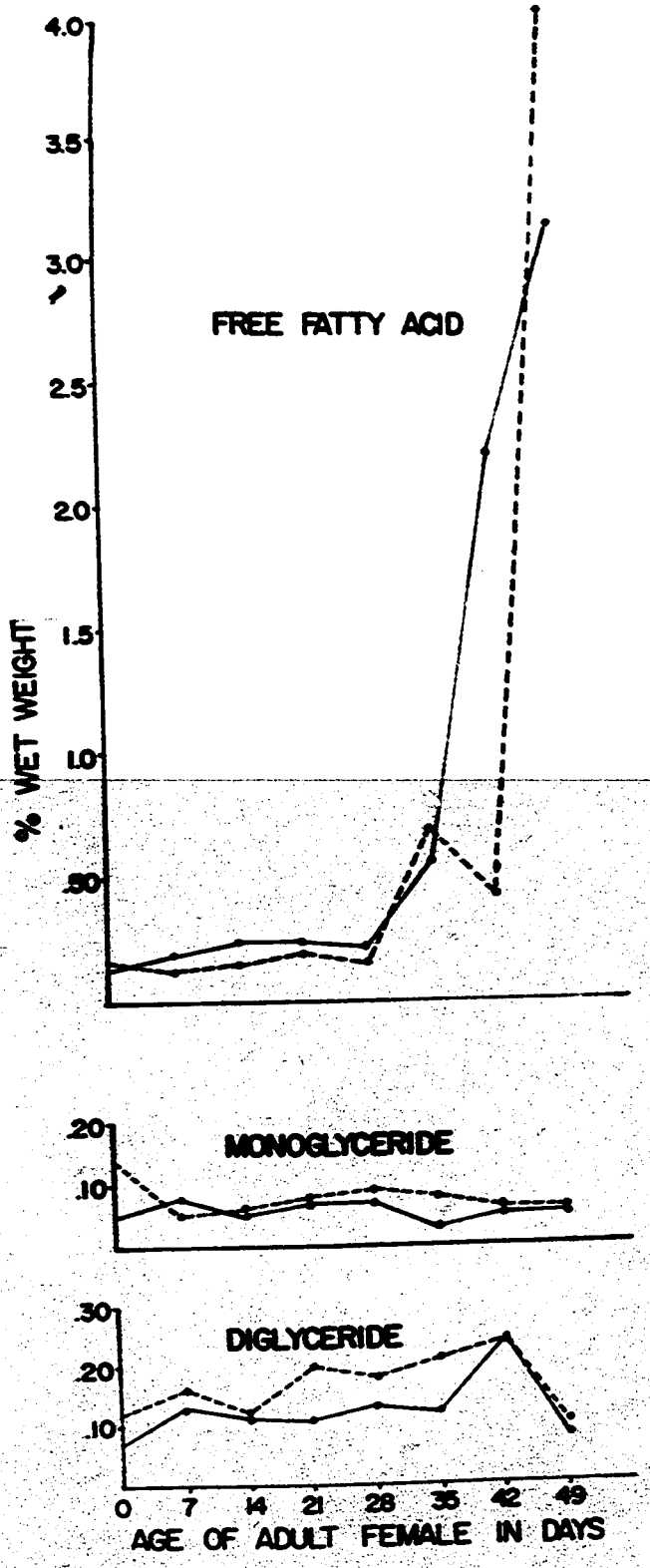

4.0
3.5
3.0
2.5
2.0
1.5
1.0
.50
% WET WEIGHT
FREE FATTY ACID
.20
.10
MONOGLYCERIDE
.30
.20
.10
DIGLYCERIDE
0
7
14
21
28
35
42
49
AGE OF ADULT FEMALE IN DAYS

FIGURE 16

FIGURE 16.

Changes in cholesterol, sterol ester and hydrocarbon in adult males.

Broken line - Batch A

Solid line - Batch B

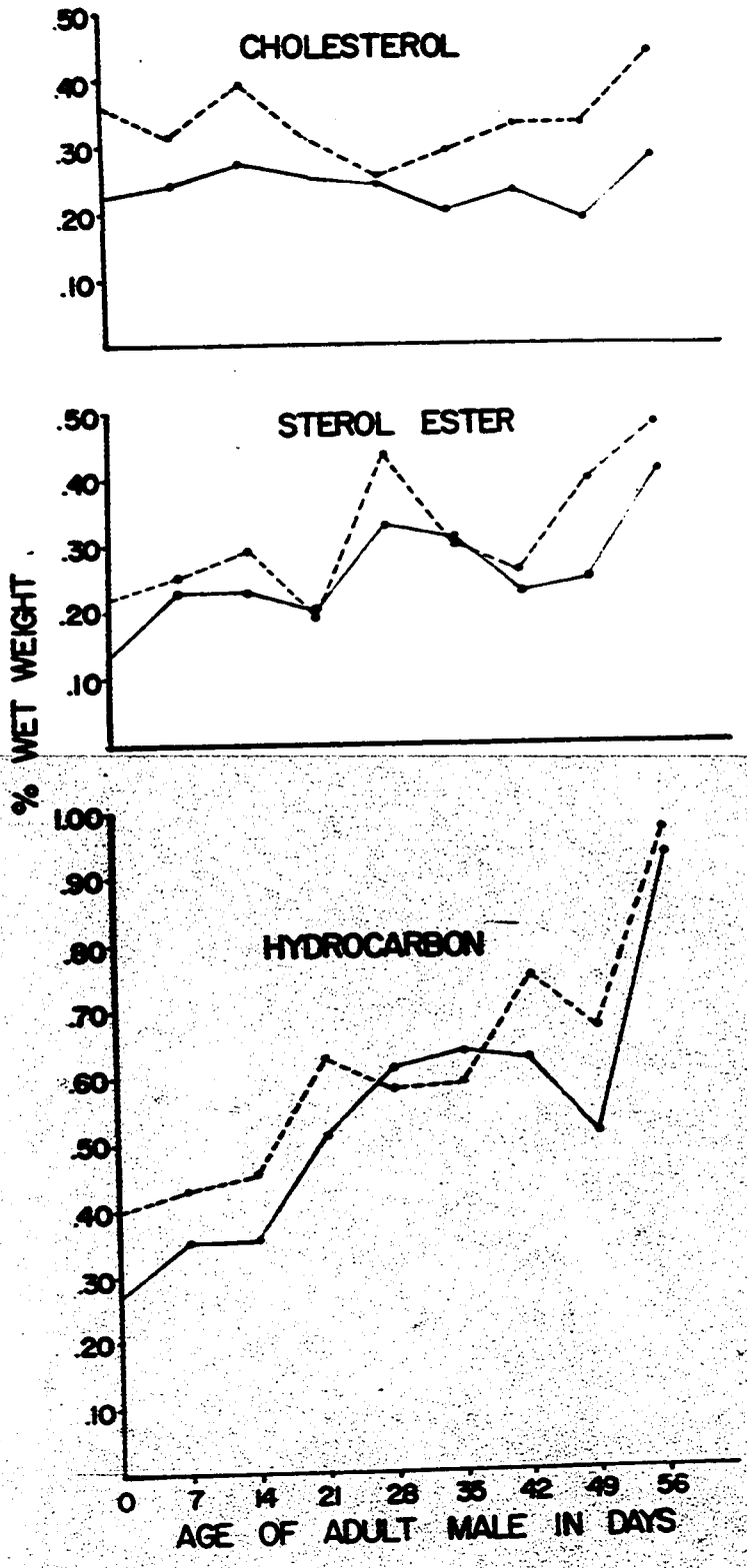

CHOLESTEROL
.50
.40
.30
.20
.10
STEROL ESTER
.50
.40
.30
.20
.10
% WET WEIGHT
HYDROCARBON
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
7
14
21
28
35
42
49
56
AGE OF ADULT MALE IN DAYS

FIGURE 17

FIGURE 17

Changes in cholesterol, sterol ester and hydrocarbon in adult females.

Broken line - Batch A

Solid line - Batch B

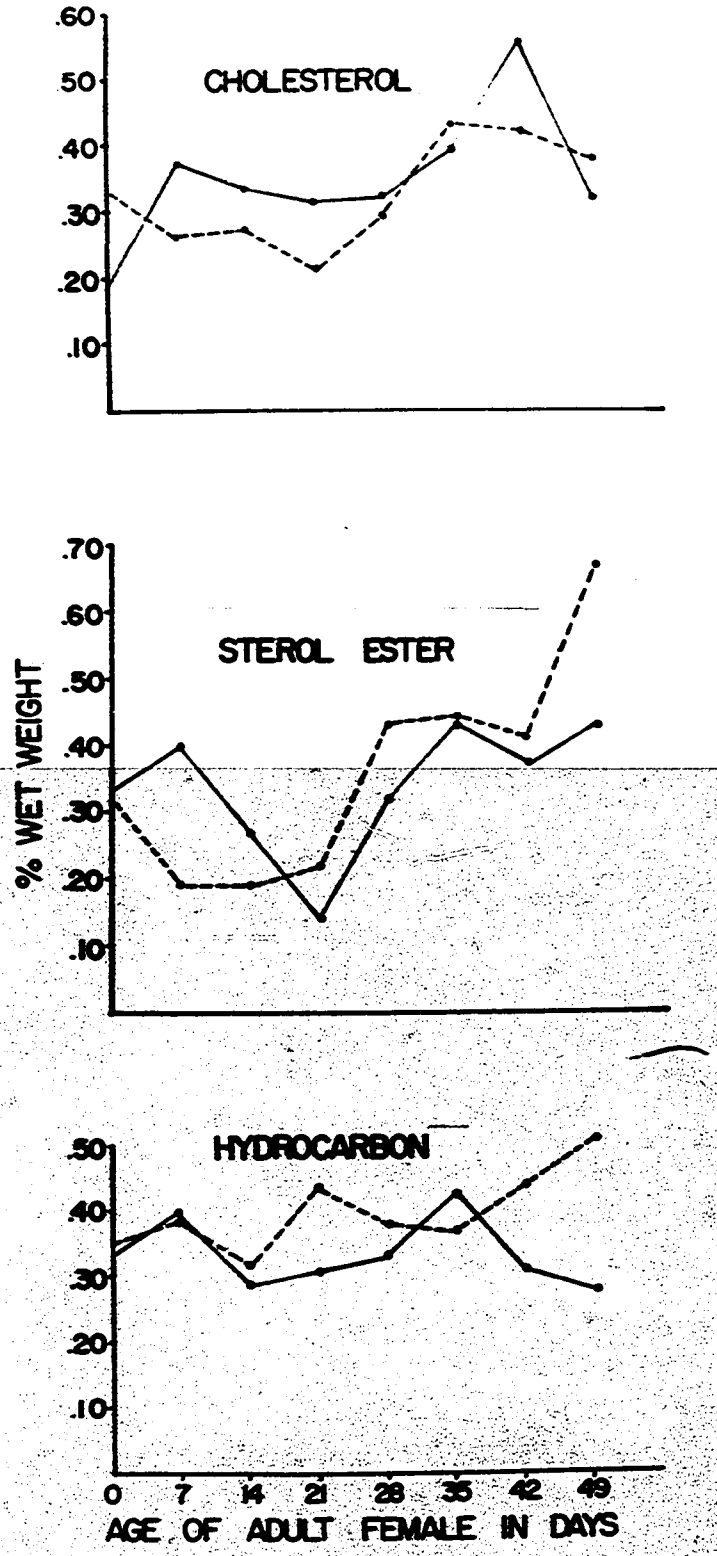

.60
.50
.40
.30
.20
.10
CHOLESTEROL
.70
.60
.50
.40
.30
.20
.10
% WET WEIGHT
STEROL ESTER
.50
.40
.30
.20
.10
HYDROCARBON
0
7
14
21
28
35
42
49
AGE OF ADULT FEMALE IN DAYS

Table 11. Distribution of major lipid fractions during embryogenesis.

Age (days)	Lipid content (% wet weight)			Lipid content (% total lipid)	
	Total lipid	Neutral lipid	Phospho-lipid	Neutral lipid	Phospho-lipid
0	18.22	15.95	2.25	87.5	12.3
7	7.60	6.25	1.29	82.2	17.0
Newly-hatched larvae	4.11	1.95	1.81	47.7	44.0

Table 12. Changes in the distribution of neutral lipid components during embryogenesis.

	(% wet weight)			(% neutral lipid)		
	Age (days)			Age (days)		
	0	7	0-day larvae	0	7	0-day larvae
Hydrocarbon	0.33	0.23	0.15	2.1	3.5	7.4
Sterol ester	0.29	0.28	0.09	1.8	4.6	3.3
Triglyceride	13.50	4.80	1.20	85.4	80.1	60.0
Cholesterol	0.39	0.21	0.16	2.6	2.7	7.8
Monoglyceride	0.44	0.13	0.10	2.8	2.2	4.8
Diglyceride	0.17	0.10	0.05	1.0	1.7	2.1
Free fatty acid	0.78	0.31	0.26	4.9	5.4	13.1

Table 13. Changes in the distribution of phospholipid during the life cycle.

Stage	Age (days)	Distribution of major fractions (% of total phospholipid)			
		S	PC	PE	SL
Egg	0	5.4	64.3	23.7	6.6
	7	13.0	50.7	23.8	0.9
Larva	0	11.4	38.7	30.4	3.6
	4	16.2	30.4	30.5	9.1
	16	15.7	40.9	29.6	0.3
	28	9.4	46.5	31.5	4.7
	40	7.9	46.2	31.9	3.6
Adult female	0	8.2	45.2	31.6	0.9
	21	8.2	49.7	32.8	1.3
	42	8.7	49.1	28.9	1.1
	49	13.9	44.6	31.1	0.7
Adult male	0	10.8	48.0	31.8	4.8
	21	9.0	46.2	28.4	4.6
	42	8.8	43.9	36.2	5.6
	56	14.4	37.0	29.0	3.7

S = Sphingomyelin. PC = Phosphatidylcholine.

PE = Phosphatidylethanolamine. SL = Solvent line components.

FIGURE 18

FIGURE 18

Changes in phospholipid components during the life cycle of the house cricket.

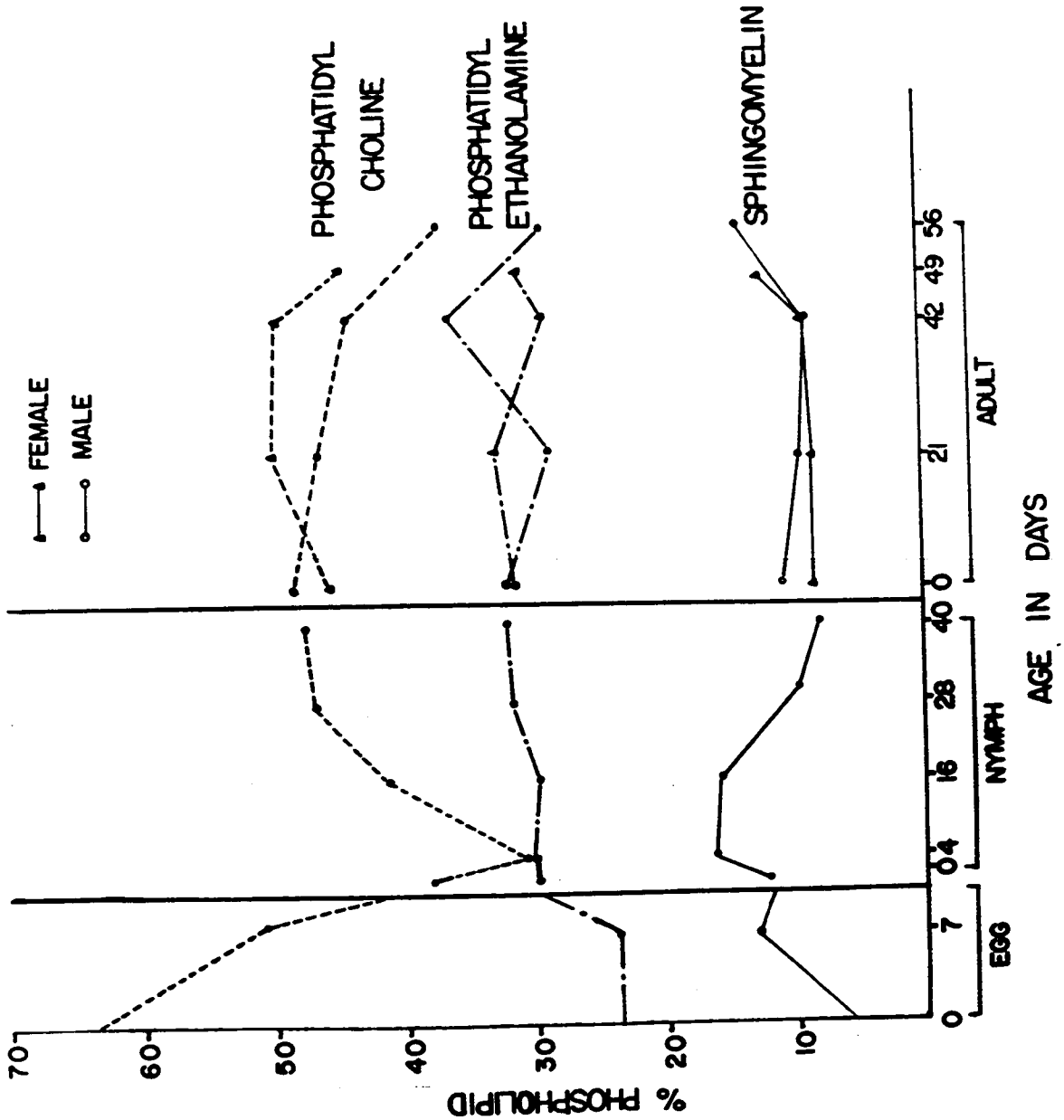

FEMALE
MALE
PHOSPHATIDYL CHOLINE
PHOSPHATIDYL ETHANOLAMINE
SPHINGOMYELIN
% PHOSPHOLIPID
70
60
50
40
30
20
10
0
7
14
18
28
40
0
21
42
49
56
EGG
NYMPH
ADULT
AGE IN DAYS

Table 14. The percentage of fatty acids of total, neutral and phospholipids from 12- and 30-day-old larvae.

Fatty acid carbon number*	Lipid extract and age (days)						
	Total lipid		Neutral lipid		Phospholipid		
	12	30	12	30	12	30	Food
12:0	trace	trace	trace	0.2	trace	trace	trace
14:0	1.0	1.0	1.1	0.9	trace	trace	0.9
15:0	-	-	-	-	-	-	trace
16:0	24.7	23.3	29.2	30.4	13.4	13.9	21.1
16:1	2.9	3.4	2.5	2.3	2.1	2.2	2.0
17:0	trace	trace	trace	trace	trace	trace	trace
18:0	13.8	12.6	14.6	10.1	13.2	12.6	5.0
18:1	23.1	21.3	23.7	24.3	12.5	12.5	22.9
18:2	30.3	34.2	25.0	28.4	54.6	54.6	40.3
18:3	4.3	4.4	3.9	3.8	4.3	4.5	7.9

* The number after the colon denotes the number of unsaturated bonds.

Table 15. The percentage of fatty acids associated with lipid classes from 12- and 30-day-old larvae.

	Lipid classes and age (days)									
	Sterol ester		Triglyceride		Diglyceride		Monoglyceride		Free fatty acid	
Fatty acid carbon number*	12	30	12	30	12	30	12	30	12	30
	(%)		(%)		(%)		(%)		(%)	
6:0	1.8	11.6	5.2	8.9	7.5	4.9	2.9	-	-	-
**X-1	2.2	15.2	7.7	13.4	8.5	5.8	3.3	trace	-	-
**X-2	1.1	4.7	2.4	3.6	4.3	3.8	2.9	-	-	-
8:0	0.6	2.4	1.4	1.9	2.6	4.5	2.6	trace	trace	1.1
10:0	-	-	-	-	trace	trace	4.3	4.9	1.1	1.4
11:0	-	-	-	-	-	1.2	-	-	trace	1.3
12:0	0:4	trace	0.4	trace	1.6	2.1	3.0	6.1	1.7	2.3
14:0 iso-	-	-	-	-	-	-	2.2	5.6	-	-
14:0	0.9	0.6	0.9	0.6	3.2	4.4	34.5	10.9	2.9	2.3
15:0	trace	trace	trace	trace	-	trace	2.8	2.7	trace	0.3
16:0	21.1	17.6	21.9	20.9	20.8	21.6	11.6	20.7	16.3	23.5
16:1	2.3	1.5	3.1	2.1	2.0	3.9	3.9	3.0	7.2	2.0
17:0	0.9	0.7	1.7	1.0	trace	trace	2.5	trace	trace	0.8
18:0	20.3	12.4	14.4	9.0	10.4	8.7	4.3	7.7	12.1	10.0
18:1	24.0	17.2	17.7	15.7	14.8	14.8	8.0	19.5	23.3	22.7
18:2	20.9	14.2	20.4	19.6	19.1	19.9	5.9	10.4	25.6	26.1
18:3	3.8	2.3	3.7	3.5	5.6	4.5	1.8	2.0	6.3	4.0
**X-3	-	-	-	-	-	-	3.8	6.9	2.8	3.3

* The number after the colon denotes the number of unsaturated bonds.

** Unidentified components. See Appendix.

Table 16. Distribution of the major lipid fractions of the haemolymph of 28-day adults.

	Lipid content of the haemolymph (%)		
	Total lipid	Neutral lipid	Phospho-lipid
Males	3.4	2.1	1.3
Females	2.3	1.6	0.7

Table 17. Distribution of neutral lipid components of the haemolymph of 28-day adults.

	Males	Females
	(% neutral lipid)	
Hydrocarbon	37.2	18.9
Sterol ester	15.5	17.2
Triglyceride	5.9	8.3
Monoglyceride	6.8	14.4
Cholesterol	11.0	12.2
Diglyceride	5.0	5.0
Free fatty acid	18.9	23.9

Table 18. The percentage of fatty acids in haemolymph total lipid of 28-day adults.

Fatty acid carbon number**	Males	Females
12:0	trace	trace
14:0	trace	trace
16:0	28.1	26.8
16:1	2.6	2.9
17:0	trace	trace
18:0	14:0	12.6
18:1	16.1	19.6
18:2	35.6	35.0
18:3	3.6	4.0

** The number after the colon denotes the number of unsaturated bonds.

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APPENDIX

FIGURE A-1

FIGURE A-1

Gas chromatogram of the fatty acids of the total lipid of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

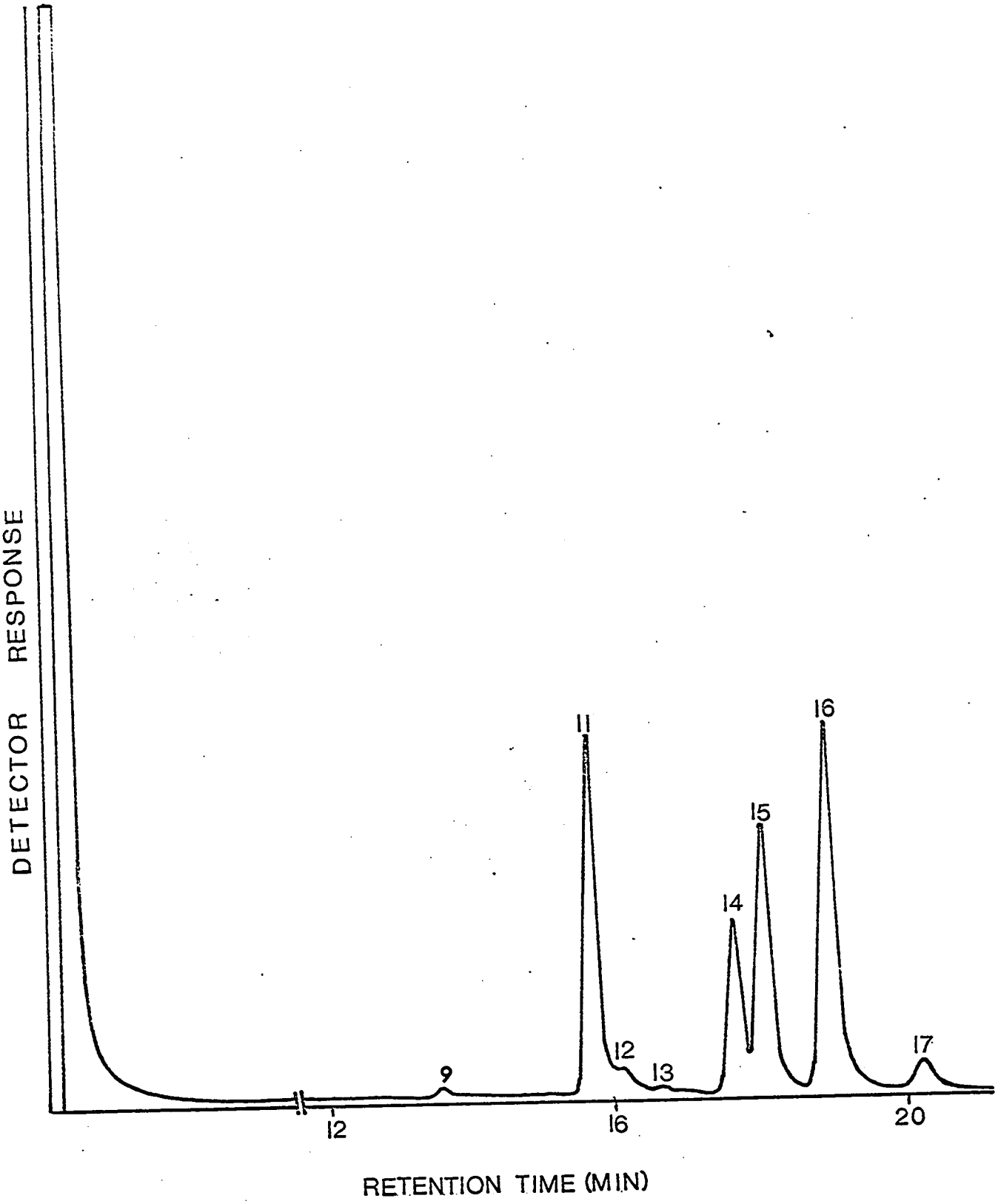

DETECTOR RESPONSE
9
11
12
13
14
15
16
17
12
16
20
RETENTION TIME (MIN)

FIGURE A-2

FIGURE A-2

Gas chromatogram of the fatty acids of the total lipid of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

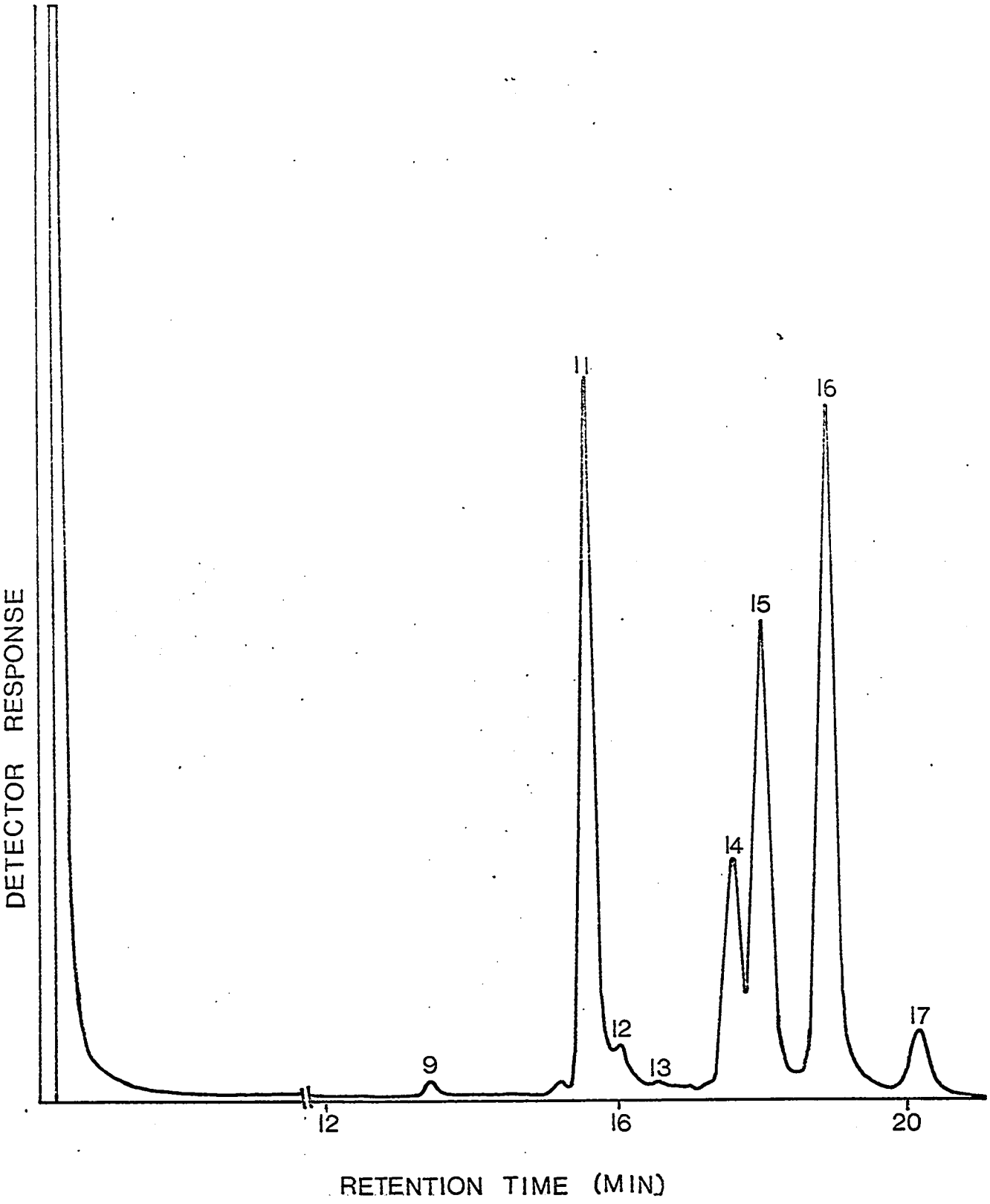

DETECTOR RESPONSE
11
16
15
14
12
9
13
17
12
16
20
RETENTION TIME (MIN)

FIGURE A-3

FIGURE A-3

Gas chromatogram of the fatty acids of the neutral lipid of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

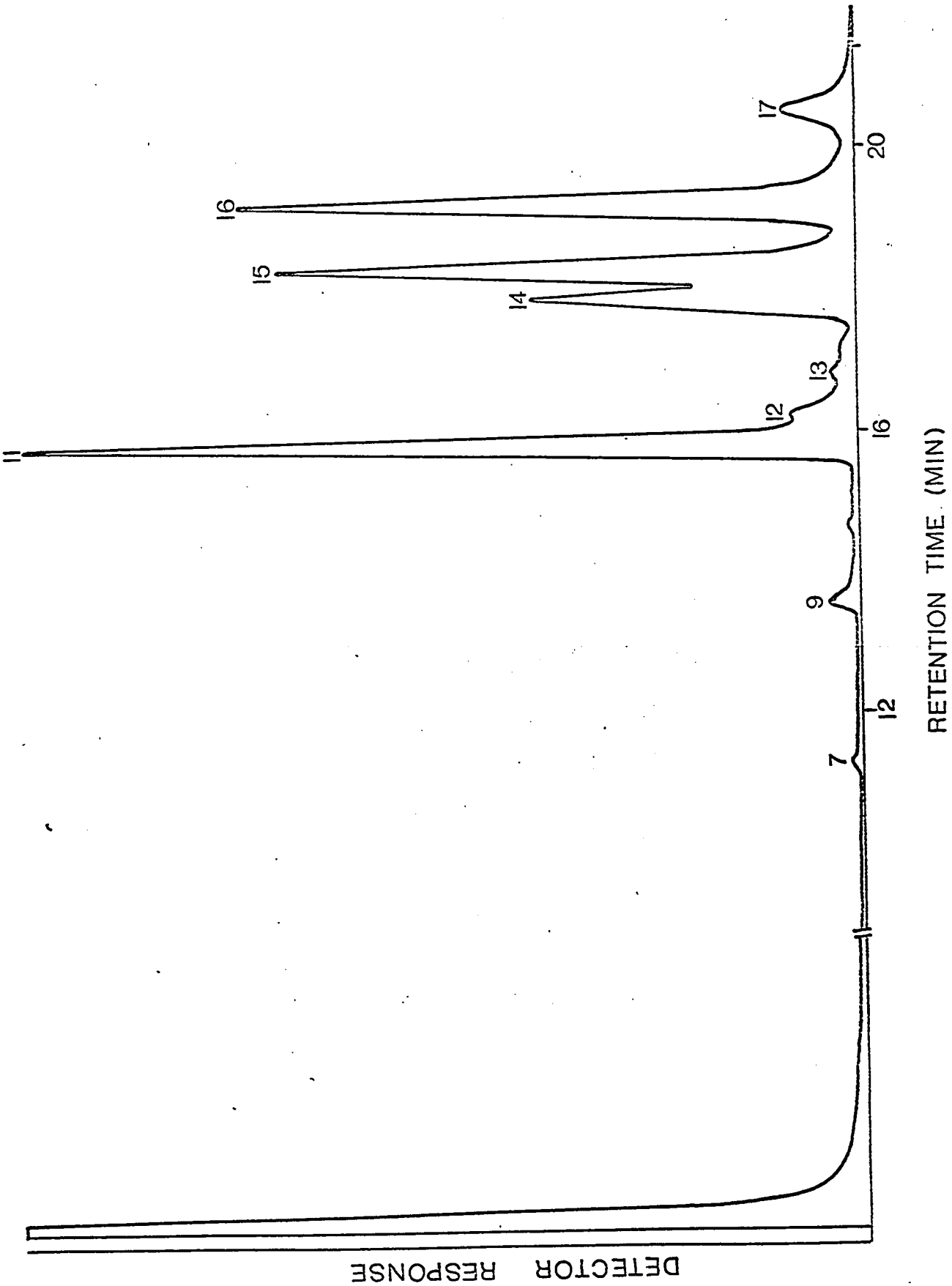

DETECTOR RESPONSE
RETENTION TIME (MIN)
7
9
11
12
13
14
15
16
17
12
16
20

FIGURE A-4

FIGURE A-4

Gas chromatogram of the fatty acids of the neutral lipid of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

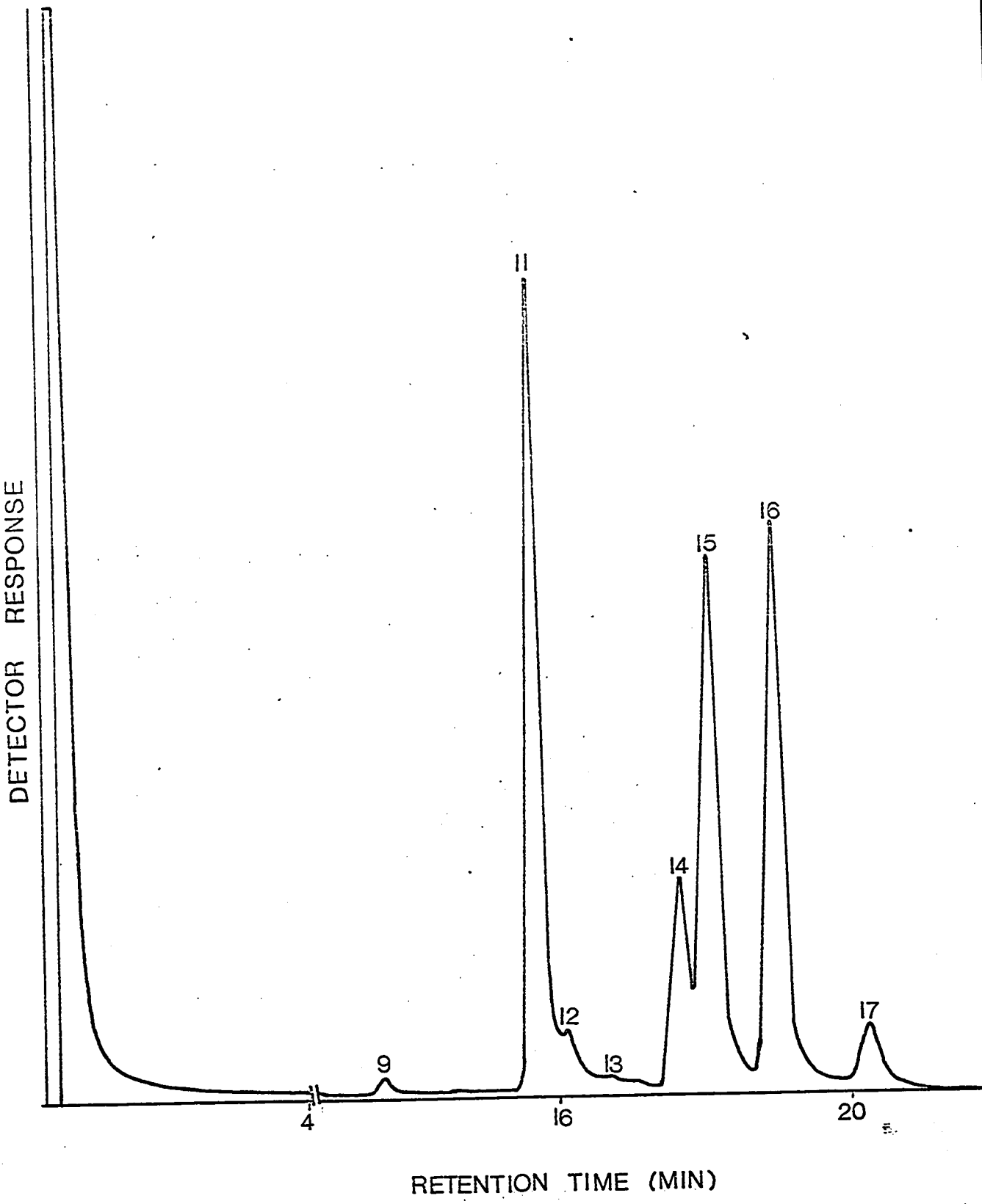

DETECTOR RESPONSE
11
16
15
14
12
13
9
17
4
16
20
RETENTION TIME (MIN)

FIGURE A-5

FIGURE A-5

Gas chromatogram of the fatty acids of the phospholipid of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number unsaturated bonds.

** Unidentified components.

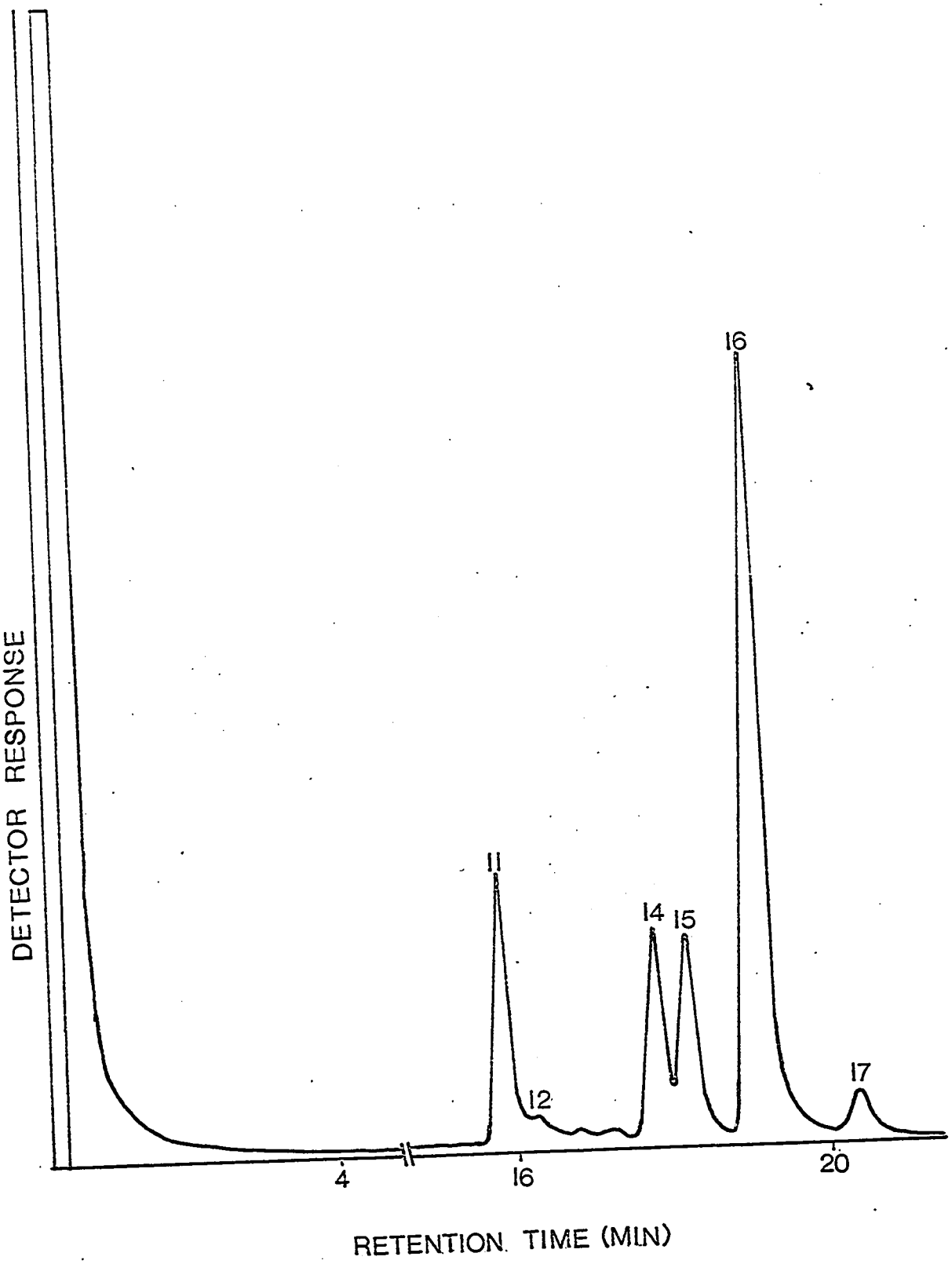

DETECTOR RESPONSE
11
12
14
15
16
17
4
16
20
RETENTION TIME (MIN)

FIGURE A-6

FIGURE A-6

Gas chromatogram of the fatty acids of the phospholipid of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified bonds.

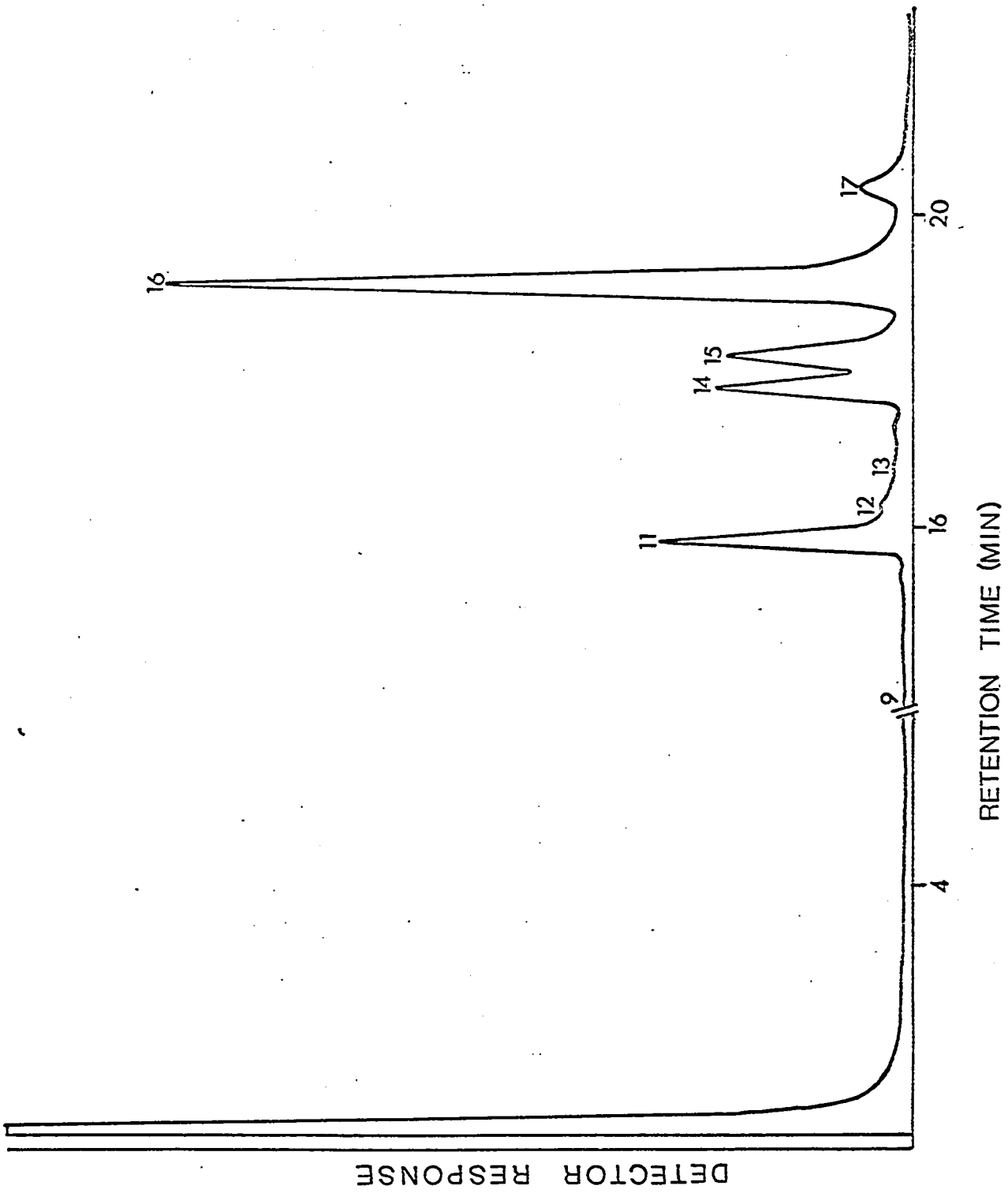

DETECTOR RESPONSE
RETENTION TIME (MIN)
4
16
20
9
11
12
13
14
15
16
17

FIGURE A-7

FIGURE A-7

Gas chromatogram of the fatty acids of the insect food.

Peak number and corresponding fatty acid.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

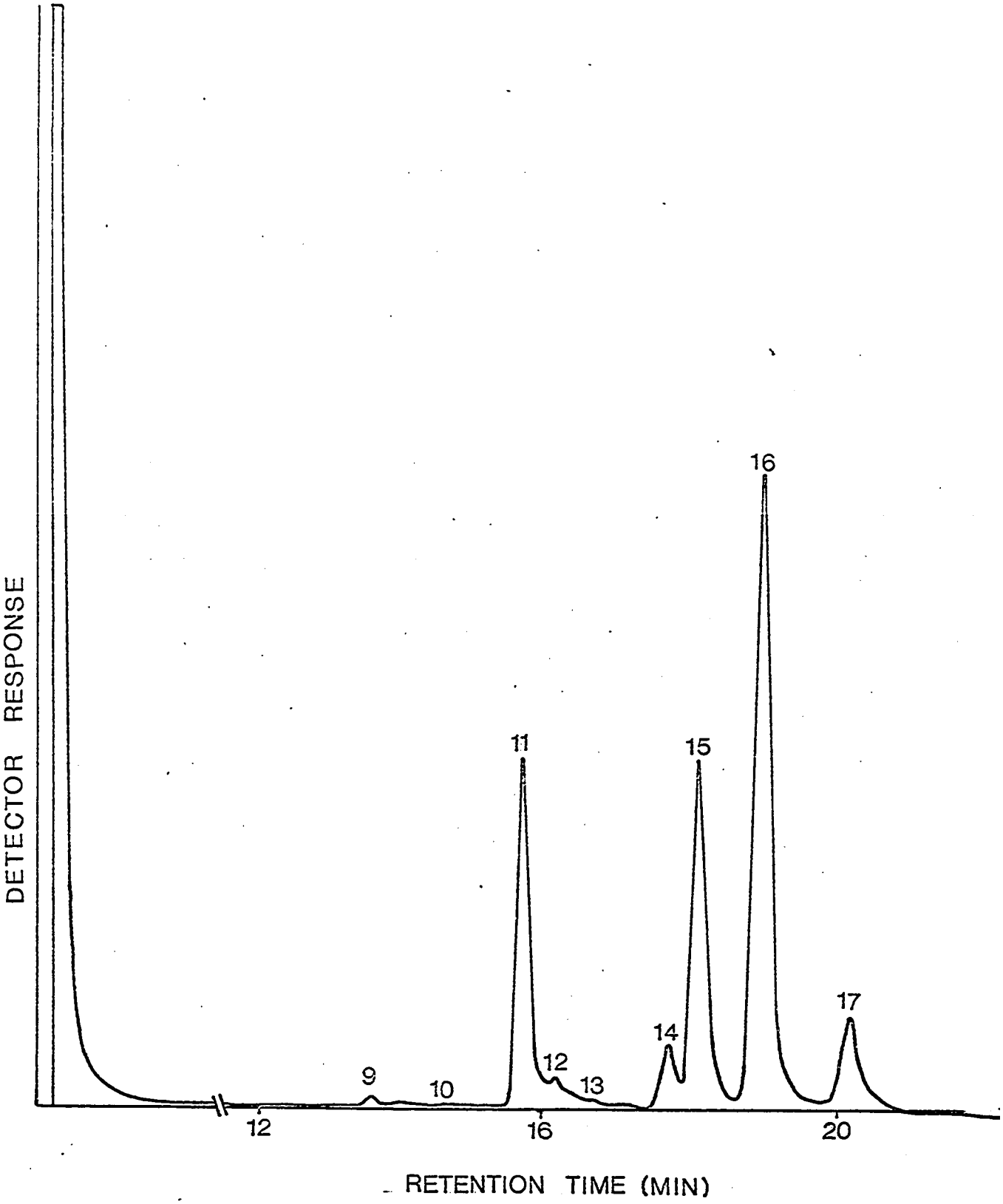

DETECTOR RESPONSE
9
10
11
12
13
14
15
16
17
12
16
20
RETENTION TIME (MIN)

FIGURE A-8

FIGURE A-8

Gas chromatogram of the fatty acids of sterol ester of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

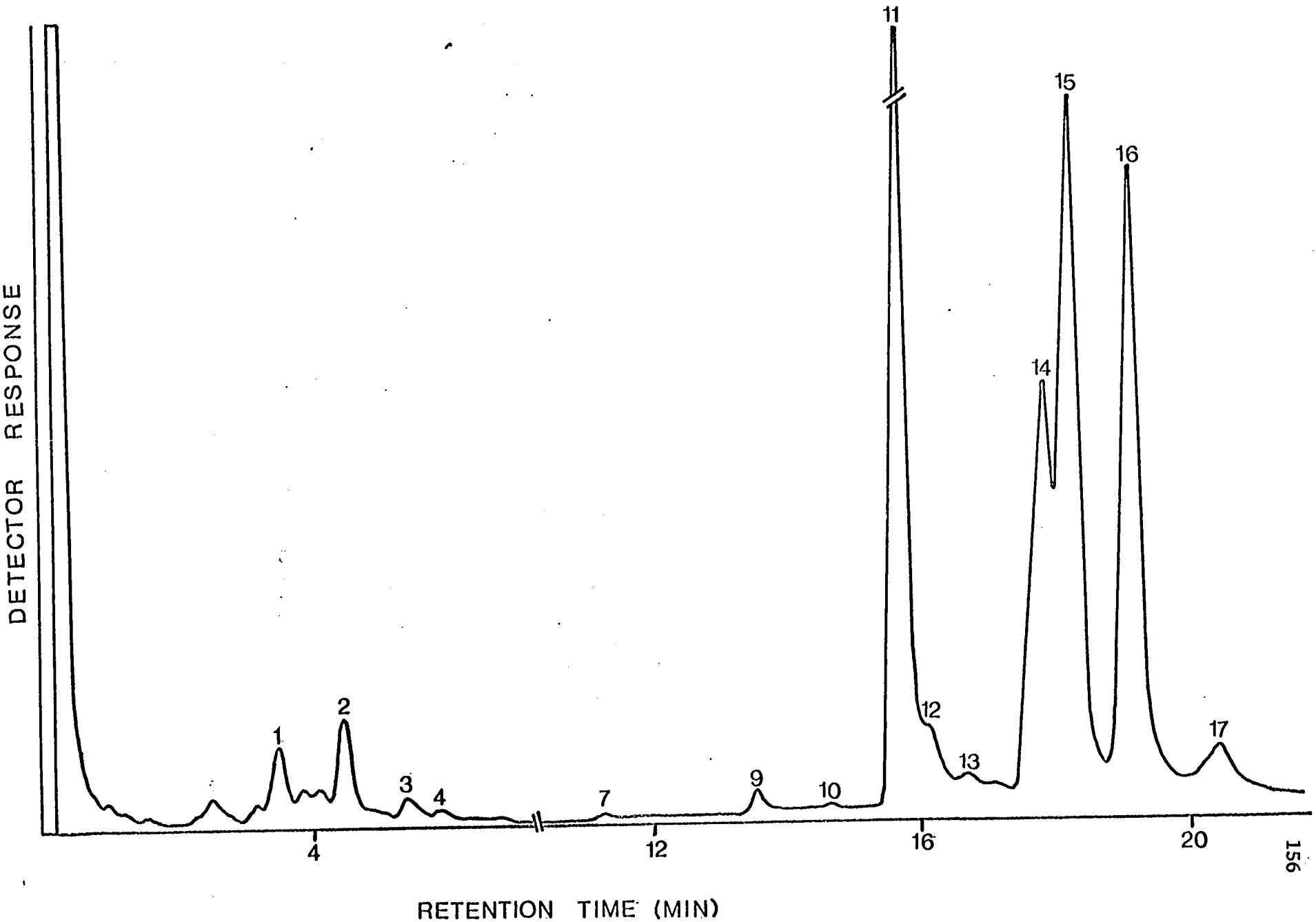

DETECTOR RESPONSE
1
2
3
4
7
9
10
11
12
13
14
15
16
17
4
12
16
20
RETENTION TIME (MIN)

FIGURE A-9

FIGURE A-9

Gas chromatogram of the fatty acids of sterol ester of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

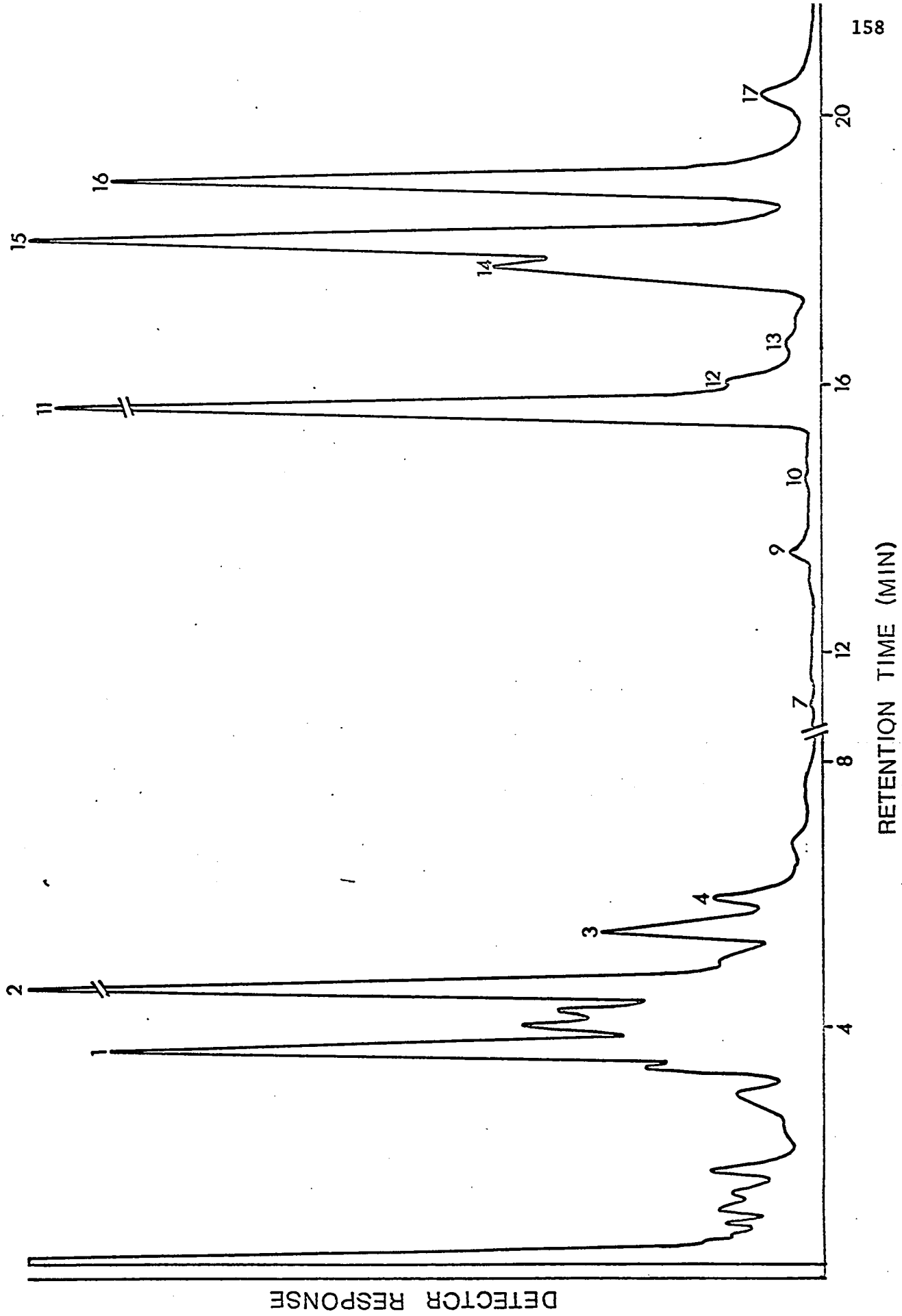

DETECTOR RESPONSE
RETENTION TIME (MIN)
4
8
12
16
20
1
2
3
4
7
9
10
11
12
13
14
15
16
17

FIGURE A-10

FIGURE A-10

Gas chromatogram of the fatty acids of triglyceride of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

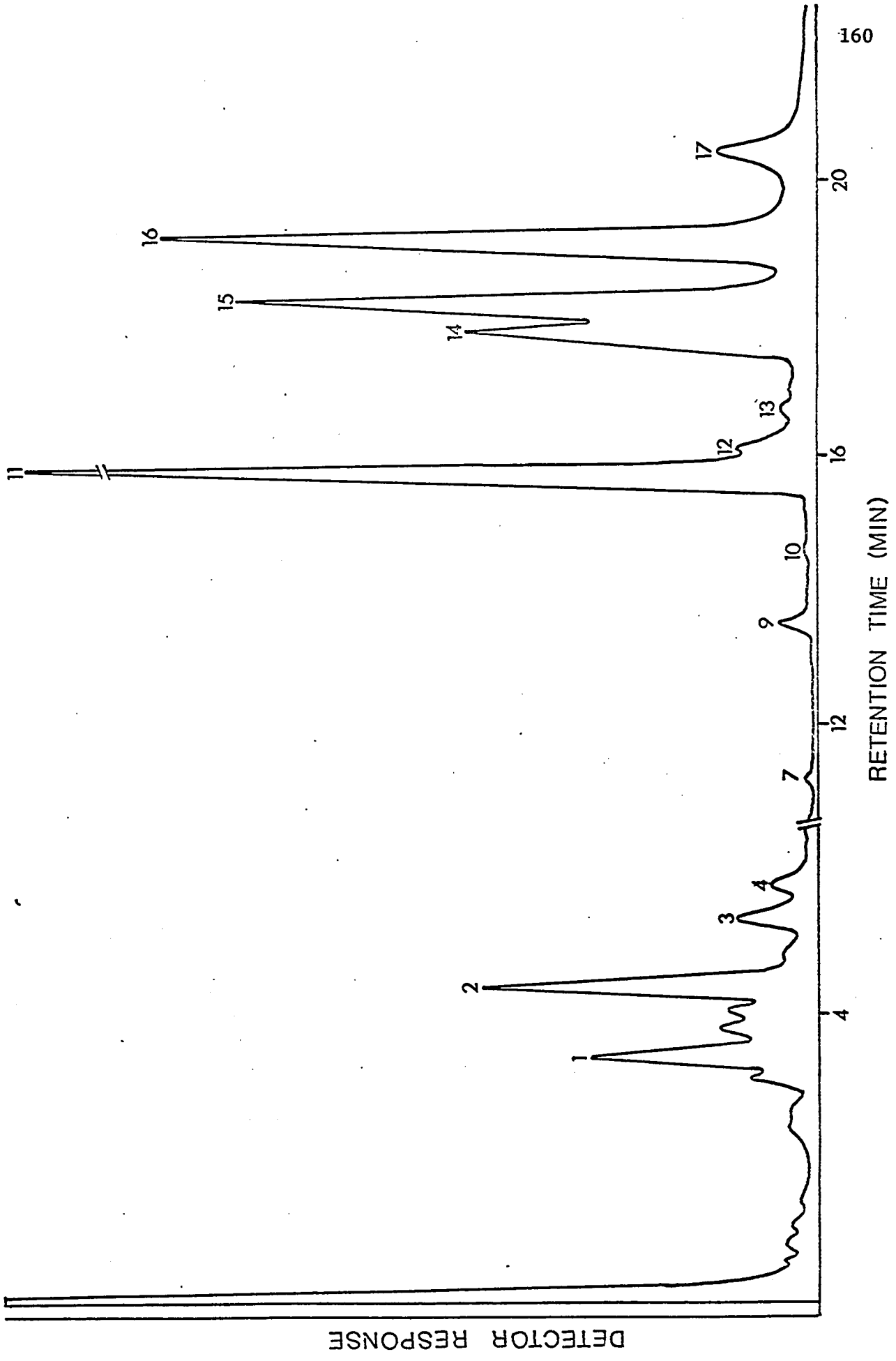

DETECTOR RESPONSE
RETENTION TIME (MIN)
4
12
16
20
1
2
3
4
7
9
10
11
12
13
14
15
16
17

FIGURE A-11

FIGURE A-11

Gas chromatogram of the fatty acids of triglyceride of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

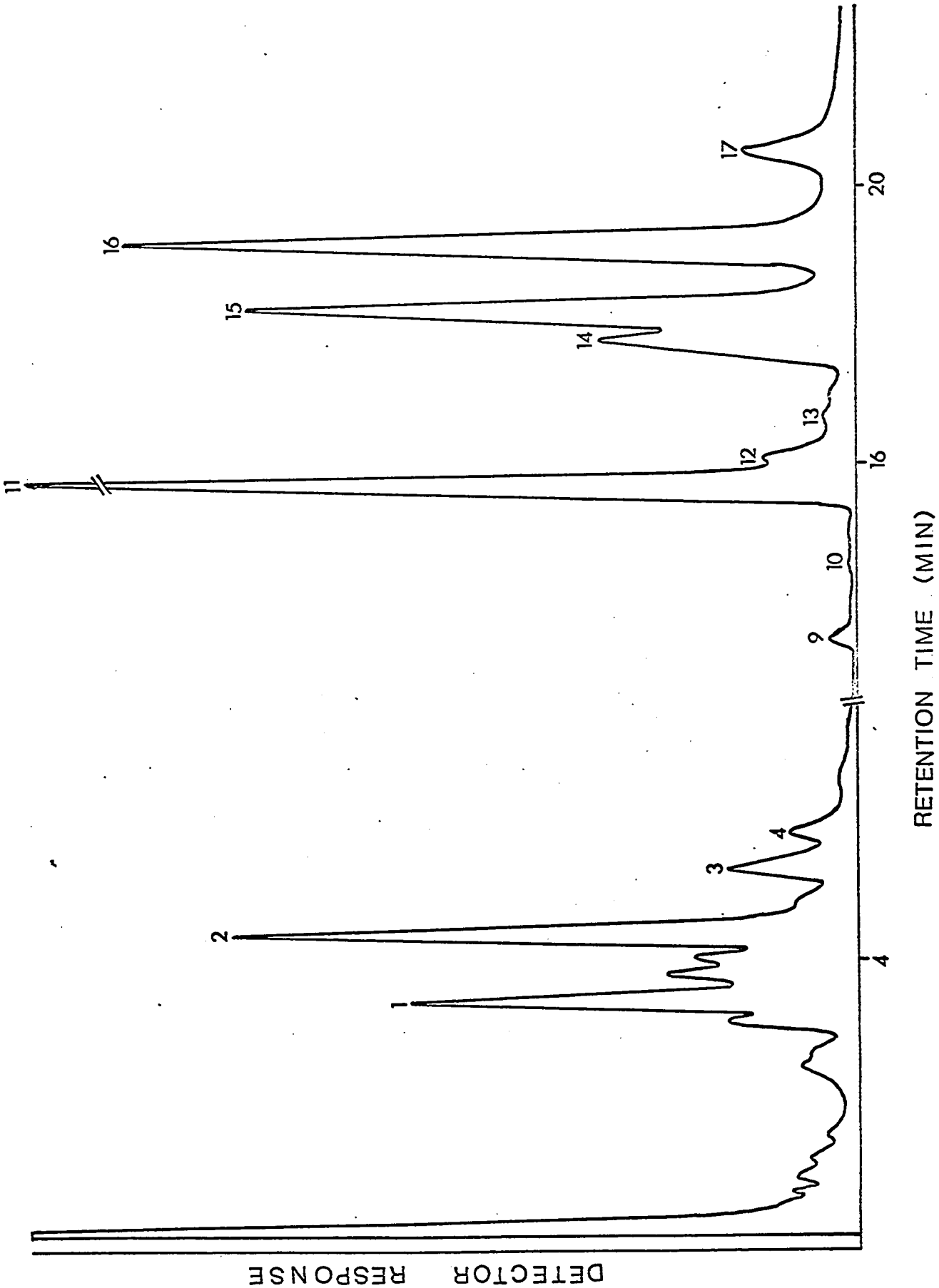

DETECTOR RESPONSE
RETENTION TIME (MIN)
4
16
20
1
2
3
4
9
10
11
12
13
14
15
16
17

FIGURE A-12

FIGURE A-12

Gas chromatogram of the fatty acids of diglyceride of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

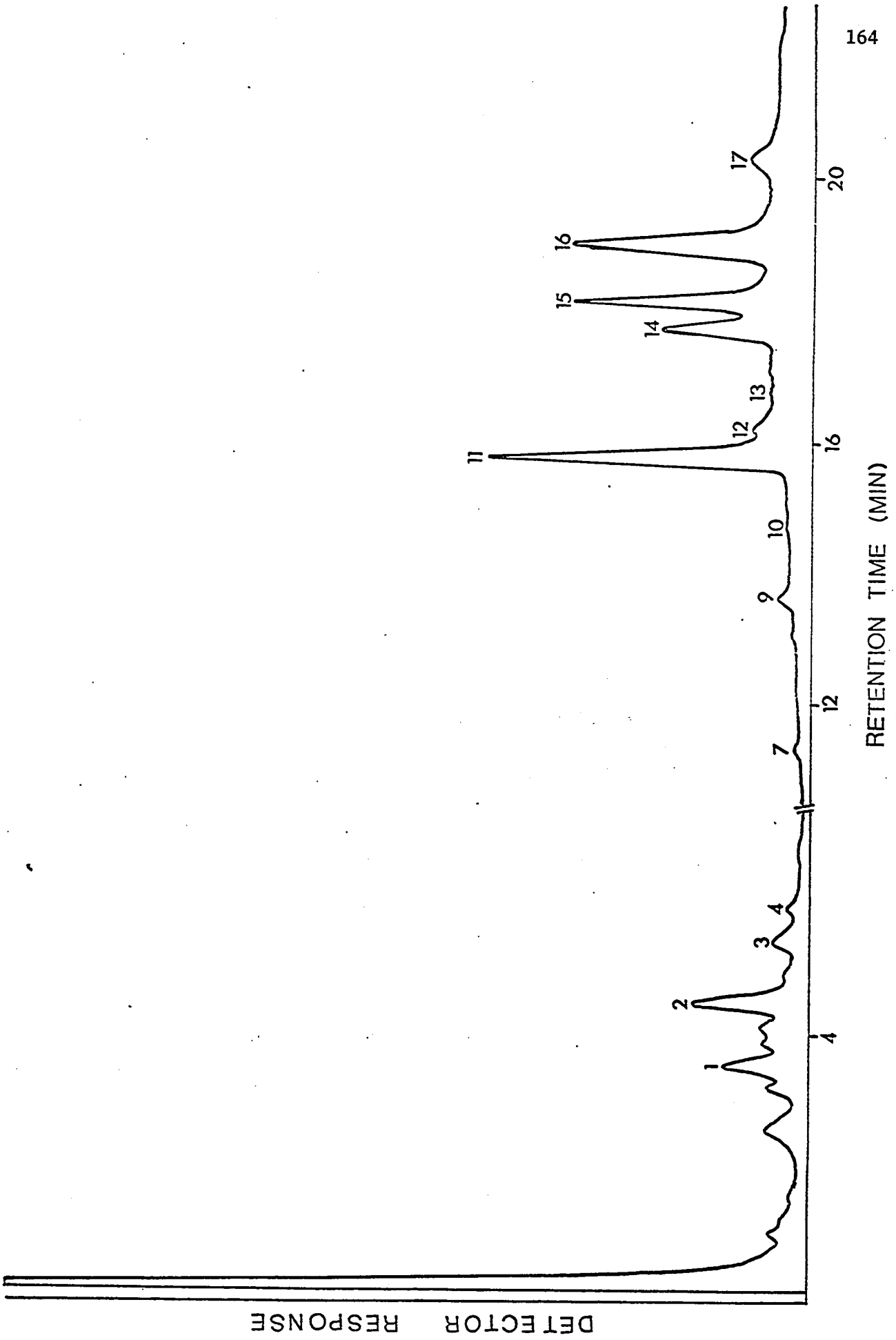

DETECTOR RESPONSE
RETENTION TIME (MIN)
4
12
16
20
1
2
3
4
7
9
10
11
12
13
14
15
16
17

FIGURE A-13

FIGURE A-13

Gas chromatogram of the fatty acids of diglyceride of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

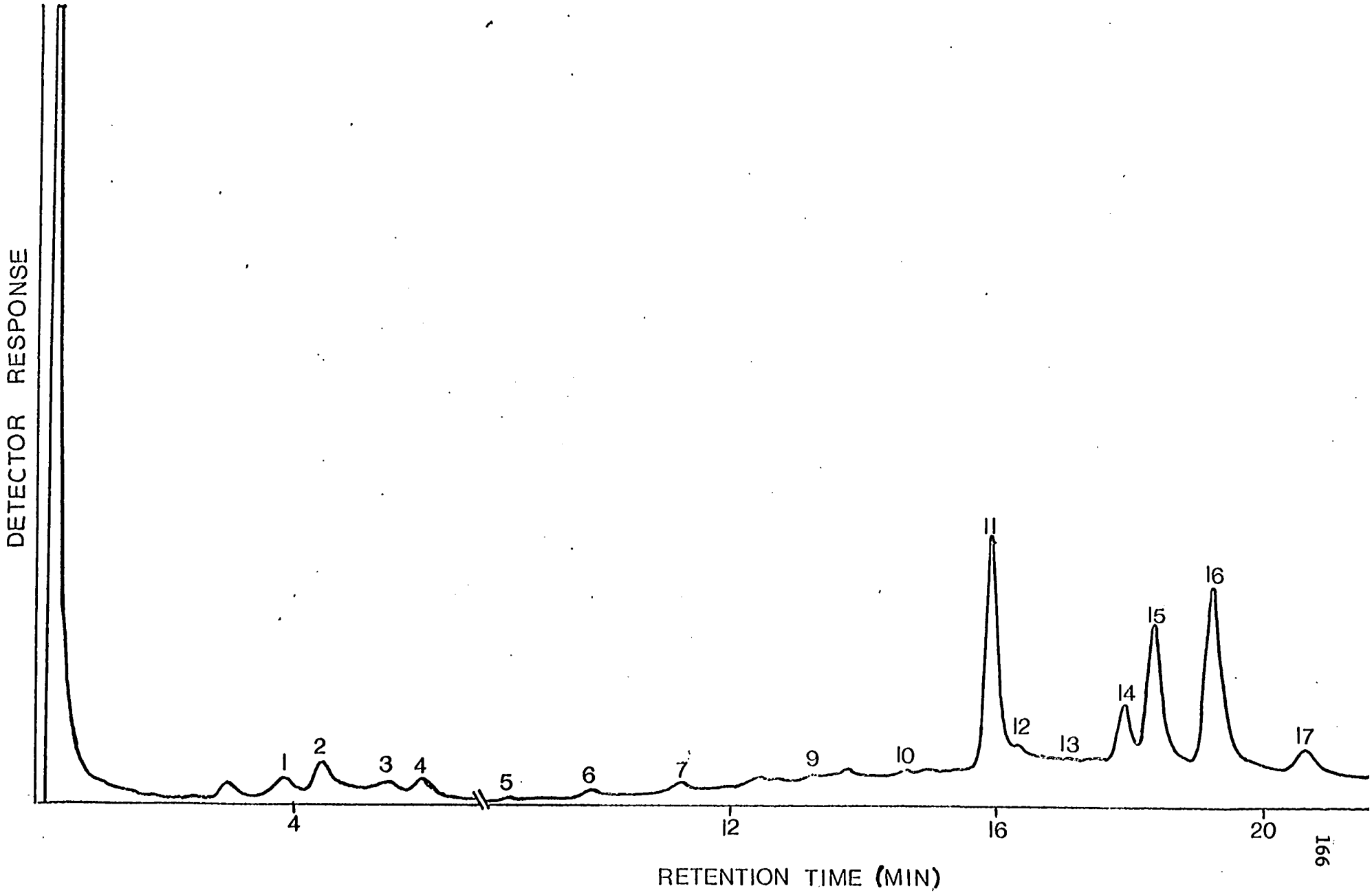

DETECTOR RESPONSE
1
2
3
4
5
6
7
9
10
11
12
13
14
15
16
17
4
12
16
20
RETENTION TIME (MIN)

FIGURE A-14

FIGURE A-14

Gas chromatogram of the fatty acids of monoglyceride of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

Note: The drifting baseline was probably due to column bleeding at elevated temperatures. The necessary triangle for the estimation of the fatty acid represented by each peak was obtained by drawing a line parallel to the X-axis through the lowest point of each peak and this line was used as the base of the triangle. The shorter side of the peak was extended to intersect with the baseline. The triangle was then completed and the area was determined. (See FIGURE A-14; Peak No. 11.).

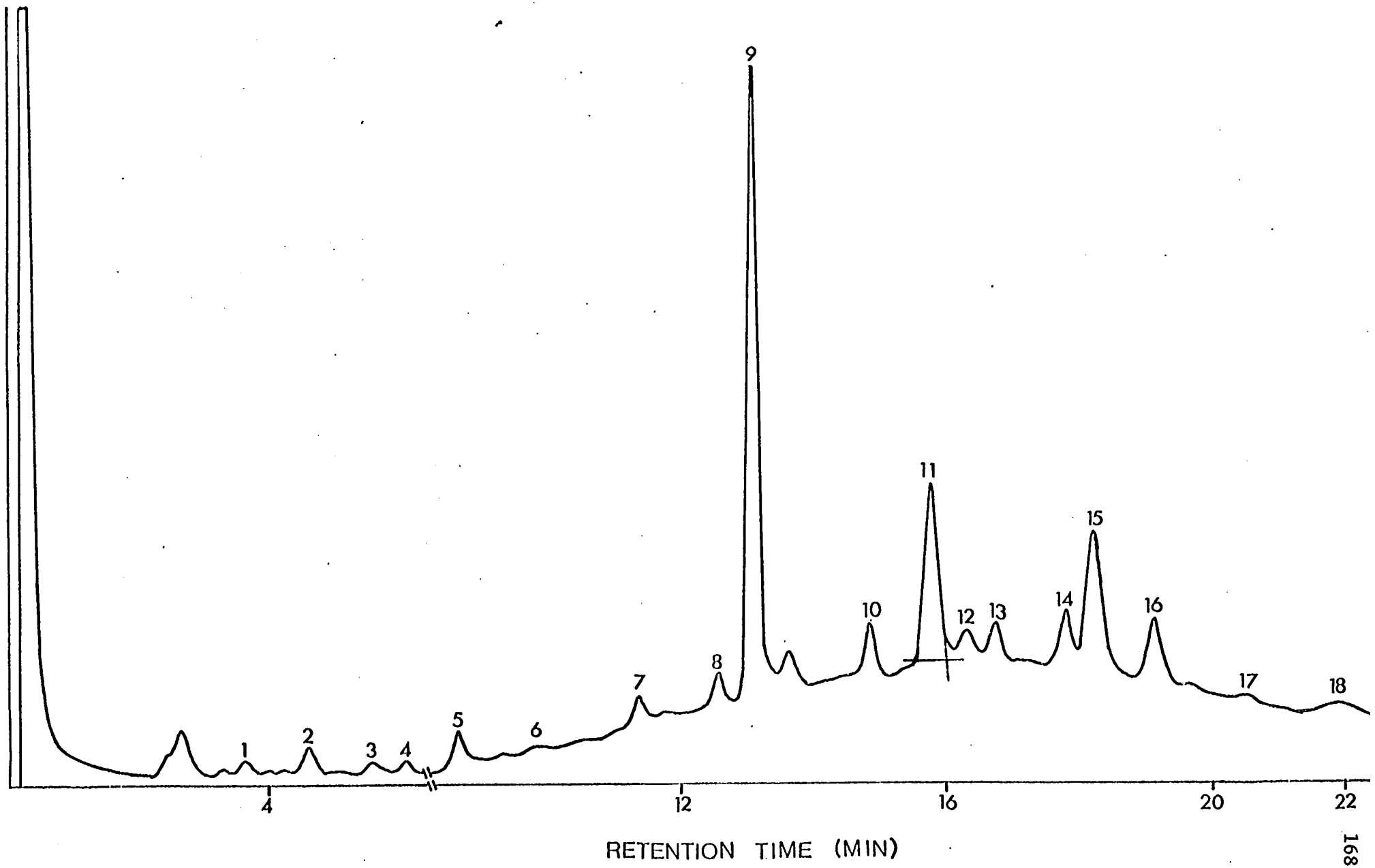

DETECTOR RESPONSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
4
12
16
20
22
RETENTION TIME (MIN)

FIGURE A-15

FIGURE A-15

Gas chromatogram of the fatty acids of monoglyceride of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

Note: The drifting baseline was probably due to column bleeding at elevated temperatures. The necessary triangle for the estimation of the fatty acid represented by each peak was obtained by drawing a line parallel to the X-axis through the lowest point of each peak and this line was used as the base of the triangle. The shorter side of the peak was extended to intersect with the baseline. The triangle was then completed and the area was determined (See FIGURE A-14; Peak No. 11).

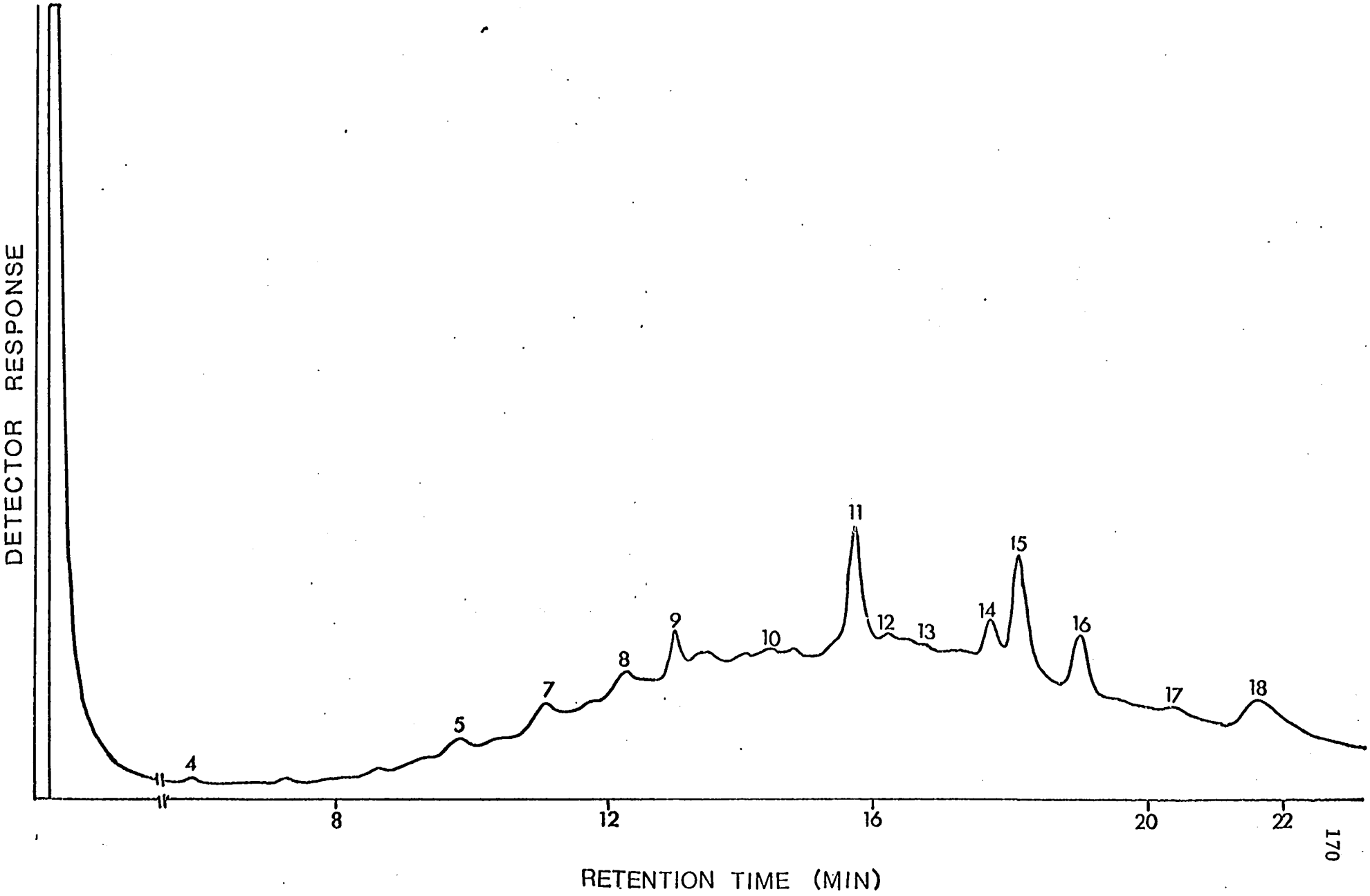

DETECTOR RESPONSE
4
5
7
8
9
10
11
12
13
14
15
16
17
18
8
12
16
20
22
RETENTION TIME (MIN)

FIGURE A-16

FIGURE A-16

Gas chromatogram of the fatty acids of free fatty acid of 12-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.

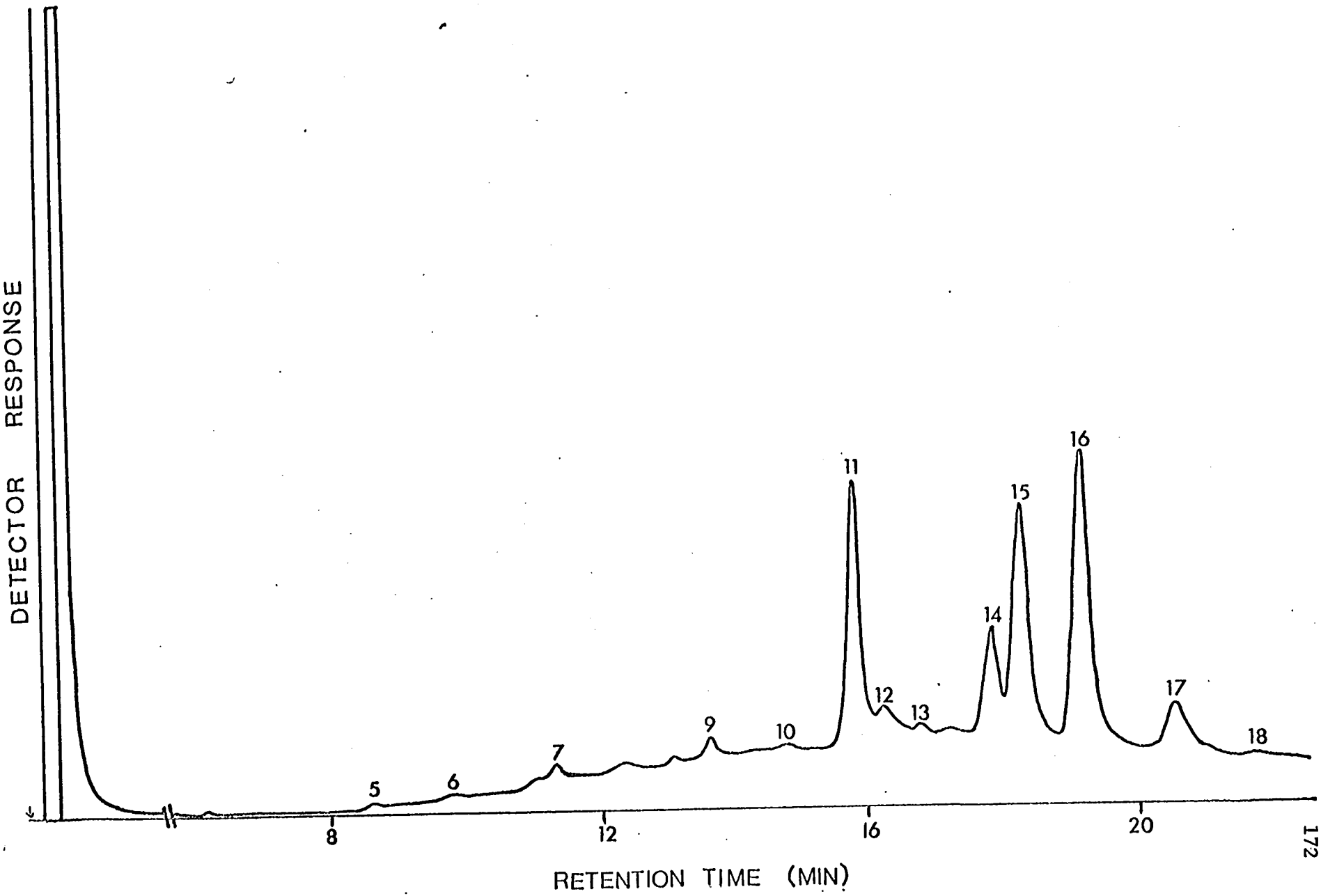

DETECTOR RESPONSE
5
6
7
9
10
11
12
13
14
15
16
17
18
8
12
16
20
RETENTION TIME (MIN)

FIGURE A-17

FIGURE A-17

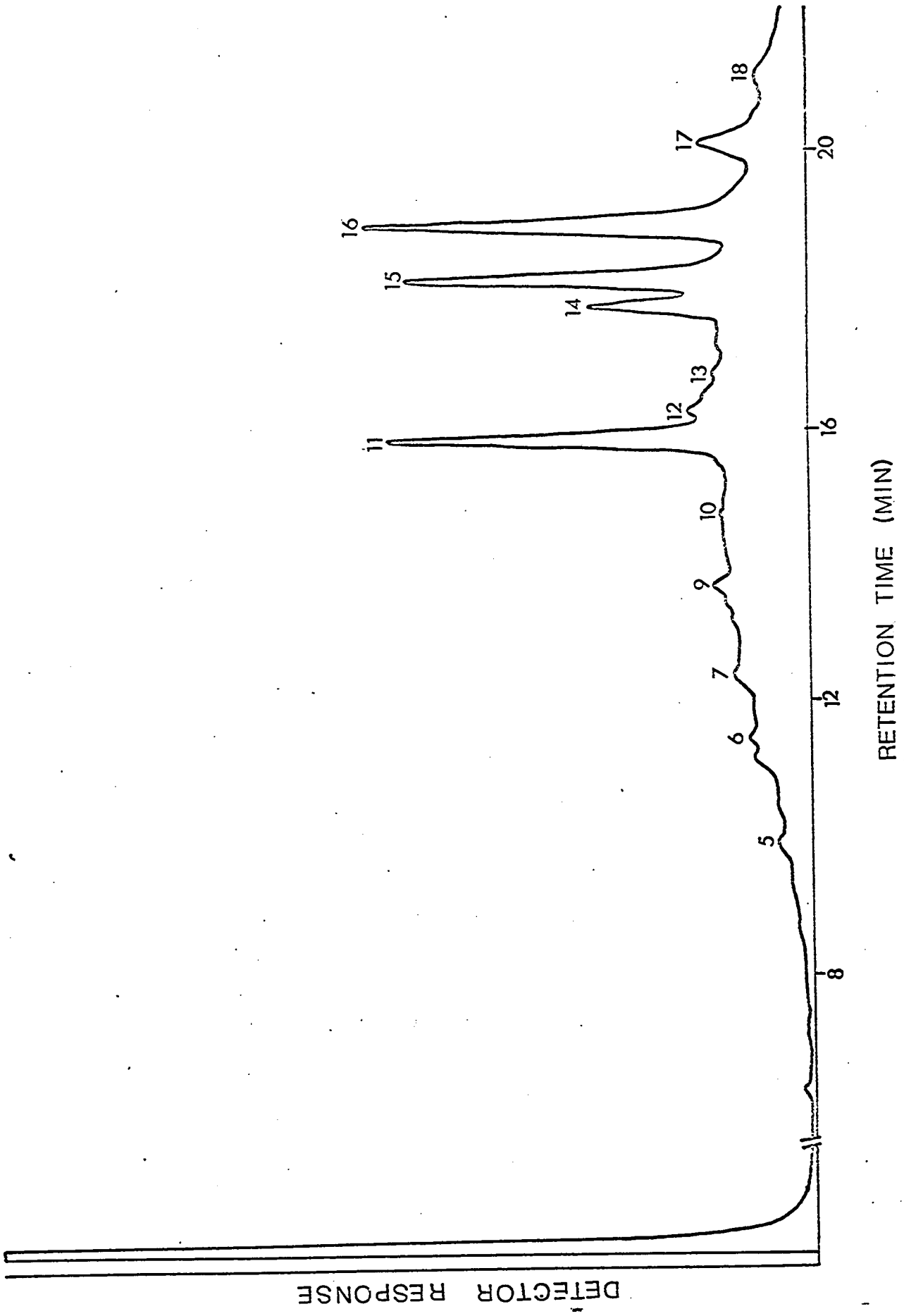
Gas chromatogram of the fatty acids of free fatty acid of 30-day-old larvae.

Peak numbers and corresponding fatty acids.*

1 = 6:0	10 = 15:0
2 = X-1**	11 = 16:0
3 = X-2**	12 = 16:1
4 = 8:0	13 = 17:0
5 = 10:0	14 = 18:0
6 = 11:0	15 = 18:1
7 = 12:0	16 = 18:2
8 = 14:0 iso-	17 = 18:3
9 = 14:0	18 = X-3**

* The first number denotes the number of carbon atoms and that after the colon the number of unsaturated bonds.

** Unidentified components.


DETECTOR RESPONSE
RETENTION TIME (MIN)
8
12
16
20
5
6
7
9
10
11
12
13
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