SOME EFFECTS OF STORAGE AT DIFFERENT TEMPERATURES ON THE LIPIDS OF THE AMERICAN ROACH AND ON THE RESISTANCE OF THIS INSECT TO HEAT AND TO DDT

by
Sam Clark Munson

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789 East Eisenhower Parkway
P.O. Box 1346
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SECTION I

INTRODUCTION

For a long time evidence has existed that the chemical structure of the lipids of an organism, either plant or animal, is influenced by the temperature at which their biosynthesis occurs; the generalization is that lipids which are produced or deposited at higher temperatures are more saturated than those produced at lower temperatures, all other conditions being equal. The melting points of the fatty acids are related to their degree of saturation as well as to the number of carbon atoms composing their chains. Naturally occuring unsaturated acids of all series are liquid at ordinary temperatures.

Organisms of warmer climates are commonly regarded as being able to withstand unfavorably high temperatures than are those of cooler zones. A type of heat coagulation (not to be confused with protein coagulation) apparently may be associated with some change in the fatty materials of cells exposed to such temperatures (Heilbrunn 1924). This and the above observations are essentially the basis for the 'lipoid liberation theory' of thermal injury at high temperatures (Belehradek 1931, Fraenkel and Hopf 1940, and Heilbrunn 1947).

It is known that oils were used as insecticides in the time of the Roman empire (Shepard 1951). Morever, many of the more effective insecticides in use today are known to have high lipid affinity. It is not, therefore, difficult to imagine that the nature of the lipids of an insect treated with such an insecticide might, perhaps influence the action of the poison. Indeed, sites of lipid-insecticide interaction in insects have been suggested.

This investigation was undertaken with a threefold intention: (1) To

determine the alterations which might occur in the saturation of the lipids of an insect, the American roach, Periplaneta americana L. held at different temperatures by determining the iodine numbers of extracted lipids. It was hoped that sufficient data would be collected to present a clear picture of the degree of alteration at such temperatures. (2) To determine whether or not roaches with different iodine numbers would vary in their resistance to death induced by abnormally high temperatures. (3) To determine whether or not roaches varying in the nature of their lipids would also vary in their reaction to the toxic effect of an insecticide such as 2,2,bis(p-chlorophenyl) 1,1,1, trichloroethane (DDT) which is known to have high lipid affinity.

SECTION II

CONSPECTUS OF THE PERTINENT LITERATURE

1. Variables Which Influence Lipids, Especially The Lipids of Insects

In this paper lipid is used in its broader meaning to include not only true fats and waxes; but, also, to include chemically related substances, the phospholipids, and other substances related by their physical properties such as common solubilities and biological relationship.

The literature dealing generally with role of the fat body and the physiological functions of fats is discussed by Wigglesworth (1950), by Chauvin (1949), and an eclectic summary by Munson will soon appear in "Insect Physiology" edited by K. D. Roeder (1952). Also a recent survey of the literature by Scoggins and Tauber (1950) covers certain aspects of the subject not relevant to the present undertaking. Here the primary concern is with factors which may cause variations in the lipids of insects; these will be thoroughly considered and compared with certain aspects of the general literature of lipids in other animals.

In the insect, as in most animals, oleic, linoleic, palmitic and stearic acids are major components of the fatty acids, with stearic predominant as compared to palmitic. Linolenic, arachidic and other higher fatty acids may be found; in some cases acids of lower molecular weight have been found; generalizations with regard to the phospholipids and sterols of insects are not yet possible. (Scoggins and Tauber 1950).

Iodine numbers (the number of grams of iodine taken up by 100 grams of lipid) furnish information with regard to the relative

occurrence of unsaturated fatty acids in the glycerides. Since the fats may be affected by diet, temperature, and other factors discussed below, iodine numbers are not too valuable unless the conditions under which they are obtained are made clear. Among such values for insects great variability may occur. Timon-David (1930) made iodine number determinations for a large number of insects representing all the major orders and found them to range from 1.5 for an aphid (Pemphigus) to 164.5 for a moth, Saturnia pyri Sch. Orthoptera varied from 68.8 for the oriental roach, Periplaneta (Blatta) orientalis L., to 122.6 for a locustid grasshopper, Oxya japonica Will. The quantities of fat expressed as percents of wet weight varied from 2.4 to 7.0 for seven Orthopterans. More recent values for other grasshoppers cited below and for other Orthopterans are very similar to these however, cockroaches appear to give somewhat higher values. Iodine values for the German roach, Blatella germanica L., under experimental conditions were found to range from 51 to 64 by Melampy and Maynard (1937); and the lipid content expressed in percent of wet weight was 5.72 for nymphs, 4.78 for female adults with egg capsules, and 1.70 for male adults. For the same insect McCay (1938) found fat to be about 15.6 to 17.1 percent of dry weight (ca. 5.24-6.1 percent wet wt.) with a water content of 64.3 to 66.3. Iodine numbers for the same roach ranged from 55 to 57. The American roach female adult has a lipid content of 28.63 percent of dry weight (ca. 9.54 wet weight) and the male 25.55 (7.12) according to the unpublished work of Schweet quoted by Scoggins and Tauber (1950). All these values exceed the 4.3 percent of wet weight reported by Timon-David for the oriental roach.

The stadium, caste, sex and phase may have an influence on the lipids of an insect. One of the more informative analyses of the lipids

of an insect in different stages of its life history is the work of Pepper and Hastings (1943) with the sugar-beet webworm, Loxostege sticticalis L. In this Pyralid moth the iodine number of 139.4 for the lipids of the first instar fell progressively with each instar to 112.8 in the prepupa and then fell precipitately to 69.8 in the fertile female. In the fifth instar, prepupa, pupa and female imagoes there was a sharp decrease in the unsaturated relative to the saturated fatty acids. An early report (Heller 1926) for the silkworm, Bombyx mori L., gives larval fat as 3.20, pupal 5.95, and adult 9.06 percent on a wet basis. For mature larvae of a Sphingid moth, Deilephila sp., the fat was 3.0 for pupae 3.83 and for young adults 6.1 percent of the wet weight. The percentage of fats and water held an inverse relationship.

In the yellow mealworm, Tenebrio molitor L., (Finkel 1948) reared at 30°C. total lipids, neutral fats and total fatty acids increased from 9.05 percent of wet weight at 50 days to 13.13 percent at 200 days. Phospholipids declined slowly relative to the increase in neutral fats, total cholesterol remained the same. The rate of growth was dependent on the temperature and humidity. An earlier report for this same beetle listed fat content as percent of wet weight and corresponding iodine numbers as follows: eggs 3.4, 114.2; larvae 12- 14, 95.8; pupa 8.9, 92.4; young adults 8.1, 94.3; old adults 4.6 (no iodine no. given). The author concluded that during metamorphosis the reserve lipids sharply decreased but the specific organ lipids remained constant. Other holometabolous insects show a similar fall during pupation; similar results having been reported for Diptera, Hymenoptera and for other Coleoptera and Lepidoptera (Scoggins and Tauber 1950). Apparently the consumption of fats during the pupal stage may or may not be selective. Thus unsaturated fats appear to be differentially used in the corn ear

worm, Heliothis armigera Hub., (Ditman and Weiland 1938); also it appears to be selectively used in a butterfly, Pieris; but in the European cornborer, Pyrausta nubilalis Hub., and Cossus sp. it is apparently non-selective (Timon-David 1930).

Drones, workers, and queens of the honeybee, Apis mellifera L., show differences in lipid content as well as in other substances (water, nitrogen, ash) and these castes vary in the larval and pupal as well as in the adult stages. (Melampy et al 1940, Scoggin and Tauber 1950). Insects may also differ with phases. Mathee (1945) investigated the fat content of two species of locusts and two species of noctuid moths and found the fat content markedly higher in the gregarious phase.

In passing it is interesting to note that in mammals fats also vary in their nature with age; young pigs and cattle have softer fats than older ones (Bloor, p. 239, 1943).

The influence of hibernation on fat content may be greatly affected by the intervention of diapause. Hibernation because of the possible occurrence of diapause does not necessarily imply starvation and in fact few reserves may b e used during hibernation. In the European cornborer the iodine number of 80.8 for caterpillars in November had fallen to 76 for pupa in June; the enormous fatty reserves which were reduced only slightly during diapause were reduced about forty-five percent in the pupa during June (Timon-David 1929). Similarly in the Colorado potato beetle, Leptinotarsa decemlineata Say the total fatty acid content of 12.6 percent of wet weight at the beginning of diapause in November remained as high as 11.6 percent in March. This was followed by a sharp drop in April to a fatty acid content in females of 3.47 percent and 3.27 for males. The utilization of the reserves appears to occur in connection with the maturation of the sex products immediately

preceding the eclosion of the adult (Busnel and Drilhon 1937a and 1937b).

During hibernation the lipid content may also fall progressively. The ether soluble extract of the chinch bug, Blissus leucopterus (Say), fell from 36.27 percent of dry weight in July to 27.19 percent in February and had further decreased to 10.25 percent in April (Schweet as reported by Scoggins and Tauber 1950). In hibernating adult mosquitoes, Culex pipiens L., the fat content of 27.9 percent of wet weight in October declined to 23.6 in December and a low of 6.3 in March (Buxton 1935).

Under actual starvation the principal reserve lost by adults of Pieris is fat which may be depleted as much as seventy percent; carbohydrates occur only in slight concentrations and non fatty loss consists chiefly of protein which may be used to the extent of about forty-one percent (Heller 1926). The case is the same with the starving mealworm, except that in this insect more glycogen and protein are used relative to the reserve fats (Mellanby 1932). The recent work of Ludwig (1950) shows that nymphs of the grasshopper, Chortophaga viridifasciata De Geer, under starvation may, after the depletion of their glycogen, lose 68.6 percent of their lipids. Cook (1944) found that the resistance of leaf hoppers, Euttetix tenellus (Baker), to starvation was correlated with their lipid content. In the German roach, under starvation, fat was found not to be greatly reduced but its iodine number diminished (Melampy and Maynard 1937). Scoggin and Tauber (1950) cite other data confirming the generality that actual starvation induces a depletion of reserve fats. The vertebrate also maintains itself during periods of inanition mainly by its stored fats as the available reserves of carbohydrates and proteins are slight in quantity and quickly exhausted so that in two or three days large mammals, and in less time smaller ones, are living on fat reserves and body proteins (Bloor 154, 1943).

The relationship between the systematic position of an organism and the nature of its lipids is not simple. Timon-David (1930) concluded from his work on many insects that the reserve fats are of very diverse physical and chemical character; the fundamental variations were related not only to the solid or liquid state of the lipids (fats, butters, and oils) but also to the relative proportions of saturated and unsaturated acids, and to the presence or absence of ethylenic acids with single, double, or triple bonds. The length of the carbon chains of the fatty acids is variable, the acids with short chains occupy a preponderant place in the acids of the aphids; morever, the proportions and nature of the unsaponifiable substances are subject to great variation. In seeking a relationship between these variations of the stored lipids and systematic position Timon-David interpreted the evidence to indicate that the relationship was one of alimentation. He concluded that the systematic position of an insect in one group or another could of itself be of no significance from the point of view of the fats the insect would have in its reserves. On the other hand members of limited groups such as families of which the representatives have the same type of alimentation may have in the reserve fats a certain uniformity of composition and thus the biochemical results may be in relationship to the anatomical structure of the feeding organs. Thus in a sense it might be said that anatomy determines the composition of the reserve fats by determining the diet of the insect.

It is essential that certain aspects of diet related to the present problem be taken into account. The nutritional availability of the fat must be considered. Its melting point must be such that it is liquid or nearly so at the temperature of the animal's body. This facilitates emulsification in water and provides an area for enzymatic action

and hence digestion. Food fats of different origin seem to be about equally available if they have sufficiently low melting points (Bloor p 59, 1943). The melting point may not be the only factor in the digestion of fats. The nature and condition of the fatty acids may be of considerable importance; soaps are more water soluble and perhaps are more readily utilized than the corresponding fatty acids (work of Levites); the splitting of fats of eight or more carbon atoms is greatly dependent on the temperature, the higher the number of carbon atoms the higher the temperature required (work of Balls, Matlack and Tucker) (Bloor, p 61-62 1943).

Also to be kept in mind is the interconversion of protein through carbohydrates to fat and fats to carbohydrates within the animal. There is much evidence that the transformation of protein to fat occurs through a carbohydrate stage; moreover, fats formed from carbohydrates may have a characteristic tendency toward hardness. On the other hand, sometimes food fats seem to be transferred to fat stores with very little change. Thus a species of animal may be properly said to have a characteristic food fat only when the animal has a variety of food most of which is not very fatty. Changes in the ingested fat may occur, and in the utilization of vegetable fats ingested by animals (work of Bhattacharya and Hilditch) oxidation of a portion of the linoleic and oleic acids may occur leaving an acid content more or less adapted to the requirements of the animal (Bloor pp 237-239 1943).

In insects there is considerable evidence that the synthesis of fats from proteins and carbohydrates occurs. According to Scoggins and Tauber (1950) Hofmann concluded that Muscida vomitoria, Weinland concluded that Calliphora (sp. ?), and Nishikata concluded that Sarcophaga carnaria, the flesh fly, were able to build body fat from

diet protein; but Bogdanow was unable to confirm the results of Hofmann and of Weinland. Evidence exists for the formation of fat from carbohydrates in the dermestids, Anthrenus museorum (reported by Abderhalden) and in Dermestes (reported by Sinoda and Kurata).

Melampy and Maynard (1937) concluded that the German roach could convert proteins into lipids; and for the same insect MacCay (1938) found that adults reared on a skim milk - whole wheat diet contained three times as much lipid as the ingested foodstuff. Schweet concluded that the American cockroach could synthesize fat from protein although to a lesser extent than from carbohydrate. Collin (1933) comparing the fatty acids of the larvae of a beetle, Pachymerus dactris, with its food fats suggested that the data indicated that the larval fats were derived in part from the food fats and in part from carbohydrates. Working with the corn ear worm Ditman and Weiland (1938) found that the prepupae and pupae of worms reared on dough stage corn contained a higher percentage of fat and this fat had higher iodine numbers than was the case of prepupae and pupae from worms reared on milkstage corn. Adults of a cutworm, Agrotis segetum and of the European corn-borer may maintain their total lipid contents provided they are fed highly concentrated glucose solutions (Kozhantshikov 1938). According to the investigation of Fulton and Chamberlain the beet leaf hopper could increase its fat content when fed sugar solutions but could not maintain itself indefinitely (Scoggin and Tauber 1950).

Melampy and Maynard fed the German roach on different diets. On diets with the iodine numbers of the fats 7, 27, and 49 the tissue fats of these roaches were 51, 59, 64 respectively. The degree of utilization seemed to agree also with the type of ingested fat. According to

the unpublished work of Schweet (Scoggin and Tauber 1950) the lipid content of the American roach showed no significant differences (except for roaches on steak with 3 percent fat) even though the fat content of the diet ranged from 1 to thirty percent. The iodine number of the fats of roaches reared on low fat diets was not correlated with the iodine number of the fats in the diet.

The iodine number for fats of the sheep blowfly, Lucilia sericata, fed on fish heads ranged from 120 to 140, the iodine number of the food fats was 113; if fed on a synthetic diet containing fats with an iodine number of 30 the iodine number of the fly fats was 60. This was interpreted as indicating a strong dietary influence (Yuill and Craig 1937); and this influence of diet on the fat of this insect has received further experimental support (Rainey 1938).

The ability of an insect to synthesize lipids may be variable between species and may be limited also. Fraenkel and Blewett (1947) found that yellow mealworms feeding on a diet containing linolenic acid had 20 percent linolenic acid in its fats, on a diet without linolenic acid - 10 percent; the Mediterranean flour moth, Ephestia kuhniella Zeller, on a diet with linolenic acid had 15 percent of its fats linolenic acid but on a diet without linolenic acid has only 1 percent of this fatty acid so essential to the proper development of this insect.

Even in warm blooded animals the chemical nature of stored fats is influenced to some extent by the environmental temperature. Zummo (1932) kept white rats at temperatures of 0^{o} and 30^{o} C and found that the iodine numbers of the reserve fats decreased with the time of exposure to the higher temperature. Also the iodine numbers at both temperatures seemed to be influenced by the diet. About twelve to fifteen days, according to the data, after rats were placed under a given

set of conditions they seemed to come to a level and thereafter showed little change. Zummo's values for iodine numbers for the rats under the varying conditions ranged from 45 to about 80. The early work of Henriques and Hansen correlates the iodine number and melting point of fats from different locations in the pig with the temperature of the region from which the fat was taken. The iodine numbers fall from 72.3 in the outermost layer of skin fat to 56.1 in omentum fat; the temperature 1 cm. under the skin in pigs is 33.7°C, 4 cm. under the skin - 39.9°C. Similar findings are reported for the beef and sheep (Bloor -242-242, 1943). Dean and Hilditch (1933) confirmed and extended this work but at the same time they concluded that the general statement that warm blooded animals and plants of tropical origin produce more solid saturated fats than cold blooded animals or plants from cooler regions is only partially true.

Lovern (1938) working with the eel, Anguilla vulgaris, found that a low fat diet caused no change in the fats of the eel, but a high fat diet exerted a not too clear influence which the investigator suggested was due to temperature effects. Lovern also reported that Brockley and Bailey found increasing iodine values for the fats of salmon as they went further north but only if the same species of salmon was considered. It is also pointed out that tropical fish do not have necessarily more saturated fats than even closely related species living in colder waters.

Only a few investigations of the relationship of temperature and the lipids of insects have been made. Ackerman (1926) reared an aphid, Rhopalosiphum prunefolia, at different temperatures and noted that the solidification point for fat globules in the body fluid increased with increase in the rearing temperature. Rainey (1938) found the iodine numbers of the sheep blowfly to be 75.8 at 15°C, 72.7 at 25°C, and 71.1

at 35°C. Ditman and Weiland (1938) found some differences in the iodine numbers of the lipids of the corn ear worm; prepupae from insects reared at 78°- 88° F had lipids with an iodine number of 70.7 at 85°-99°F the iodine number was 65.8. The iodine values for the phosphatides of the bluebottle fly, Calliphora erythrocephala (Meig.) and the sheep blow-fly, Phormia terra-novae R.D. (Fraenkel and Hopf 1940) undergo quite a fall with rise of temperature. Iodine numbers (averaged from the data of Fraenkel and Hopf by the present author) are 91 at 18°C, 75.6 at 27° and 65.5 at 36°C for Calliphora and 85.2 at 12°, 77.2 at 27°and 70.4 at 30-31°for Phormia. It should be noted at this point that some of the iodine values quoted in preceding paragraphs on stages of development, diet, etc. may in fact have been influenced or modified by temperature. Pepper and Hastings in their work on the sugarbeet webworm collected the insects from the field where they must have been subjected to fluctuating temperatures although, undoubtedly, the variations reported in this excellent work are due, as its authors ascribe them, to the different instars.

By way of summary, it is seen that the lipids of insects, as well as other animals undoubtedly may be influenced by the following factors: stage of development, caste, sex, phase; hibernation, starvation, migration, systematic position, diet, and temperature of the ambient medium. Therefore, in any physiological study involving the lipids as many of these factors as possible should be made constant. As a generalization one may then say that the lipids of an organism are determined by the interaction of (1) the genetic characteristics of the species (2) the nature of the ingested lipids and (3) temperature.

There is a viewpoint with regard to the lipids which enters into the present investigation. In selecting the phosphatides, Fraenkel and

Hopf (1940) were choosing for investigation the lipids considered to be of more vital importance, that is to say, those which entered more directly into cellular metabolism, and were thereby avoiding any differences which might be attributable to selective utilization of the stored lipids. This differentiating concept of the stored lipids being more or less variable in character and the phospholipids being more or less constant, dates back to the work of Mayer and Schaeffer in 1914 who found that overfeeding or inanition did not greatly modify the lipid phosphorous content of organs (Bloor 1943, p 247-250). Terroine and his affiliates (1930) studied the constant lipids, phospholipids and free cholesterol, as well as the variable content, i.e. the neutral fats. The former were relatively constant both quantitatively and qualitatively for a specific organ. Parenthetically, it may be said that the phospholipids of the insect as such are practically uninvestigated. Phosphatides of sugarbeet webworm amount to about 0.2 - 0.4 percent of the total fats (Pepper and Hastings 1943). Bloor (p 229, 1943) gives the unpublished data of Stoneburg on the phospholipids of the grasshopper, Brachystola magna 1.025 percent in thoracic muscle, 1.24 percent in thigh muscle, cholesterol 0.045 percent of wet weight.

While the vital role of the constant elements or specific organ lipids is in no way a matter of disputation, one may well think that the differences between these and the reserve fats have been perhaps overstressed to create the conception of one set of lipids playing a vital protoplasmic role in the cell and another set being held in reserve to await the time when some emergency would necessitate their mobilization for use as an energy source. In contrast to this concept Schoenheimer and his associates (1940) have by the study of the metabolism of the white rats by use of tracer substances produced

rather startling revelations. Fatty acids are freely and continuously interconverted, oleic from stearic, palmitoleic from palmitic but linoleic and linolenic do not seem to be formed by desaturation. Lengthening and shortening of the carbon chain apparently occurs with ease. These processes occur not only in the neutral fats but in the phospholipids. The fats in a liver may mostly have been formed within the last day; there is a constant and rapid turnover.

2. The 'Lipoid Liberation' Theory

Among the various theories listed by Heilbrunn (1947) and by Prosser (1950) which have been proposed to account for the death of organisms exposed to high temperatures are: protein coagulation, enzyme inactivation, viscosity alteration, inadequate oxygen supply and water loss. One of the more inviting theories has to do with the lipids of the cells. Heilbrunn (1924) working with Arbacia eggs described a heat coagulation other than protein coagulation and which occurred at lower temperatures than protein coagulation. This heat coagulation was apparently in some way associated with some change in the state of fatty materials of the cells as it was increased by small concentrations of ether. Heilbrunn pointed out the correlation between the melting point of the lipids of organisms and the sensitivity of organisms to heat. Belehradek (31) extended these observations.

While many experiments on the acclimatization of animals have been performed, still very little has been done by laboratory experiment to test the role of lipids in thermic injury. In their well designed and executed work published in 1940, Fraenkel and Hopf set out to test the 'lipoid liberation' theory, that is the theory that heat injury whether reversible or irreversible is caused by the melting of lipids in the cell

or cell membranes. The blowflies, Calliphora erythrocephala and Phormia terra-novae were reared at low, medium and high temperatures. For Calliphora the temperatures were 12°, 27° and 30-31°C for Phormia 18°, 27° and 36°. For Phormia 15°C was also used; but the authors used two methods of determining the iodine numbers and the results are not exactly interchangeable. The iodine numbers (vide supra) for the phosphatides were higher for the lower temperatures. Moreover, flies reared at lower temperatures and thereby having higher iodine numbers seemed less resistant to killing temperatures. The authors concluded that the blowfly larvae reared at higher temperatures not only become more resistant to killing temperatures but the melting point of the fats in their cells is adapted; and the authors suggest that while the results lend support to the lipoid liberation theory, death at moderately high temperatures hardly is due to a mere melting of protoplasmic lipids, although such may, as it were, trigger a sequence of events.

In a subsequent investigation Hopf (1940) subjected the same two species of flies to high temperatures and found that in both species there was an increase in the lipidphosphorus, the inorganic phosphorus, and in the adenylpyrophosphate phosphorus in the haemolymph. On the basis of his complete data Hopf postulated a preferential release of phosphatides compared with other fats. As phosphatides are known to form rather stable complexes with proteins he concluded the phospholipide release was a metabolic process rather than a physical dissolving of tissue materials. He did conclude, however, that the changes which he described, represented an adaptation of the organism towards a change in its environmental conditions.

Heilbrunn, Harris and Lefevre (1946) have presented strong evidence

that in the vertebrate heated tissues may liberate toxins which cause changes in tissues at a distance. The toxin seems to be a thrombin-like substance possibly interfering with a sodium /potassium ratio. At present there is no evidence that this could apply to the insect.

In contrast with the small amount of work which has been done to test the validity of the 'lipoid-liberation' theory much study has been devoted to acclimatization. Of this, however, little is germaine to the present paper; a few papers may be profitably considered. Mellanby (1939) studied chill coma in the oriental roach and found that cold in activation was appreciably lowered if the animals were previously subjected to cold acclimatization. Twenty-four hours was sufficient to produce the acclimatization.

Sacharov (1930) working with hibernating beetle larvae, Melolontha hippocastani and Plagionotus arcuatus found water lower and fat content higher in cold resistent insects. Kozhantshikov (1938b)found no obvious correlation between fat content and cold hardiness in several species of lepidopterans; but suggested that unsaturated fatty acids may be of importance in cold hardiness. Slifer (1932) found the iodine number for the fatty acids of the eggs of seven species of grasshoppers to range from 128 to 167. Also the eggs of nine species of grasshoppers which lay eggs in the fall to hatch the following spring yielded fatty acids melting between 25.5 to 30.5^{o}C, but three spring laying species whose eggs hatch in the subsequent summer yielded egg fatty acids melting in the range 37.0 to 39.5^{o}C. She concluded that factors other than environment influence the fats laid down. Ditman and Weiland (1938) point out that reduced water content, increased fat and reduced saturation of the fat are factors, generally associated with the ability of an insect to withstand hibernating conditions.

Thus, it seems the lipids of an insect may be of importance in its survival of chilling as well as heating conditions.

3. Toxicity and Physiological Action of DDT

The exceedingly great insecticidal effectiveness of DDT has instigated a vast amount of investigation by many workers in an attempt to discover the physiological basis of its effectiveness. One of the earliest theories of its action was set forth by the discoverers of its toxic properties, Lauger, Martin and Muller (1944). This attributed the effectiveness to the presence in one molecule of the bis(p-chlorophenyl) methylene group which conferred high lipoid solubility. Martin and Wain (1944) were of the opinion that the p-chlorophenyl groups were responsible for the lipoid solubility and that the trichloromethyl group was responsible for the toxicity by undergoing dehydrohalogenation. They further theorized that there might be chemisorption and interference with some enzyme system. They pointed out that an effective poison should withstand translocation to the site of physiological action. In passing it may be noted that the dehydrohalogenation theory is not suitable for universal application for effective chlorinated organic insecticides as some of them do not undergo such breakdown; in fact Martin and Wain (1949) list two fairly effective compounds which do not. Also increasing the number of chlorine atoms may in some cases decrease the toxicity of some chlorinated hydrocarbons for some arthropods (Metcalf 1948, Dormal et al. 1950).

Busvine (1946) working with analogues of DDT observed that the greater the departure of the compounds from the DDT molecule the less the toxicity; and he suggested that the entire molecule is involved in a steric relationship to a vital enzyme, the toxicity thus being attrib-

utable to the molecule as a whole. Meanwhile, in an address delivered in 1945 and published in 1946 by Lauger and his associates (1946) redefined the 'lipoid solubility component-toxic component theory' to "____architecture of the molecule".

The theories of lipoid solubility have been attacked on the basis that some less effective analogues of DDT are more lipoid soluble. Brown (Chapter 1, 1951) points out that while lipoid solubility and the contact toxicity of DDT and its analogues show no direct relationship it may well be that nearly all of these are above the threshold of liposolubility.

These theories of lipoid action may be considered from the viewpoint of penetration of the molecule through the integument of the insect or into the lipids of the cell. Half a century ago Overton (1900) studied the penetration of various dyes into the plant cell and noted the probable involvement of the lipids of the cellular membrane; there has arisen a theory of narcosis known as the Overton-Meyer theory. This postulates that narcosis occurs when a chemically indifferent substance reaches a definite molecular concentration in the cell lipids (Meyer and Hemmi 1935). Gavaudan and Poussel (1947) considered DDT to be such an indifferent narcotic acting on a lipoid substrate. The thermodynamic activity for DDT calculated from c/c_o where c is the active concentration i.e. the minimum lethal concentration and c_o is the aqueous saturation concentration gave values in agreement with those determined by Meyer and Hemmi (1935) as characteristic of indifferent narcotics. In considering the literature on the penetration of DDT through the cuticle a review of all the literature on the cuticle of insects is not necessary. Richards (1951) lists the lipids of the integument and these seem to be primarily waxes. An exception is in the oriental cockroach

in which the cuticle is covered with a mobile grease (Beament 1945) spreading at all temperatures. Webb and Green (1945) working with the sheep ked, Melophagus ovinus L. found that high carrier efficiency was correlated with a high rate of penetration through beeswax, a high partition coefficient of the solvent between beeswax and water; and that certain solvents increase the rate of penetration by carrying the insecticide through the lipid layers of the epicuticle.

With regard to the penetration of DDT through the cuticle Tobias, Kollros, and Savit (1946) found that the toxicity of externally applied DDT and injected DDT for the American roach were not very different; this indicated a rapid and effective absorption of the insecticide. For adult roaches fifteen micrograms by injection gave a fifty percent mortality at ninety-six hours; application of thirty micrograms externally gave a fifty percent kill in seventy-two hours. Richards and Cutkomp (1946) observed that animals with a chitinous cuticle were especially susceptible to DDT, and thought that the chitin might selectively concentrate the DDT by adsorption. Hurst (1949) reports that for Calliphora erythrocephala (Meig.) penetration of the cuticle by DDT is increased with temperature as its lipid solubility is augmented, but that paralysis follows only after the temperature is lowered. He thought that DDT in the cuticular lipids acts on the peripheral network. He found a decrease in the storage capacity of the cuticular lipids for DDT by the adsorption displacement of the more mobile components on to an inert adsorbent powder, alumina.

Hoffman et al (1951) using radioactive tracers found that 31 to 40 percent of DDT applied to houseflies is absorbed and Lindquist et al. (1951) found that 31 to 71 percent of absorbed DDT is metabolized in susceptible flies, but 63 percent was detoxified by resistant flies. DDT

or its metabolites appeared in sundry organs but in some only after several days. Apparently from 26 percent to 34 percent may be absorbed by internal organs, the remainder being distributed throughout the cuticle (1951 Lindquist, Roth, Hoffman, Butts). Sternberg, Kearns, and Bruce (1950a) found DDT readily absorbed by both nonresistant and resistant strains of houseflies. However, the more resistant strain appeared to be able to degrade the DDT to DDE, the ethane analogue. (Sternberg and Kearns 1950a). Perry and Hoskins (1951 a and b) found that the synergistic action of piperonylcyclonene which greatly increases the mortality of resistant houseflies treated with DDT (but not susceptible ones) concluded that this is not due to increased absorption of DDT but to the prevention of degradation to DDE by the resistant strains.

Because of the striking symptomatic picture in the DDT - poisoned insect, early conjectures arose that it might affect the nervous system. In 1945 Yeager and Munson by a series of experiments with the American roach demonstrated that DDT in high concentrations could cause its characteristic symptoms by an action on a motor nerve between its origin in the ventral nerve cord and the termination of its fibers in a muscle. Shortly after the publication of this work Tobias and Kollros (1946) with somewhat similar experiments confirmed and extended the above observations; and described in detail the symptomatic picture in the intoxicated roach. By a study of action currents Roeder and Weiant (1946) found that although DDT did cause symptoms when applied to motor nerves in high concentrations, in exceedingly low concentrations it could cause spike discharges in afferent fibers of the roach. Bodenstein (1946) found that in Drosophila the neuromuscular system of the wings and legs were involved in spasms long before the muscles of the abdominal wall; moreover, no tremors were present if the insects were

narcotized with phenobarbital.

Welsh and Gordon (1947) suggested that continuous activity induced by DDT action on arthropod axons might cause metabolic exhaustion and death; the same authors (Gordon and Welsh 1948) presented evidence that the toxic effect of DDT could be modified by the concentrations of calcium, magnesium and potassium ions in the physiological saline, and suggested that the calcium in the nerve surface was interfered with.

Roeder and Weiant (1951) again with oscillographic recording found that trains of impulses appear in afferent fibers of the crural nerve of the cockroach only when a definite period of time has elapsed after the injection of DDT through the leg. This time of onset seemed unaffected by temperatures between 12^{o} and 32^{o}C. Moreover the effect of injecting 3ppm DDT could not be removed by washing repeatedly with saline. The authors suggest that DDT is immediately bound at the surface of sensory neurones and that the time interval between application and appearance of the impulse trains is occupied by the solution of DDT in a lipoid layer below the neurone surface and that the appearance of the impulse trains in the afferent fibers indicates the reaching of a required concentration in the lipoid layer. A constant time interval between knockdown and death for DDT-treated houseflies Musca domestica L. has been observed by Yeager and Munson (1949) who interpreted this to mean, probably, that once the accumulation of DDT at certain tissue sites attains a certain level the lethal action of the poison may go on independently of the DDT concentration.

Some investigators have sought histologically detectable effects of DDT on the nervous system of insects but the results are not in good agreement (Metcalf 1948). A recent paper (Chang 1951) reports the virtually complete disintegration of the Golgi apparatus in ganglionic

ganglionic cells of the American roach. In this connection it is well to recall the well known fact that the Golgi bodies, whatever they may be, are readily soluble in lipoid solvents; stain by some quasi-selective lipoid stains; and among other things have been considered a site of cellular oxidation. So far, however, no one has produced any evidence that DDT can exert any direct physiological effect on the ganglion in the insect.

The effect of DDT on the metabolism of insects has been investigated from several angles. Merrill, Savit and Tobias (1946) found no significant change in the water content of poisoned American roaches but found a precipitate drop in glycogen and glucose. Immobilization of these roaches by anesthesia eliminated almost entirely the carbohydrate depletion but did not prevent the death of the insects. Pyruvate content fell as poisoning progressed but this could be almost completely prevented by the administration of glucose. Only, gross estimation of fat reserves were made; and no significant change in non-protein nitrogen was produced.

Ludwig (1946) reported that in the larvae of the Japanese beetle, Popillia japonica Newman, poisoned by DDT glycogen was almost depleted but there was no loss of protein. Increased oxygen consumption corresponding to the period of symptomatic hyperactivity occurred and the Respiratory Quotient indicated oxidation of carbohydrates and fats the first two days and the oxidation of fats thereafter. Ludwig suggests that the hyperactivity exhausts the reserves of the insects -- in effect causing death by starvation. Lord (1949) poisoned the sawtoothed grain beetle, Oryzaephilus surinamensis (L.), with DDT and some of its analogues, and found the oxygen uptake of DDT-poisoned beetles to be the same as starving ones. He considered death to occur from a de-

pletion of reserves. Lord (1950), also poisoned the flour beetle, Tribolium castaneum (Hbst.), and found that several organic insecticides including DDT increased the oxygen uptake before killing the insects.

Buck and Keister (1946) found in DDT-poisoned blue-tailed flies, Phormia regina (Meigen) a greater water loss than occurred in starving but otherwise normal blowflies; also the R.Q. of 0.90 forresting controls and 0.93 in active controls indicated a utilization of both carbohydrate and fat but in DDT-poisoned flies an R.Q. of 0.96 indicated high carbohydrate utilization. Ludwig, cited above, found much metabolization of fat in the DDT-poisoned Japanese beetle.

Fullmer and Hoskins (1951), comparing resistant and susceptible strains of houseflies, found that they normally respire at equal rates. Application of successively larger dosages of DDT to groups of susceptible flies caused the respiration to increase after delays varying with the dosage; after passing a maximum there was a decline proportionate to the previous rise and after several hours to a day respiration reached zero. Resistant flies increased their rate much less and lower maxima were obtained. If piperonyl cyclonene was added to the DDT the respiration curves resembled those of susceptible flies but still had lower maxima. These authors concluded that the critical process in DDT poisoning of houseflies is the accumulation of a portion of the absorbed DDT in regions where it causes the typical muscular activity. If the concentration in these regions is kept down by detoxification the insects are resistant, but if detoxification is inhibited the insects are made susceptible.

March and Lewallen (1950), comparing the DDT-resistant and

non-resistant houseflies, found no difference in the two strains with regard to survival of unfavorable temperatures except that females at 45° were more resistant. Dahm and Kearns (1951) determined the total phosphorus, phosphoarginine, pyruvic acid, reducing substances expressed as glucose equivalents, and non-protein nitrogen in houseflies, and although they found highly significant differences between normal flies and flies held six hours without food and water, they apparently found no variations attributable to DDT.

The possible action of DDT on enzyme systems must be considered. It was inevitable that the startling symptomatic picture in DDT-poisoned insects should stimulate an investigation of the acetylcholine-cholinesterase system. Tobias, Kollros, and Savit (1946 b) found that in American roaches and houseflies prostrated by DDT the acetylcholine of the nerve cord increased by 200 per cent. Previously Richards and Cutkomp (1945) used several cholinesterases as substrates for the measurement of cholinesterase activity in bee brains and roach nerve cords. They found that DDT did not inhibit cholinesterase when added to the roach cord in vitro but because of the low solubility of DDT in water considered their evidence as inconclusive. The increase in cholinesterase activity in these cords seems to be restricted to the interganglionic connectives.

Babers and Pratt (1950) found cholinesterase activity lower in resistant strains of houseflies than non-resistant until the fifth day after emergence and that the males had much greater cholinesterase activity than the females. Also, these authors (1951), finding differences in the cholinesterases in the heads of houseflies, cockroaches and honeybees, emphasized the necessity for several substrates in the study of cholinesterases. Metcalf and March (1950) had found the cholinesterases

of the housefly, bee, and mouse to differ. Earlier papers on the normal occurrence of acetylcholine in the insect are cited by these authors. Apparently phosphorus containing organic insecticides have a more definite effect on cholinesterases than DDT has (Metcalf 1950, Chamberlain and Hoskins 1951).

The cytochrome system seems to be more affected by DDT, perhaps, than does the above system. Munson and Yeager (1946) injected a series of compounds into the American roach and compared their speed of kill and symptomatic effects with DDT. Among the compounds causing symptoms similar to DDT were some known to be oxidized by the cytochrome oxidase system and three of these, pyrogallol, and, especially p-phenylenediamine and alpha-naphthol, have long been used in the detection of indophenol (cytochrome oxidase). These facts along with the toxicity results prompted these authors to conclude: "---an effect upon the cytochrome oxidase system in the insect might be a part of the mode of action of DDT." According to Lauger et al. (1946) compounds which are fat solvents non-miscible with water but which are hydrophilic (as chloroform) will produce narcosis, while fat solvents non-miscible with water and hydrophobic (benzene, chlorinated benzenes, carbon tetrachloride, etc.) will produce tremors. Also, miscible alcohols such as alcohol and acetone will produce tremors. To the present author this has never seemed an adequate explanation of the effect of the compounds just mentioned.

Hurst (1945) in a stimulating paper discussed the "narcotic" of DDT and speculated about a possible effect on the polyphenol oxidases present in the integument. Also, Hurst (1949) comparing DDT and fat solvents recalled similarities in symptomatic tremors and referred to the known fact that fat solvents exert a nonspecific action on intracellular

respiration by inhibiting the dehydrogenase systems such as succinic dehydrogenase which reduces cytochrome oxidase; and these reducing systems show a high temperature coefficient of activity. As experimental results he reported that the average rate of oxygen uptake of 23.6 cmm/gm/min fell after DDT paralysis of Calliphora to 7.8 cmm/gm/min. Injected succinate induced complete, and injected glucose induced partial recovery; but lactate and pyruvate were ineffective. Also if flies were injected with succinate they were much more resistant to DDT.

Sacktor (1950) says that DDT and its methyoxy analogue inhibited the cytochrome oxidase activity of the enzyme from mammalian heart and from house flies. With reference to cytochrome oxidase activity in two strains of flies he reports that the resistant strain had greater cytochrome oxidase activity than the non-resistant strain.

Apparently DDT has no measurable effect on glutathione (Forgash 1951).

Lindquist, et al. (1945) obtained a negative temperature coefficient for knockdown and kill of houseflies exposed to DDT, that is the knockdown and kill at lower temperatures was greater than for higher. The temperature at which the flies were kept previous to the treatment was not given. Potter and Gillham (1946) used a beetle, Tribolium castaneum and found that environmental temperature before the test did not influence the results but cooler conditions after spraying did influence the speed of toxic action of DDT to these insects. The time of exposure to the "conditioning" temperature before the experiments were too brief to permit any comparison with the results of the present investigation. Other investigators, including Hafliger (1948) who used honeybees, Woodruff (1950) who used the milkweed bug,

Oncopeltus fasciatus (Dallas), have reported a negative temperature coefficient for DDT. Woodruff used an injection technique.

Fan Cheng, and Richards (1948) found that with injection of DDT the temperature coefficient was positive but that for externally treated larvae of the yellow fever mosquito, Aedes aegypti (L.), and a midge, Chaoborus sp. the coefficient was negative in the range 16^{o}-20^{o}C. Although these authors used pretreatment at various temperatures the times of exposure were too brief to be comparable with the results of the present investigation. A proved explanation of this negative temperature effect is still unprovided (Richards 1951).

It is neither necessary nor desirable here to recapitulate the excellent and voluminous literature on the interactions of DDT and the mammal. In his 1945 address Lauger (1946) described symptomatic effects on the nervous system of the rabbit and stated that(based on a personal communication from Dr. H. O. Calvery) it was known that if DDT were fed to the lactating bitch, the poison would appear in the milk fats. Telford in 1945 reported that the milk of DDT-fed goats was poisonous to rats and houseflies. White and Sweeney (1945) described the degradation of DDT to di (p-chlorophenyl) acetic acid in the rabbit and the excetion of this compound in the urine. Vaz, Pereira and Malheiro (1945) reported an alleviation of the toxic effect of DDT on dogs by injecting calcium gluconate. Judah (1949), also, reported that calcium gluconate can afford some relief to the DDT intoxicated mammal. The effects of DDT on the nervous system of mammals, its storage in fats and its degradation and excetion have been repeatedly confirmed for most domestic and laboratory mammals under diverse experimental conditions. The reader interested in this phase of DDT action is referred to the summary by Brown (1951 pp 504-518).

The time is not yet propitious for final conclusion with regard to the lethal physiological action of DDT; but certain phenomena with regard to its insecticidal action seem reasonably clear. It posseses sufficient lipoid solubility and the proper steric configuration to permit it to pass easily through the cuticle of the insect. It can, and does set up repetitive discharge volleys in the nervous system of the insect either by direct action on the calcium-lipoprotein complex at the surface of the nerve fibers or by some unclarified method of indirect stimulation. Whether or not one considers it an indifferent narcotic depends on the weight one gives to this phase of literature. Its partition coefficient is such that it meets the physical requirements of an indifferent narcotic. It may well be an indifferent narcotic and yet have its lethal action be due to an entirely different action.

Another question arises: is the death of the insect due to a depletion of its energy reserves or may it occur subsequent to the disruption of an enzyme system? Neither of these possibilities is yet excluded. Finally what are the factors which give one strain of a species more resistance than another? Detoxification and excretion probably provide part of the answer here; but likely do not provide the whole answer.

One thing, however, is certain:- at many points the lipids of the insect and DDT with its high lipid affinity are in contact. In penetrating the cuticle, in entering the lipoprotein membrane of cells, in possible adsorption onto lipoprotein surfaces, in storage in lipids and probably in its relationship to temperature and to enzymes DDT may possibly have its action modified by the nature of the lipids of the insect to which it is applied.

SECTION III

THE EXPERIMENTAL INVESTIGATION

1. Conditions Common to All the Experiments

The animal used in these experiments were American cockroaches of which the ancestors were captured at the National Zoological Park several years ago. Since that time the colony has been maintained under the same general conditions as during the time of these experiments. The roaches, confined to two large glass aquaria, were fed exclusively on "Milkbone" dog biscuits produced by the National Biscuit Company. Six samples taken from six different cartons of this food were analyzed for the iodine number of the fats using the same procedure outlined below. All of the individual values were near to the mean of 74. No other analysis of any sort was made on the food. The analysis on the carton, stated as guaranteed by the company, was as follows: minimum crude protein 21.00 percent; minimum crude fat 1.50 percent; maximum crude fiber 2.50 percent; maximum ash 6.25 percent; maximum moisture 12.00 percent; minimum nitrogen free extract 55.00 percent. The listed ingredients were unbleached wheat flour, milk, soybean oil meal, meat meal, wheat germ, bone meal, irradiated brewer's type yeast, fish liver oil (0.03 percent), vegetable oil (0.24 percent), calcium carbonate, baking powder, dried fermentation solubles and salt.

The room in which the roach colony was kept became very warm occasionally during the summer. However, with the advent of cooler autumn weather it was possible to maintain a relatively constant ($\pm$ 2°C.) temperature as desired by means of a thermostatically controlled electric heater. In addition to this there was available for

controlled temperature conditions a "cool" refrigerator set at 17°C and fluctuating not more than one degree from this setting; and a "warm" box made from an old refrigerator into which an electrical heating unit controlled by a very sensitive thermostat was placed. This fluctuated less than a degree about the setting point. Except for the heat death experiments the temperatures reported in this paper were obtained by means of the above equipment.

Groups of roaches for pretreatment at various temperatures preceding the determination of iodine numbers, reaction to heat, or poisoning with DDT were taken from the stock colony and groups of about thirty were placed in glass storage jars (14 cm. x 14 cm. x 24 cm.) supplied with the dog biscuits and water. Each jar was balanced with regard to sexes and only large nymphs were selected randomly and placed alternately if two jars or by rotation if a series of jars were set up at the same time in order to obtain approximately equivalent groups with regard to size. No nymphs which would have weighed less than 400 mg. were used; as it was intended to use (and as was done) in the subsequent experiments, no nymphs weighing less than 500 mg. Many nymphs passed through the imaginal moult. A few adults were used in the experiments but all of these were very young adults and wherever adults were used it is called to the reader's attention.

2. The Determination of the Iodine Numbers of the Lipids

All reagents used in the extraction of the lipids and in the determination of the iodine numbers were of the quality specified for this type of work.

At the very outset the problem of selecting a method of extraction of the lipids arose. It was decided that a simple and rapid method requiring simple equipment would be most suitable for this investigation

as the primary purpose was to obtain a sample of the total lipids of a small amount of material. A method which secured a representative sample and avoided or minimized enzymatic destruction and oxidation of the lipids would be satisfactory.

Roaches of which the body weight was determined, were placed in an Erlenmeyer flask (1 to 10 roaches per flask); and were killed by pouring boiling ethyl alcohol over them. It was hoped that the high temperature would not only kill the roaches; but as fat splitting enzymes are generally inactivated by such a high temperature (Bloor 1943 Chapter II) would also avoid any enzymatic destruction of the lipids. The roaches were then minced with dissecting scissors and the fragments were ground in a mortar with the alcohol in which they were killed poured over them and ten to thirty cubic centimeters of sand which had been washed in water, in alcohol, and in ether was added to the contents of the mortar. The roaches were then ground to break up the tissues. The alcoholic brei was transferred to a large beaker. The Erlenmeyer flask, the mortar and pestle, and the scissors were rinsed with hot alcohol which was poured into the beaker. A volume of alcohol was used to give a ratio of about ten of alcohol to one of tissue in the beaker. It will be noted that the alimentary tract was included in the brei. Some workers keep the experimental animals on a starvation regime to cause an emptying of the gut contents. It was not considered that this would greatly influence the results with roaches and would also introduce the factor of starvation so this was not done.

The beaker with the mixture in it was placed in a water bath and its contents gently boiled for about half an hour. The contents were then filtered over fat free filter paper and the residue was washed

with a volume of hot alcohol about half the original amount in the beaker. The rinsing alcohol was applied in two portions; the first one was poured over the residue after dripping from the funnel had ceased and the second portion to which was added twenty to thirty cubic centimeters ether was poured over the residue after the first wash alcohol had gone through.

As no vacuum evaporation equipment was available, the filtrate was collected in a flask and the alcohol evaporated by standing the flask in boiling water. Care was taken not to evaporate to dryness, a point on which Bloor (1943, p43) recommends great caution. After the alcohol was evaporated the material remaining was taken up with ether for rectification. This was filtered and the filtrate collected in 35 cc test tubes. From these tubes the ether was evaporated by manually agitating the tubes in warm water. When the tube contents were down to about two milliliters this was poured into a ten milliliter glass vial which had been carefully weighed. The test tube was rinsed with about one milliliter of ether and this was added to the vial. Final evaporation of the ether was accomplished by agitating the vial in boiling water; after which the vial was dried and reweighed to obtain the weight of the fat sample contained therein. This method will undoubtedly extract practically all the lipids of the animals but in order to get all these lipids in the final vial more rinsing along the way is necessary in order not to lose lipid by having it adhere to the glassware. This additional rinsing was done in three of the experiments only - this will be discussed below. It is the feeling of this investigator that the above method is a satisfactory one for obtaining a representative sample of the total lipids of the insects, and in this investigation by total lipid such a sample is meant. However, at two points especial care must be

exercised: (1) the alcohol solution must not be evaporated to dryness and (2) the evaporation of the ether from the test tube must be done slowly as the ether will easily boil over.

The Wijs method was used for the determination of the iodine numbers. This method depends upon chlorine to hasten the taking up of the iodine by the unsaturated lipids. In preparing the Wijs reagent the chlorine was generated by heating hydrochloric acid and potassium dichromate together in a generator. All the other reagents met the specifications of and were prepared in accordance with the procedure given by Jamieson (1943); and the determinations followed his directions except that the sample of lipids was occasionally smaller than recommended. This is undoubtedly a source of some error in the results. Careful attention was given to the end point and to the breaking up of the chloroform stratum in the titration flask. Two blanks were always used for each series of titrations.

The design of the particular experiments may now be considered.

Experiment 1. This was in some ways a preliminary and exploratory experiment. Three groups of roaches were used: Group A was stored at 34°C, group B at room temperature, and group C at 17°C. The previous history of the roaches had been one of high room temperature and during the course of the experiment the room temperature was about 31°C and, undoubtedly, had been this high for several days previous to this experiment as the room kept at a high temperature generally. The original idea was to let the B group of roaches as "room temperature" be subjected to fluctuating temperature conditions. Two days before the first analyses were run for this experiment the idea of letting the temperature for the B group fluctuate was abandoned; the laboratory

radiator was turned off and the thermostatically controlled electric heater was turned on with the thermostat set at 27°C. In effect then the roaches in all three groups had been at a high room temperature of about 31°C for many days before the groups were set up and except for the last two days group B continued at this temperature for experiment I; but groups A and C were at the temperatures of 17°C and 34°C respectively. At the end of one week three lots from each group were killed, their lipids extracted and the iodine numbers determined. The lots, weights, sexes, instars, iodine numbers etc. for this and all the other iodine number determinations are shown in Table I. In this first experiment, especially in group A the groups were mixed nymphs and adults. In subsequent experiments except for one determination such mixed groups were avoided.

Experiment 2. The A at 34°, B at 27° and C at 17°C groups of experiment 1 were continued for another week after which three lots from each group were set up and the iodine numbers determined.

Experiment 3. The above groups were continued for a third week, divided into lots and the iodine numbers determined.

Experiment 4. The iodine numbers of five lots of roaches held at 29°C for two weeks were determined. Their previous temperature had been 23°C for several weeks. In this experiment an attempt was made to obtain a quantitative value of the lipids. Expressed as percent of wet weight the following values were obtained: for the male lots 6.3, 5.3, 5.9; for the female lots 5.0 and 6.9.

Experiment 5. The roaches previously at 23°C for over three weeks were kept at 30°C for two weeks and then the iodine number for their lipids were determined. The quantity of the lipids for the lots in table 1 were for the male nymphs 6.1, 7.4, 7.0 and for the female nymphs

4.1, 4.6 and for the one group of female adults 4.3 percent of wet weight.

Experiment 6. A group of roaches previously at a temperature of 32°C for several weeks were kept at 39°C for twelve days after which they were subdivided into four groups for which the iodine numbers of the lipids were determined.

Experiment 7. This experiment was undertaken to determine not only the iodine number of the lipids of roaches at 23°C for over four weeks but also to determine the extent of variability in quantity of total lipids and iodine numbers determined for individual roaches. Five male and five female nymphs were used. The lipids as percent of wet weight were for males 9.5, 4.6, 7.0, 9.5, 7.7 and for females 7.4, 8.2, 8.1, 5.2, 9.6.

Experiment 8. A group of roaches were submitted to a somewhat erratic diurnal variation; the nights being spent at 17°and the days being passed at 34°C. It came out that a total of 5.88 days were spent at the higher temperature, 7.28 days at the lower temperature. Previous to the treatment these roaches had been at a temperature of 27°C for two weeks. The group of roaches was divided into six lots for which the iodine numbers of the lipids were determined.

Table 1. Iodine numbers for lipids extracted from the roach

Exp. No.	No. Roaches* males	No. Roaches* females	Avge. wt. in mg.	Temp. °C	Time at Temp.	Iodine No.	Avge. for lots
1A	3,3A	3,1A	750	34	1 wk.	62.5	
	4	4,2A	750			61.1	
	4	2,2A	725			55.8	59.1
B	4	5,1A	680	ca.	many	57.7	
	4	6	750	31	wks.	60.9	
	6	4	750			55.8	58.1
C	4	6	770	17	1 wk.	63.2	
	7	3	720			61.4	
	6	4	710			65.8	63.5
2A	5A		680	34	2 wks.	57.6	
	5		740			62.3	
		5	720			60.5	60.1
B	5		700	27	12	74.2	
	5		700		days	72.0	
		5	740			63.7	68.0
C	5		660	17	2 wks.	71.6	
	5		740			72.3	
		5	720			70.7	71.2
3A	5		800	34	3 wks.	58.5	
	2,2A		800			63.0	
		6	750			64.9	62.1
B	5		760	27	19	71.8	
		5	780		days	72.7	
		5	780			69.5	71.3
C	5		640	17	3 wks.	69.9	
		5	780			74.2	
		3	700			77.3	73.8
4	5		840	29	2 wks.	67.7	
	5		880			68.7	
	5		860			67.1	
		5	800			67.9	
		5	840			66.7	67.6
5	5		820	30	2 wks.	64.2	
	5		840			64.2	
	4		880			61.8	
		5	800			67.3	
		5	920			64.6	
		3	930			67.5	64.9
6	4A		810	39	12	55.5	
		4	610		days	56.5	
		4A	1050			63.2	
		4A	1200			52.7	57.0

Table 1 cont'd. Iodine numbers for lipids extracted from the roach

Exp. No.	No. Roaches males	females	Avge. wt. in mg.	Temp. °C	Time at Temp.	Iodine No.	Avge. for lots
7	1		985	23	4 wks.	75.1	
	1		710			72.3	
	1		945			66.0	
	1		680			71.4	
	1		560			72.8	
		1	720			67.7	
		1	835			69.6	
		1	670			66.9	
		1	560			69.0	
		1	540			68.0	70.0
8	5		800	17	alter.	68.3	
	5		780	&	ca.	62.6	
	5		720	34	2 wks.	65.5	
	5		760			69.3	
		5	740			72.4	
		5	760			69.7	68.1

* A=Adults, otherwise Nymphs

3. Experiments on Resistance to High Temperatures by Roaches

Experiment 9. This was in the nature of preliminary investigation. Seven nymphs which had been kept at 30°C, group A for three weeks and seven nymphs that had been kept at 23°C, group B, for about five weeks were placed in shell vials covered at the end with wire mesh. Four roaches from the A group and three from the B group were placed alternately on the sliding tray of a Columbia paraffin oven; the tray was moved into place and the door partially closed so as to leave an aperture about one inch wide through which the roaches could be observed. The temperature in the oven as indicated by the thermometer rose rapidly to 65 - 68°C. Suddenly and simultaneously the three roaches of group B went into violent activity; the group A roaches remained only mildly active for about thirty seconds longer and then these four became violently active. Then the activity of the roaches of both group A and B gradually abated with the onset of heat rigor with no manifest difference between the two groups. Under this setup it was not possible to watch the roaches, the temperature and the time very well, but the average time for cessation of motion was slightly less than ten minutes from the time the insects were put into the oven. The four remaining roaches of group B and the three remaining roaches of group A were then treated similarly; and the same phenomemon was observed. Three roaches of each group recovered slightly but were discarded the next day.

Experiments 10 through 13. A and B groups of roaches which had been conditioned at different temperatures were used. These animals were placed in 125 cc Erlenmeyer flasks closed with a wire mesh cap. One roach was placed in each flask. In all these experiments A and B group roaches alternated both within and between rows arranged on the middle shelf of a thermostatically controlled electrically heated oven,

which in addition to the outer door had an inner glass door through which observations could be made. Although the oven heated moderately rapidly and the thermostat was sensitive, there was no blowing fan to distribute the heat so the temperature cannot be regarded as even throughout the oven. Nevertheless, the arrangement of the flasks, and the fact that in different experiments the group A and B roaches were shifted in position, provided comparable treatment for the two groups. The temperature inside the oven was read from a thermometer lying beside the front row of flasks. Nymphs were used in all experiments except experiment 13 in which young adults were used. Approximately half the number of roaches in each A and B group were male and the others female. Data for experiments 10 through 13 are given in table 2.

Table 2. Mortality at different high temperatures of roaches previously exposed to different storage temperatures.

Exp. No.	Group	Storage Temp. °C	Time at Storage Temp. wks.	Treatment Temp. °C	Period of Treatment mins.	Number of roaches	Number killed
10	A	34	3	ca. 43	29	5	2
	B	23	3	ca.43	29	5	2
11	A	34	3	ca. 45	50	9	8
	B	23	3	ca. 45	50	9	8
12	A	34	2	ca. 43	35	11	5
	B	23	8	ca.43	35	11	4
13	A	34	3	ca. 41	210	11	9
	B	23	8	ca. 41	210	11	11

4. DDT Toxicity to Roaches Held Previously at Different Temperatures

Originally it was planned to use both DDT and Chlordane but this plan was abandoned in favor of a more extensive investigation of DDT alone. Nymphs only were used in the experiments and these were stored as A and B groups as under the preceding experiments. After sufficient time to bring the lipids to the characteristic level for a particular temperature, that is two weeks or longer, the insects were used in the particular experiments.

The weight of each nymph was determined in milligrams and the DDT was applied in proportion to body weight. After treatment, each roach was placed in a shell vial covered with a wire net cap held in place by a rubber band. The insects (both A and B groups), without food or water, were kept at a room temperature of about $23^{\circ} \pm 2^{\circ}C$ and were observed at proper intervals; and their toxic symptoms were recorded until death supervened. Death was considered to have occurred when the insect gave no response whatever to prodding with a blunt needle or to violent jarring. No untreated animals were kept as controls in these experiments as the point of primary interest was the comparison of groups A and B in each experiment. It has been the past experience of the investigator, confirmed by repeated experiments, that roaches under starvation conditions survive an average of about 750 hours (Yeager and Munson 1945 b). In some experiments controls on the carrying medium were run.

In each experiment the A group roaches were the ones stored at the higher temperature, the B group roaches kept at the lower temperature. According to the results of the experiments on iodine number determination the A groups would have lipids with an iodine number around 60, the B groups around 72. Care was taken to balance the

groups with regard to males and females; about half the number of roaches in each group being male and the other half females.

Two mechanical devices were employed in administering the poison. One consisted of a 0.1 ml. serological pipette graduated to 0.001 ml. To the delivery end of this was affixed, by means of a segment of rubber tubing, a drawn out glass needle; to the other end was attached a long piece of rubber tubing with a medicine dropper inserted in the free end. The pipette was held horizontally by two clothespins fastened to a board stand. By pressure of the breath of the operator the fluid in the pipette could be expelled from the glass needle onto or into the animal. This method is quite satisfactory for medium volumes. (Yeager and Munson 1945 b).

A method for applying smaller volumes was desired, so into the jaws of two clothespins fastened to a stand was inserted a 1 ml. tuberculin syringe with a no. 27 needle, and the whole rig was clamped into the blade holder of a Spencer rotary microtome. A cork in the vise of the microtome served to ram against the plunger of the syringe. By varying the micra setting and the number of revolutions desired volumes could be obtained. Several precautions, however, are necessary for good results. In injection work the ram should be left in the top position pressing the cork against the plunger for a few seconds to overcome any back pressure exerted by the animal; in external application the fluid is ejected with great force so that the animal must be held in such a position that the entire dosage goes into it. In either form of application it is well to rotate the driving wheel once just before the application in order to bring the fluid to the end of the needle (this is especially necessary with a volatile carrier such as acetone), and also to rotate the wheel once just after the application if an

injection procedure is used in order to remove any tissue or blood which might clog the needle.

All insects which received external application had the dosage delivered onto their abdominal terga; all animals were injected through the coxa-femoral joint of the left metathoracic leg. The leg was pinched by a pair of forceps as the needle was withdrawn in order to minimize the chance of haemorrhage.

The DDT used was technical DDT with a setting point of about 89°C. One may, if he wishes, express the dosages in this paper in approximate concentrations of DDT with 108.5 to 109.0 C melting point DDT by multiplying the doses in this paper by 0.7 as about 70 percent of technical DDT is the proper isomer (Frear 1948 p60-1).

The fundamental details for the injection experiments are shown in table 3 and the external application experiments in table 4. The experiments are numbered and arranged in these tables in an order suitable for their comparison. They were conducted chronologically in the following series: 14, 17, 15, 18, 19, 22, 20, 21, 16. By improvement in technique the last five experiments are considered more reliable than the first three.

In experiment 14 an emulsion was injected. DDT and lanolin were weighed, melted together and this was emulsified by agitation through a syringe needle into a sodium chloride solution, with a small amount of the detergent "Alconox" added. The ingredients were in the following quantities: NaCl 1 g/100cc, lanolin 5 g/100cc, "Alconox" .5 g/100cc and DDT 1 g/100 cc. In preliminary experimentation the emulsion held up quite well and it exhibited (without DDT) only mild toxicity to roaches; eighteen injected roaches gave a mean survival time of 484 hours; while four roaches injected with it containing DDT gave a mean

survival time of 54 hours. On this basis the experiment proper was undertaken

A volume of 0.01 ml/gram of body weight was injected by means of the serological pipette apparatus. Roaches were injected alternating between the A and B groups. Toward the latter part of this experiment it was noted that the emulsion began to break rapidly in the pipette. This undoubtedly accounts for some of the longer survival times recorded for this experiment in table 3.

In the other two injection experiments (15 and 16) the dosage was achieved by using a solution of DDT in acetone up to 59/100cc; and application was by means of the microtome hookup. Control roaches for experiments 15 and 16 were injected with the same volume acetone (0.0016 ml.) as were the DDT injected roaches; and showed no indication, whatever, of toxicity. Preliminary to running experiment 17, the first in the series of external application experiments, five roaches were treated with 20 ml^3/gram of body weight of acetone. One roach died at 72 hours but the rest survived 552 hours. On this basis the application of DDT using acetone was made with the serological pipette apparatus. In the remaining experiments, however, application was made with a small volume of acetone delivered by means of the microtome apparatus.

Table 3. Survival time of roaches previously held at 34° C (group A) and 23° C (group B) when injected with DDT.

Exp. No.	14		15		16	
DDT micrograms/gram	100		160		150	
Groups	A	B	A	B	A	B
Survival time in hours	38	42				
	39	50				
	42	66	48	48		
	45	72	48	48	29	62
	52	86	48	48	36	72
	62	86	72	48	40	72
	66	110	72	72	51	72
Median	(77)*	123	72	96	51	80
	86	136	72	96	51	80
	88	312	72	120	51	96
	144	324	96	120	86	96
	168	420	240	120	96	96
	384	744	672	216		
	816	816				
	936	864				

* ( ) interpolated

Table 4. Survival time of roaches previously held at 34° C (group A) and 23° C (group B) when treated externally with DDT.

Exp. No.	17		18		19		20		21		22	
DDT micrograms/gram	200		80		100		60		60		30	
Groups	A	B	A	B	A	B	A	B	A	B	A	B
Survival time in hours			72	120								
		20	72	168								
	26	46	120	192	48	120	120	288	120	184	144	120
	30	50	144	192	72	240	192	312	144	288	168	648
	36	50	168	192	96	312	216	312	216	384	216	720
	44	62	192	192	120	336	216	480	240	504	384	748
	62	74	192	192	120	336	240	528	240	528	648	792
	74	96	216	216	144	360	240	624	288	552	672	816
Median	(89)	96	216	216	168	456	264	648	288	624	696	816
	103	100	264	216	216	552	264	672	312	672	772	864
( )=cal. median	122	100	264	240	216	648	312	720	312	744	792	912
	125	103	264	264	240	696	312	744	336	792	792	960
	125	104	264	264	264	720	336	>840	336	>816	816	1008
	125	112	288	264	>840	744	360	>840	388	>816	844	1032
	140	120	312	528	>840	>840	672	>840	744	>816	960	1032
		136	312	720								
			504	816								

SECTION IV

ANALYSIS AND DISCUSSION OF THE RESULTS

1. The Lipids of the Cockroach at Different Temperatures

The lipids obtained by the procedure outlined above were a viscous straw-colored oil. Timon-David described the oils extracted from the oriental roach as having a pungent odor; the odor of the lipids from the American roach had only a faint odor. No determinations of solidification as such were run but it was noted that at about 22°C the oils with the lower iodine numbers showed some indications of setting.

Immediately after the iodine numbers in the three groups in experiment 1 were determined it was seen by means of these values that if different temperatures were to produce significant differences in the iodine numbers of the lipids, longer periods of exposure to the temperatures were required. Group C, was somewhat higher than the other two groups. The three groups were continued at their respective temperatures and the determinations of experiments 2 and 3 were made. While the group B and C roaches show a rise in iodine numbers, the group A roaches within experimental error do not, but remain essentially at the level produced by the pretreatment room conditions (about 31°C for several days). Although it seemed obvious by inspection that the A groups of experiments 2 and 3 were different from either the B or the C groups; it was perhaps a little questionable whether the B and C groups might differ from each other. By calculation the combined A groups gave a mean of 61.0 with a standard error of the mean (computed for small numbers) of 1.08; for the combined B groups 70.7 and 1.52; for the combined C groups 72.7 and 1.16. Comparing the three groups by the standard error of the difference

between two means confirmed the conclusion that the A groups were extremely different from the B and C groups but that the latter were not statistically different from each other.

The conclusion is then obvious: between 17° and 27°C the degree of saturation of the total lipids of the roach under the same conditions is the same and between 27° and 34°C there is a rapid fall in iodine number. Actually if one considers the not quite as well established data for group B in experiment 1 it is seen that the drop from around 72 to sixty for the iodine numbers actually occurs in the range 27°- ca. 31°C.

The iodine numbers for the B and C groups show a steep rise during the first and second weeks and a leveling off between the second and third week, thus indicating an approximately dynamic stabilized condition at about the end of the second week. Experiment 8 in which the roaches were fluctuated from 17° to 34°C indicates the same rate. These insects having been for several weeks at a temperature of 27°C before the treatment began would have had an iodine number of about 72. Had they been kept for two weeks at 34 degrees their expected iodine number would have been about 60. As it worked out a summation of the total number of hours spent at the 34°C temperature was slightly less than six days and their mean iodine number of 68.1 is within experimental error what one would expect if the roaches had simply been stored at 34°C for about six consecutive days. Aside from confirming this rate of change over, experiment 8 establishes that the mechanism by which the iodine numbers are altered is quickly responsive to change in temperature and, moreover, is thrown into and out of operation each time the temperature falls or rises. A single brief exposure does not take the iodine numbers from one level to the other, in other words

there does not seem to be a "triggered" reaction.

The data of Zummo (1932) with white rats also indicates that a shift in iodine numbers seems to be completed in about twelve to fifteen days. This, with the observation above is of considerable theoretical interest. If the storage lipids of an animal are to be considered as sharply set aside from the protoplasmic lipids there seems to be very little cause for a change in their iodine numbers. The rather rapid shift in the numbers may be interpreted to be in line with the concept of Schoenheimer that all the materials in an animal are in a constant state of flux.

In order to determine more completely the response of the lipids to temperature between 17° and 27°C, between 27° and 31°C, and above 34°C experiments 4, 5, 6, and 7 were run. The arithmetic means for these experiments with the arithmetic means from the groups in experiments 2 and 3 are plotted in Figure 1.

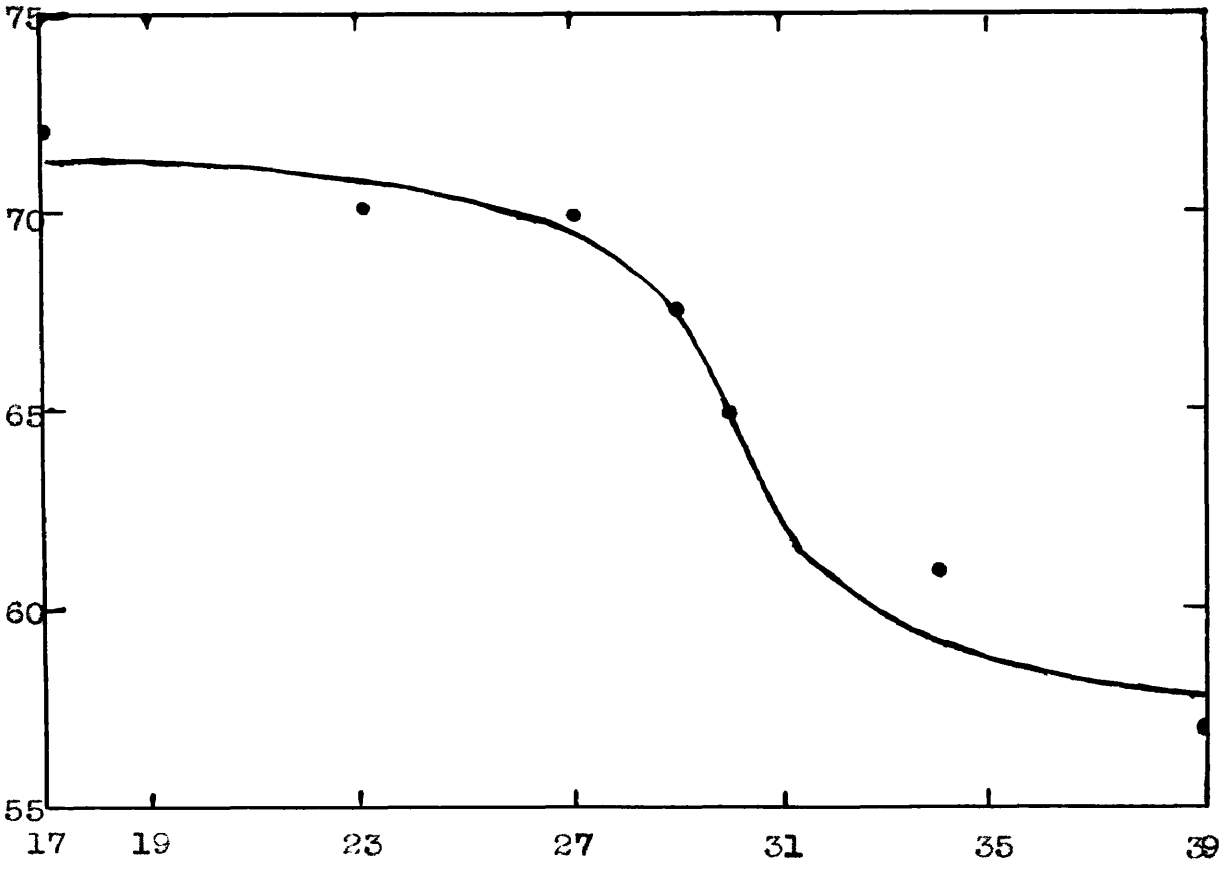

Figure 1. Iodine numbers (ordinates) of lipids of roaches held at different storage temperatures (abscissas)in degrees centigrade.

The curve in Figure 1 is worthy of attention. It exhibits a virtual plateau from 17° to 27°C; the slope then changes rapidly and the curve falls precipitately until it starts leveling off at about 31° or 32°C. Unfortunately the author found in the literature no data with which this curve might be compared. The data of Henriques and Hansen deals with different sites within an animal and is not comparable; Zummo kept his rats at two extreme temperatures. The data of Fraenkel and Hopf while consisting of a considerable number of iodine number determinations can not be used as these are distributed among two insects and two methods, and thereby do not provide over three points with any one animal for one method. The selected temperatures include one low, one medium, and one high and lines connecting these might be considered as straight line or only mildly curving. If only the values for, say, 17°, 30° and 39°C in figure 1 are considered they could be interpreted as lying on a straight line.

The nature of the curve is such as to invite discussion. Perhaps, it may be the result of differential absorption of the food fats dependent on the temperature but considering the low fat content stated to be in the dog biscuits (not less than 1.5 percent) and the fact that this fat ran iodine numbers higher than those obtained on the average at any temperature from the roaches one is inclined to reject an explanation of differential absorption. The form of the curve is suggestive of an enzyme optimum. It might be postulated that the animal is forming hard fats from the carbohydrates in its diet and that these fats are being desaturated at temperatures around 30°C and below by a dehydrogenase system. The answer will have to await more data; conjecture is hardly warranted at the present time.

Some incidental observations may be made. In table 1 it may be

seen that the determinations for males and females vary randomly. As the iodine numbers for 17°, 23° and 27°C are statistically equivalent the determinations for the sexes in experiments 2 B and C, 3 B and C and 7, legitimately, may be grouped for comparison. For eleven determinations involving males the mean is 71.9; for an equal number of determinations for females it is 70.0. Thus within the experimental error of the determinations no difference is shown. The few iodine numbers involving adults in table 1 are mostly for mixed groups, but the few exclusively adult values do not seem to differ from those for nymphs. However these adults were very young adults; in most cases they were not over a week old.

Experiment 7 may be considered to have given relatively good measures of the total quantity of lipids. The mean values for the five male nymphs and for the five female nymphs are exactly equal, 7.7 percent of wet weight. This is slightly lower than Schweet's value for adult American roaches but exceeds all other reported values for other species of roaches reported in the literature. Also it is in line with quantitative reports for Orthoptera in general. It may be seen by table 1 that the iodine values for individual roaches in this experiment do not vary much more than those for pooled values in the other experiments, which indicates that the variation is more a matter of technique than of actual biological variation in the insects.

In this investigation it was not intended to study the influence of temperature on rate of growth or development; but it was qualitatively obvious that the roaches at higher temperatures matured much more rapidly than roaches at lower temperatures. For example, although they, presumably, were on the average of the same age and weight at

the time the experiments began the roaches in groups A and C in experiments 1, 2, and 3 developed at quite different rates. In the three week period in which they were stored fifteen out of thirty roaches in the A groups moulted to adults; in the C groups none, as adult roaches seem to lose weight near the last moult the A groups only slightly outweigh the C groups.

2. The 'Lipoid Liberation' Theory and the American Roach

While the preliminary observations in experiment 9 indicated some difference in the reaction of roaches with presumably different iodine numbers to unfavorably high temperatures the more definite data in table 2 indicate no differences. In all the experiments the few surviving roaches appeared to be about equally seriously injured and roaches of comparable A and B groups were indistinguishable by their symptoms.

While the conditions of these experiments might have been improved by the heating unit in the oven having a circulating fan, still conditions for the compared groups were essentially the same. Therefore, on the basis of these experiments it must be concluded that no very wide margin of difference existed and that an iodine difference of about ten digits in the total lipids of the American roach does not appreciably influence the ability of this animal to withstand temperatures sufficiently high to be lethal.

3. The Alteration of the Resistance of Roaches to DDT

While the results with experiments 14, 15, 17, and 18 might appear somewhat dubious, there is in all of them a tendency for the A group roaches to be more susceptible to the DDT than the B group roaches. In experiments 16, 19, 22, 20, and 21, in which the technique of

administration was superior, the tendency becomes an overt difference. For example, in experiment 19 when 85 percent of the A group had succumbed, only 24 percent of the B group were dead; in experiments 20 and 21 when 92 percent of the A groups were dead, only 24 percent of the B groups had been killed. In fact, at this time, 388 hours after application of the poison, most of the B group insects in these two experiments were in a fairly vigorous condition. Finally, in experiment 22 with the smallest applied dosage (30 micrograms/gram body weight), while 30 percent (four) of the insects in the A group had succumbed, only 8 percent (one roach) had died in the B group, and the death of this animal seemed more likely due to an unsuccessful moult than to lethal action of the DDT.

There are, then, two demonstrable facts: 1. Roach nymphs kept at 34° C for a few weeks have total lipids with an iodine number around 60, while roach nymphs kept at 23° C for a few weeks have total lipids with an iodine number around 72. 2. The 34° C roaches appear to be less resistant to either externally or internally applied DDT than the 23° C roaches.

There is not any proof, of course, of a direct connection between these two facts, yet, assuming relevancy the question arises: How can the more saturated lipids cause an applied dosage of DDT to be more toxic? The answer probably lies in the solubility of DDT in the lipids. If DDT is less soluble in the more saturated lipids, it will be more toxic in the sense that it may accumulate in a greater amount at a site of toxic action rather than be taken up in reserve fats. Expressed in another way, the 34° C roaches have in effect been poisoned by DDT at a temperature which, with regard to their lipids, is physiologically lower than the temperature for the 23° C roaches, although the roaches

were actually poisoned and held at the same temperature ($23^{o} \pm 2^{o}$ C) after treatment. The DDT may then be considered to be showing a "negative coefficient" in the 34^{o} C roaches.

For this explanation to hold it is necessary for the solubility of DDT in the reserve fats to be more readily influenced by temperature than its solubility or, perhaps, its rate of adsorption at some site of lethal action. The slope of the curves for the solubility of DDT in various solvents, determined by Gunther and reproduced by Frear (p 63, 1948), clearly indicates that the solubility of DDT in some solvents increases markedly with increasing temperature; with others it does not change much in a comparable temperature range. Indeed, compared to some of its more lipid soluble analogs DDT may be more toxic because it is not too lipid soluble.

Moreover, the experiments above in which the more massive doses were applied seem to be more different in survival rate in the earlier part of the experimental time than in the latter periods. As the insects were kept after treatment with the DDT under conditions of starvation, this trend could be due to DDT being expelled from the depot fat as this became mobilized for energy. This, in effect, would add to the amount of DDT available for action in the poisoning process.

Evidence is accumulating in this laboratory that the volume of total lipids in adult roaches is a very important factor in resistance to death by DDT. It is intended that this aspect of the lipid-DDT action correlation be exhaustively investigated especially with regard to age and sex differences, in the next few months.

The author wishes to express his appreciation to Dr. E. N. Cory, Professor of Entomology at the University of Maryland, for his kindness and understanding during the past few years, and, especially for his

encouragement during the period in which the above research was accomplished.

SECTION V

SUMMARY AND CONCLUSIONS

Total lipids have been extracted from the nymphs of the American roach stored at different temperatures and the iodine numbers for these lipids determined.

Between 17° C and 27° C iodine numbers range around 72 and show no statistical difference; between 27° C and about 32° C the iodine numbers fall rapidly to around 60 and show little further decline with increase in temperature.

When shifted from a suitably high to a suitably low temperature, or vice versa, the alteration of the iodine numbers appears to be approximately complete in about two weeks. This rate of change is consistent with the idea of Schoenheimer that the lipids of an animal are in a constant state of flux.

The shift from one iodine number level to another under the stimulus of temperature, apparently, is not a "triggered" reaction.

Male and female nymphs show no quantitative difference in their total lipids, the amount being about 7.7 percent of wet weight for both sexes. This value is in line with those for other arthropods, including other species of roaches, as reported in the literature.

As an incidental observation it was noted that the roach nymphs mature much faster at the higher than at the lower temperatures.

Groups of roaches, stored for a time interval sufficiently long to insure different iodine numbers for different temperatures, show no difference in their resistance to high lethal temperatures under the experimental conditions of this investigation. This investigation, while in no sense disproving a possibly very vital and fundamental role of lipids

in the resistance of animals to high temperature, does not lend support to the 'lipoid liberation' theory.

Groups of roaches, stored at temperatures sufficiently far apart to insure differences in the saturation of their total lipids, show a marked difference in their resistance to the toxic action of DDT; those roaches with the more saturated lipids, as evidenced by lower iodine numbers, show the lower resistance to DDT applied externally or injected. The difference in response to DDT is more evident in the lower doses than with the more massive ones.

SECTION VI

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