STUDIES ON INSECT MUSCLES [1])

by

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CONTENTS

I. INTRODUCTION AND PROBLEM

The great hygienic and economic importance of insects continuously leads man to search for their effective control. To achieve this chemical methods have been used to an ever-increasing extent. Insecticides are produced and consumed on a large scale and in great variety. The production and consumption of these substances take place under the influence of extremely complex interactions between consumers on the one hand and the chemical industry on the other. Such a far-reaching control can be handled effectively only by a well-developed technology, by government action, and last, but certainly not least, by an extensive scientific investigation into the numerous problems which are largely to be found in the field of applied entomology.

Such an investigation must of necessity range over a great many special subjects, one of which is the relation between toxic action and chemical structure. A clear insight into this problem could, among other things, provide important directives for a purposeful synthesis of new and improved insecticides. Here again the investigation divides itself, and we are confronted with different aspects of toxicological research.

The spectacular results obtained by the use of DDT attracted much attention. Several authors observed that not only DDT, but many other halogenated hydrocarbons employed as insecticides, acted or at least appeared to act on the muscles or the nervous system of representatives of various animal groups.

However, a detailed insight into the mode of action is still lacking and it seemed worth while to make investigations in that direction.

The toxicological work with muscles and nervous system of insects aimed at, is naturally preceded by a physiological study, as a knowledge of the normal functions is indispensable. Compared with the data known of vertebrates the physiology of insects, including their nervous and muscular system, is still in its initial stage. There are a number of reasons for this, one of which may perhaps be its short history. Further, the slow development of insect physiology can partly be attributed to the small size of insects, although increasingly improved techniques are gradually overcoming this disadvantage.

This limited physiological knowledge, since it is closely related to as limited a knowledge of anatomy and histology of insects (which relatively speaking is perhaps not less than that of other groups of invertebrates) is the reason that nearly every physiological—

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and hence also toxicological–investigation must be preceded by a parallel study of anatomy and histology.

Real progress towards an insight into the mode of action of halogenated hydrocarbons in nerves and muscles is hindered by our lack of fundamental knowledge. A programme has therefore been stipulated: to make a combined study of the anatomy, physiology and toxicology of muscles, sense organs and nervous system of one insect.

Following this programme it was firstly DRESDEN and NIJENHUIS who considerably increased the anatomical knowledge of the second thoracic leg of the american cockroach *Periplaneta americana* (L). Linking up with their work, together with that of other co-workers, a refined myographic technique was developed which was especially adapted for use with the subject chosen.

Thus far an anatomical and technical basis had been found suitable for further physiological work. Finally, a choice had to be made with respect to the toxicology. Since at the time an investigation concerning the metabolism of–and the resistance to–γ-HCH was in progress (K. VAN ASPEREN and F. J. OPPENOORTH) there were good reasons to continue the studies by investigating the action of γ-HCH on the nervous and muscular system.

My investigation to be carried out was divided into three parts because of methodical differences: Experiments on the muscles, the sense organs and the central nervous system. This thesis deals with the muscles.

II. MATERIAL AND METHODS

A. THE SUBJECT

The choice of *Periplaneta* as a subject was determined by the following two considerations:

The first is formed by a number of practical advantages, which–although sufficiently well-known–will be mentioned here.

a. An appreciable amount of anatomical and physiological data concerning this insect have been described.

b. It is a large insect and therefore easily dissected.

c. The insects are generally available and in our case they could be supplied in unlimited quantities.

d. They are easily kept in the laboratory.

There are few subjects that have the same practical advantages as *Periplaneta;* one of them, however, is *Locusta.*

In choosing between these insects in favour of *Periplaneta,* secondly, the theoretical consideration holds that of the two insects the cockroach is regarded as the least specialized; in other words, it is a general type of insect, of which one may expect the general physiological characteristics.

The cockroaches were obtained from the Zoological Gardens in Amsterdam, but owing to lack of space they were not bred.

They remained in a favourable condition for experimentation in a thermostat at 25°C, amply provided with water, but with just sufficient food.

The food mentioned by DRESDEN (1949) appeared to be satisfactory.[1]) Contrary to his prescription the powder was not mixed with water, but was given dry. The food containers and the cage consequently remained much cleaner.

Just sufficient food is defined as a quantity which is eaten in an hour and given once or twice a week. If the insects are fed too abundantly, the gut is overfilled and the insect will grow fat. An overfilled gut as well as too much fat can be quite troublesome during preparation.

It is well known that the females generally have more fat and haemolymphe and are therefore less easily prepared. For this reason males were preferred.

B. MYOGRAPHY

The myographic method for investigating the muscles of the leg of *Periplaneta* has already been described by DRESDEN (1956). As this technique played an important part in obtaining our results, a short description will be useful.

The apparatus consisted of a metal dissection table covered with a cork plate on which the insect could be fixed. The table is mounted on an adjustable

[1]) It consisted of a mixture of wheatmeal 1,000 g; whole milk powder 900 g; dried yeast 100 g; Vit.C 500 mg; "Davitamon A" 10 ml.

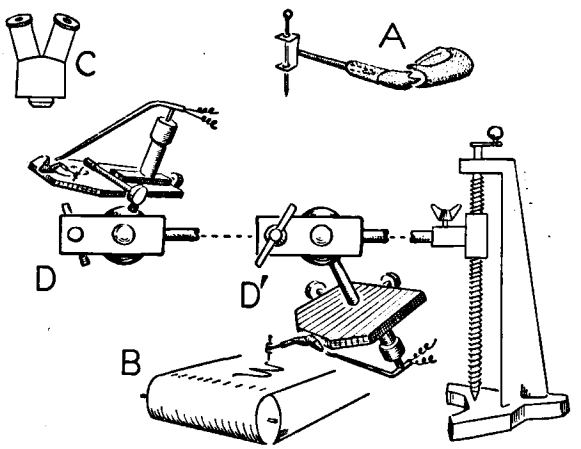


Fig. 1. The technique for recording muscle contractions.*)
A, second thoracic leg of *Periplaneta* with inserted writer.
B, smoked paper and kymograph drum.
C, dissection microscope.
D, dissection table with micromanipulator and insect under the microscope.
D^1, the same adjusted on the recording paper.

support by means of a solid ball bearing socket. The whole is a rigid assembly and is capable within limits of being set to any required position.

A micromanipulator carrying tapered electrodes of fine platinum wire was mounted on the table. There was also the important detail of a writer consisting of a piece of straw which supports a freely moving writing pin of metal or glass. It can be inserted into a half-cut femur in which it quickly fastens through drying haemolymphe and tissue. The weight of the writer is barely heavier than the cut part of the leg (11 mg and 7 mg respectively).

Since the joint concerned is a pure hinge, the tip of the leg can only describe an arc. As an arc lies in a flat plane and the contraction of the muscles may cause the femur to move through an angle of more than 120°, the use of a cylindrical kymograph-drum is impossible. The writing is done on a flat piece of paper stretched between two kymograph-cylinders.

The plane of the paper is horizontal because the writer will function only on a horizontal surface. With a correct adjustment the segment of the leg with its writer should move parallel to the paper, the pin being perpendicular to it. Thus, the friction on the smoked paper is determined only by the weight of the vertical pin.

Unevenness in the paper or small incidental contractions of the musculature of the body cannot always be avoided. They sometimes cause a vertical component in the movement of the segment of the leg and direct it outside the horizontal plane. These possible inconveniences are eliminated by the movability of the pin.

The insect was narcotized by carbon dioxide, decapitated and after removal of the first and third pairs of legs, it was pinned onto the cork of the dissection table, ventral side up. The preparation is preferably kept intact as much as possible. But in the case as referred to above, an overfilled gut can be removed with little difficulty, through a longitudinal incision in the lateral side of the abdomen. The remaining pair of mesothoracic legs were clamped by bent pins in such a manner that the leg to be examined, and especially its praecoxal part with the nerves were easibly accessible for the operation. The writer was fixed and the electrodes were brought into position.

The whole arrangement, i.e. dissection table, insect with writer and electrodes, could now be handled as one rigid unit and could be adjusted on the kymograph.

The dissections were carried out with ophthalmological scissors, jeweller's forceps and needles ground into small knives.

If necessary the preparations were moistened with saline[2]). All experiments were performed at room temperature of 18°–20°C.

[2]) 9.32 g NaCl + 0.77 g KCl + 0.50 g $CaCl_2$ + 0.18 g $NaHCO_3$ + 0.01 g NaH_2PO_4 to 1 l H_2O.

*) I acknowledge the Editors of NATURE for permission to reproduce the figures 1, 5, 6, 8 and 15 which were published earlier.

The relative shortenings of a muscle can be measured as follows (Fig. 2):
Through the end D of the recorded part DF or reconstructed part D'F' of a contraction curve, a line DE is drawn parallel to the direction of the muscle BC (the lateral displacement of the muscle is supposed to occur parallel). From the other end F of the part DF, a perpendicular FG is dropped on line DE. The line segments DG and D'G' thus constructed are proportional to muscle shortenings belonging to them. In some cases where the direction of the muscle was not noted down it was taken arbitrarily perpendicular on the base of the myogram.

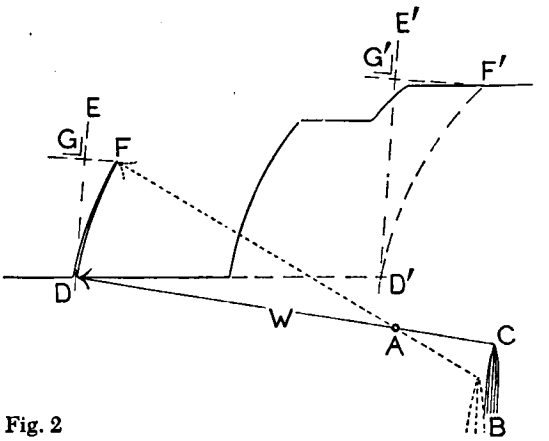


Fig. 2

C. ELECTRONEUROGRAPHY

When examining the electrical activity of motor nerves and also later of the sense organs (BECHT, 1958), we greatly profited by the dissection table. Instead of the erect, adjustable support, we made use of a small fixed support, because throughout the experiment the subject was continually observed under the microscope. The ball joint and the support clamping screw allowed sufficient space for movement. To avoid electric-magnetic "hum" the support was placed on an earthed metal plate on which separate wire-gauze screens could also be placed. By this arrangement the subject stayed easily accessible.

By means of the platinum electrodes attached to the micromanipulator, action potentials of the exposed nerves were recorded. In the first experiments the action potentials were led via a simple balanced pre-amplifier to a PHILIPS GM 3156 oscilloscope. Later, we had an excellent amplifier at our disposal; it was designed by Mr. VAN PROOYEN, of the Medical Physical Department T.N.O., at the Hague. For investigations into the reflex function this amplifier had a very high rejection factor for in-phase signals; the recording was made with chlorinated silver electrodes.

D. PROTECTION AGAINST DRYING OF THE PREPARATIONS

In some electro-physiological methods for investigating insects the object is partly immersed in saline (HOYLE, 1955). The method described here, however, is a "dry" one with all its advantages and disadvantages. Undoubtedly the advantage of the dry method is the easy manipulation and accessibility of the preparation, even when the electrodes have been adjusted. An obvious disadvantage is the desiccation of the exposed tissue. With myography this desiccation is not so very disturbing because the necessary but nevertheless short-circuiting fluid is still permissible, on account of the available intensity of the stimulus. When recording the fairly small sensory action potentials, all short-circuiting liquid must be carefully removed; however, a thin nerve is difficult to keep in good condition for longer than a few minutes in the usually relatively dry atmosphere of a working-room. Hence, paraffin oil is often applied to prevent desiccation. In our experiments the oil flowed away from the preparation far too quickly. Oil would be useful only in methods capable of retaining it e.g. with a bath, but this loses the advantage of the dry method. The difficulty was overcome by covering the tissue with a thin layer consisting of a soft jelly-like mixture of vaseline and paraffin oil (melted four parts vaseline and six parts of paraffin oil; the desired consistency was obtained at 18–20°C. Other ratios of mixture may be used at other temperatures). Treated in this way, isolated sense organs measuring some mm's only, otherwise very vulnerable, appeared to survive for many hours. Covering dissected muscle tissue with the mixture made it easy to record the action potentials with common platinum wire electrodes.

E. TOXICOLOGICAL METHODS

In most of our experiments the insects were poisoned by the injection of an emulsion containing insecticide. Tissues were sometimes covered with the emulsion, and in a few cases poison dissolved in acetone was applied to the integument.

Emulsions with γ-HCH or DDT[3]) were obtained according to DRESDEN (1949) and OPPENOORTH (1956).

[3]) γ-HCH puriss, PHILIPS ROXANE, Weesp, Holland
p-p' DDT puriss, ORGANON, Oss, Holland.

A solution of γ-HCH or DDT in oil was emulsified in saline containing 1% gum arabic.

The emulsions were not ground in a mortar as was described by the above authors but made in a Waring blendor. The latter method is quicker and yields nearly the same stable emulsions. These were kept for at most a few days.

For uniformity it appeared to be desirable to keep the injected volume constant. The quantities of the components were chosen such that 20 mm^3 could be used for all injections independent of the dose of poison.

The dose of insecticide was varied by changing the amount of oil in the emulsion, or by making use of different poison-concentrations in the oil. For this purpose some stock solutions were made of 0.005–1% poison in pea-nut oil.

It is conceivable that the site of injection exerts influence on the regularity of the circulation of the injected poison, and hence on the reproducibility of the symptoms of poisoning. In order to obtain preliminary information on this point three groups of five insects each were injected with 10 μg HCH in 20 mm^3 emulsion per insect (L.D. 50 = about 5 μg). The three groups were injected in the coxa, the thorax and the abdomen, respectively. The course of the symptoms was noted down. The thoracic and coxal injections appeared to provoke the symptoms more quickly and their succession was less scattered than with the abdominal injection. Injecting into the coxa is not easy, however, and the leg to be investigated is apt to be damaged. In all other experiments we therefore applied the thoracic injection; we could again establish the relatively slight scattering in the time of appearance of the poisoning symptoms.

The injections were carried out in CO_2-narcosis under the dissection microscope. An extremely fine needle was used (especially manufactured; external diameter 0.25 mm), which was introduced into the left or right half of the thorax, just beneath the pronotum.

Our experiments were carried out as follows: A certain amount of poison was administered. Since the circulation is completed in a few minutes, it was assumed that this made complicated experiments with perfusion unnecessary. After a certain stage of poisoning had been reached, the preparation was made and examined. If necessary, correction was applied for the duration of preparation.

III. THE MUSCLE PHYSIOLOGY

A. THE LITERATURE ON THE PHYSIOLOGY OF LOCOMOTORY MUSCLES

In the introduction we have seen that the starting point of our investigation was not a clear-cut causal problem but a programme based on our lack of knowledge of physiology and toxicology of the insect neuromuscular system. Bearing this in mind we will give a review of the literature.

Compared with other systematic groups of invertebrates, we have rather meagre and fragmentary knowledge of the physiology of neuromuscular systems of insects. This is reflected in a series of recent reviews. PROSSER (1946) in his section of neuromuscular integration deals principally with crustaceans investigated by WIERSMA and co-workers. The review of WELSH and SCHALLEK (1946) differs from PROSSER's in its treatment of neurotoxic insecticides, but they agree with him that "of the several classes of arthropods far more is known concerning the neurophysiology of the crustaceans than any other class".

The literature reviewed by KATZ (1949) is concerned mainly with *Crustacea, Cephalopoda* and *Coelenterata.* There is a schematization of the functions of the neuromuscular system of *Crustacea.*

WIERSMA (1941, 1952, 1953) has written extensively on many aspects of the efferent innervation of muscle, but he also dealt largely with crustaceans.

We shall now treat the literature especially on insect locomotory muscles.

As early as the last quarter of the nineteenth century stimulatory physiological investigations were made into insect muscles; this was adequately discussed by earlier authors such as KAHN (1916) and HEIDERMANNS (1931).

In his examination on the contraction of the extensor and flexor tibiae of *Locusta,* KAHN himself did not study anatomy. He was supported in this only by BAUER's work (1910) on *Dytiscus,* which support was, of course, limited [BAUER gave an excellent and extensive description of the musculature of the head, thorax, legs and abdomen of *Dytiscus marginalis*; later it formed a part of the monograph of KORSCHELT (1923)]. The muscles mentioned, mainly the extensor tibiae, that is the powerful jumping muscle, were stimulated with an inductorium; this was mostly done by inserting needles through the femur. In this way

KAHN determined the duration, form, superposition and fatiguability of the contraction, and the influence of temperature. Attempts were made to determine the rate of propagation of the contraction wave. In the last experiments curare was injected, resulting in a complete paralysis, but with maintenance of direct excitability of the muscles. This observation could not be confirmed by subsequent authors, and will be referred to later on.

KAHN's interest in the physiology of innervation appeared from his determination of the time of neuromuscular transmission. The results obtained were compared with those of the frog; they show certain differences. From the recent work of HOYLE with the same locust muscles also to be discussed later on, we know that the innervation and hence also the possibilities of response to stimulation are very complicated, and that conclusions from the results found should be treated with reserve.

Not until fifteen years later did a group of workers start again on the comparative physiology of the contraction of insect muscles: HEIDERMANNS (1931) on wing muscles of *Aeschna* and his pupils SOLF (1931) on leg muscles of *Decticus* and *Gryllotalpa*, KRAEMER (1932 a, 1932 b) on leg muscles of *Dytiscus* and *Lucanus* and, finally, CREMER (1934) on wing muscles of *Aeschna*. The influence of temperature and load were especial centres of interest.

As regards the anatomy of *Dytiscus*, KRAEMER was supported by KORSCHELT. The monograph by KORSCHELT contained HOLSTE's chapter on innervation written in 1910, in which, however, no attention was paid to the details of the neuromuscular junctions.

The stimulation was carried out "directly" by means of large electrodes, sometimes at abnormally high voltages (CREMER 8–40 V). For the recording far too-slow common myographical methods were used. The application of this method aroused much criticism but no improvements were made (HEIDERMANNS, 1932).

FREDERICQ (1928) determined the chronaxie of some insect muscles.

During the preceded period the problems had remained about the same as KAHN's. The methods of electric stimulation and of myography still showed considerable deficiencies; a saline, for instance, was not available.

Because of these rather faulty techniques and insufficient knowledge of anatomy, relatively few differentiated results were obtained; results that may be summed up as more or less general properties of skeletal muscles of the insect, for instance, the behaviour of a muscle at different temperatures. In their generality these results greatly resemble those achieved with vertebrates, but it is not always clear in how far these are acceptable. A number of quantitative data on this point can be found in HEIDERMANNS (l.c.) and WIGGLESWORTH (1950).

With the finding of the so-called "multiple" innervation of muscles in arthropods, a new period of investigation was arrived at; it was characterized by the study of the structure and the electrical and mechanical properties of the different neuromuscular systems.

In 1920 VON BUDDENBROCK wrote on the occurrence of tonic muscles in *Dixippus* and studied their rate of metabolism, but not until 1932 did RIJLANT record two different electrical responses pertaining to active contraction and tonus in the muscle of a number of arthropods.

FRIEDRICH (1933)–a pupil of VON BUDDENBROCK–posed the problem of the double innervation in insects. He was apparently inspired by the (at the time already frequently investigated) BIEDERMANN phenomenon (1887)–opening at weak and closing at strong stimulations of a lobster claw–which was explained by double innervation. He was acquainted with the histological data of MANGOLD (1905) and HOFFMANN (1914), concerning double innervation; the data of MARCUS (1922) and MARCU (1929) were also available at that time. A considerable technical improvement was obtained by the introduction of very light recording apparatus. The lack of anatomical knowledge again caused an insufficient technique of stimulation by which the claimed result of an activating and inhibitory innervation was greatly weakened.

In 1933 WIERSMA started his work on crustaceans and has continued it to the present date, originally with VAN HARREVELD, later on with many other coworkers. WIERSMA was fortunate in choosing the legs of crustaceans, not only for isolation but also for identification of separate axons; the anatomical isolation appeared to be possible, in contrast with what had hitherto been known of insects. The identification led to distinguishing axons concerned with fast and slow muscle contractions and inhibiting functions. Each of these functions can be supplied by the activation of one single motor nerve fibre. This means that a number of muscles are "multiple" innervated, i.e. they are provided with more than one function and therefore with more than one type of nerve fibre. The importance of this work to comparative physiology can hardly be overestimated. According to PROSSER (l.c.), it is "the most extensive

and definitive work ... on the isolation and identification of single motor nerve fibres". The striking results of this investigation are bound to have had a substantial influence on insect physiology and will probably continue to do so.

In this connection mention should be made of an important and highly appreciable proposal by FURSHPAN (1955) (quoted after WIERSMA, 1957: page 145). "Since there exists a good deal of variation in type of innervation and effects of stimulation in different muscles, a new terminology ... with a slight variation in definition ... will be presented here. Instead of multiple innervation, an older term which covered at the same time the fact that many nerve endings are present and that more than one axon makes connection with a muscle fiber, the term *multiterminal innervation* will be used to describe the fact that one axon has a considerable number of endings on a muscle fiber. To indicate that more than one motor fiber innervates a muscle, the term *polyneuronal motor innervation* will be used (subdivisions like dineuronal motor innervation, etc. can be derived from this)."

PRINGLE (1939) succeeded in distinguishing between a fast and a slow system in the extensor tibiae of the metathoracic leg of *Periplaneta americana*. A correct anatomical description is given of the leg muscles and their innervation to be found in the thorax and coxa, but unfortunately we are without the anatomical details of the object of the physiological examination proper, the extensor tibiae present in the femur. This limits the interpretation of his results.

He obtained electrical records from this muscle by "electrodes inserted through the chitin at opposite ends of the femur, without any dissection". The efferent nerve (*3 B*) of the muscles was electrically stimulated or left connected with the active ganglion whereby the derived potentials should be distinguished in two types with regard to frequency and in amplitude.

In other experiments, two types of muscle contractions—fast and slow—were obtained by accident or by the artifice of repeated drying and moistening, or by shifting the electrodes along the nerve. These contractions were recorded isotonically with a very sensitive optical method. The effective stimulation frequencies for the slow system were between about 50 and 350 c/s. This means, as may be assumed to be known, that no contraction occurs at stimulation frequencies of less than 50 c/s. The fast system showed separate twitches up to 20 c/s and a smooth tetanus at more than 70 c/s. No indication could be found of the existence of inhibitory fibres.

Microscopic examination of the muscle revealed no difference in the locus of the different contractions.

PRINGLE assumed that the two types of electrical and mechanical phenomena take place in the same muscle fibres, which means that they are innervated dineuronally. This assumption was discussed especially in connection with observed electrical phenomena; in my opinion, too little account was taken of the way in which the phenomena were derived.

It would have been interesting to test the observations on other assumptions. For instance, the examined muscle is homonomous with the compound muscle *142*[4]) of the mesothoracic leg. It is conceivable that the muscle is also a compound one and that several parts of it yield different mechanical and electrical responses which cannot be further localized by the method used. As a second possibility, the same reasoning applies to the separate muscle fibres inside one muscle.

It was WILSON (1954) who recorded intracellularly two types of muscle action potentials from the metathoracic flexor tibiae of *Periplaneta*, after stimulation of the efferent nerve. The muscle is homonomous with the compound muscle *143*[4]). One of the electrical responses is a fast all-or-none phenomenon, which is presumably conducted; the other is a slow facilitating potential probably local. Because of the spatial distribution of the inserted electrodes and the responses pertaining to them, this author assumed that both types of action potential originate from different types of separate muscle fibres.

ROEDER and WEIANT (1950) simultaneously recorded the electrical and mechanical events during the twitch of the tergal remotor of *Periplaneta americana* (muscle *162*, CARBONELL, 1947). This muscle appears to be a unit of the fast type. They obtained the accurate time course of stimulus, nerve impulse and muscle action potential derived from the periphery of the muscle. Although several interesting data were gathered on the electrical aspect of the neuromuscular transmission, these are of little importance for the purposes of our paper.

While the older literature (see e.g. KAHN) contains several data on action of curare on insect muscles, ROEDER and WEIANT found the substance inactive as a means for discriminating between the function of muscle and nerve fibre. This is in agreement with other more recent investigations (see ROEDER, 1953; HARLOW, 1958). This important expedient in the

[4]) In accordance with CARBONELL (1947) numbered by DRESDEN and NIJENHUIS (1953).

examination of neuromuscular systems in vertebrates is probably of little or no value for the insect physiology.

In conection with the apparently isometric recording technique described in this publication we are interested in the fact that a piezo-electrical method was employed. The resolving power of this method for successive contractions was fairly great, but it was not suitable for a correct reproduction of the time course of the force.

RIPLEY and EWER (1951) found peripheral inhibition in a limited number of preparations of the levator tarsus of *Locusta*. They obtained their results by means of the usual mechanical myography by stimulating the "main nerve trunk" of the leg. HOYLE (1955) made the objection that because of an incorrect stimulation technique due to polarisation of the electrodes an artefact would arise, which was wrongly taken for inhibition. The fact that the phenomenon in question was observed only in the levator tarsus and not in one of the other three examined types of muscles supports RIPLEY and EWER's interpretation of their experiments.

Important contributions to the knowledge of neuromuscular phenomena were made by HOYLE (1953 a, b; 1955 a, b). He gave a detailed description of the anatomy of the extensor tibiae of the metathoracic leg of *Locusta* (i.e. the powerful jumping muscle) and its innervation. From histological investigation it appears that a number of the muscle fibres receive at least two motor axons, while the presence of a third must be considered highly probable for functional reasons. Much attention has been paid to the electrical recording technique, less attention to the mechanical one. The nature of the contractions is described as well as the simultaneous intracellular recorded muscle action potentials.

Relaxation is rapid, following all kinds of slow and fast fibre stimulation. It makes it highly probable that a similar, if not the same contractile material, is involved in the two kinds of activity, and that the difference between slow and quick contraction is to be found in the way in which the contractile mechanism is activated.

In about 30% of the cases one muscle fibre appears to be able to give different types of action potentials in response to stimulation of the fast or the slow nerve fibre, thus providing physiological evidence of the dineuronal innervation.

The following conclusions may be drawn: One axon supplies large, fast contractions which are supposed to be used for "hopping" and "jumping"; the second axon, depending on the supposed kind of nerve endings, gives rise to small twitches and more or less slow contractions. Stimulation of the third axon causes an increase in the resting potential of the muscle fibres and has probably an important "preparatory function" for the insect. It may be interesting to note that WIERSMA (1941, page 260) assumed the existence of motor nerve fibres with such a conditioning function.

BUCHTHAL, KAISER, ROSENFALCK and WEIS-FOGH published, partly in co-operation, a long series of extensive investigations mainly on the mechanical and biophysical aspects of muscle contraction, including the flight muscles of locusts. The most recent communication (1957) is of interest, especially in this sort review, as it gives a description of an excellent new type of myograph which was used to study isolated flight muscles of locusts. It enabled the authors to record changes in length and force simultaneously or in quick succession. The results are expressed quantitatively but at the moment they are not yet relevant to our subject.

Since the differences in contractile properties of the muscular system of arthropodes had been recognized, questions arose on the finer structures that might be correlated with them. Remarkably enough, the interest in the histology of the neuromuscular junctions has so far been fairly small, though in this field more data should certainly be available. Most of the investigations are concerned rather with the structure of the muscle tissue itself, or with its difference in structure of different genera, than with the comparison of the different functioning muscle tissues in one insect (see e.g. WIGGLESWORTH, 1950). The adequate anatomical descriptions are often lacking; this invalidates earlier investigations connected with the problem because the function and structure cannot be correlated.

For general orientation, mention may be made of KRÜGER's extensive monograph dealing with another group of animals. "Tetanus and tonus of the striated skeletal muscle of Vertebrates and man" (1952). In it are described—largely with the aid of the data in the literature—differences in histological, physiological, biochemical and physico-chemical characteristics. He believed these differences could be correlated to the tetanic and tonic functions of muscle.

Deviating experimental results, also with Vertebrates, were obtained by KUSCHINSKY et al. (1956).

A number of investigators have determined especially the length of sarcomeres of functionally different muscles. JASPER and PEZARD (1934) found that the fastest muscles of some crustaceans possessed the narrowest striation, that is, the shortest length of sarcomere. Later on, other authors also found this in some insects.

Some inter- en intra-specific structure differences in insect muscles were discovered by EDWARDS et al. (1954) using the electron microscope.

An interesting field of investigation is probably awaiting here.

From the literature described it is seen that since about the early twenties and thirties there has been interest in the neuromuscular physiology of insects, but that our knowledge of it is still fragmentary compared with that on the crustaceans. We know for certain that there are fast and slow neuromuscular systems, but their mutual relations in the muscle are not uniform, as we learned from the examples given by WILSON and HOYLE. The histological knowledge to support these data is still slight. Peripheral inhibition, the widely known phenomenon of the crustaceans, has seldom been observed in insects. Further, in some cases we have tried to indicate shortly how sufficient anatomical knowledge, and naturally a good technique, may contribute considerably to the solution of the problems.

In our laboratory, investigations have been made in both directions. DRESDEN AND NIJENHUIS (1953) described the mechanism of motion of the second thoracic leg of *Periplaneta americana* and some supplementary anatomical data belonging to it; they could draw on PRINGLE's data (l.c.) and on the excellent and extensive description of the thoracic and leg musculature by CARBONELL (l.c.). Furthermore, studies in great detail were made of the course of the sensory and motor nerves as far as their end organs (NIJENHUIS and DRESDEN, 1952, 1955), and finally of the number of nerve fibres occurring in them (DRESDEN and NIJENHUIS, 1958).

With the help of this knowledge and the myographical technique mentioned the physiological properties of some muscles could be examined, as will be shown in the description below.

B. EXPERIMENTS ON THE PHYSIOLOGY OF LOCOMOTORY MUSCLES

1. General information

The muscles which have been studied by means of the technique described are the depressors and levators of the coxo-trochanteral joint of the second thoracic leg.

On the anterior and posterior side of the trochanter a condylus is situated which is enclosed firmly in the distal edge of the coxa. Accordingly, the joint is a dicondylic hinge or ginglymus. Every contraction of one of the depressors or levators trochanteris results in a turning movement around the joint axis of the distal part of the leg. In our neuromuscular preparations the latter consists of trochanter and femur with the inserted writer.

The description, definition and numbering of the muscles—with little alteration—have mainly been derived from CARBONELL (l.c.), that of the innervation from PRINGLE (l.c.) and from DRESDEN and NIJENHUIS (l.c.).

The muscles concerned are the depressors *135, 136* and *137* and the levators *138, 139* and *140*. The term depressor and levator is of real significance only in a functional morphology of the whole animal. In our study, which deals principally with observations on dissected legs, the terms extensor and flexor—which in fact are generally used—are preferred for clarity and convenience. The publications just mentioned are illustrated by very valuable and beautiful anatomical drawings. Linking up with these, other sketches of a more functional significance are added to support the description following.

2. Making and properties of various nerve muscle preparations

EXTENSORS

136. Posterior coxal extensor of the trochanter. Broad muscle which originates on the dorsal wall of the coxa, near the rim.

137. Anterior coxal extensor of the trochanter. It arises from the meso-ventral part and meso-ventral angle of the coxa.

These two muscles are separated by those of the *135* group. The extensors converge to three apodemes attached by small tendons to three dentiform apophyses at the medio-proximal edge of the trochanter; *136* and *137* on either sides, *135* in the middle (Figs. 3C and D).

A neuromuscular preparation of *136* and *137* is obtained as follows: All the nervous connections between the leg and the ganglion are dissected. This is done firstly to save the axon to be used from rupture due to mechanical strains; secondly, to prevent the

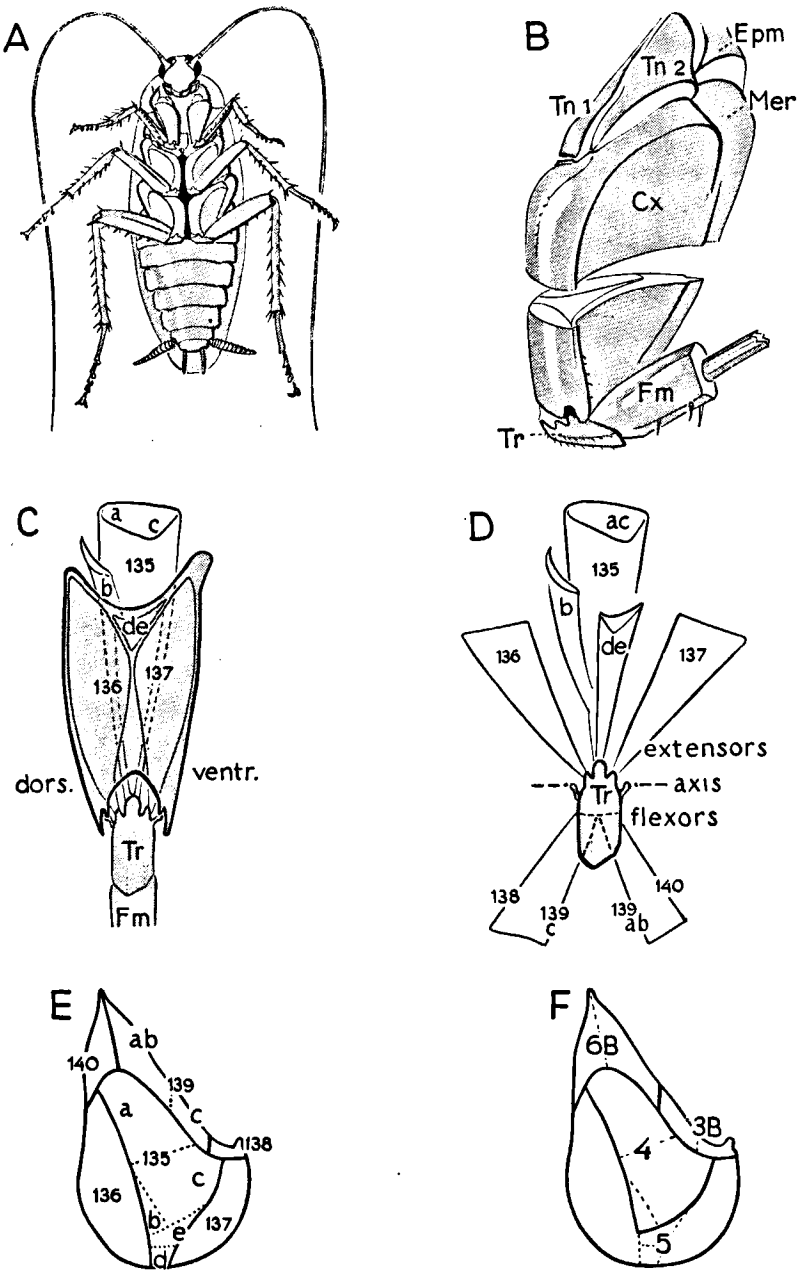


Fig. 3. A. The cockroach, *Periplaneta americana* (L), seen from ventral side.
B. The left mesothoracic leg, seen ventrally. Diagram of cross-section (E, F) visible halfway the coxa.
C. The left mesothoracic leg seen from the medial side. Diagram of position of the extensors and their insertion on the trochanter.
D. Diagrammatic sketch of all muscles inserting on the trochanter spread in a flat plane.
E. Diagram of cross-section of the coxa; position of the muscles.
F. Idem; position of the muscle groups innervated by one nerve.

Epm	= epimeron	Cx	= coxa
Mer	= meron	Tr	= trochanter
Tn1, Tn2	= first and second trochantin	Fm	= femur.

The length of muscle *135 ac* is about 9 mm, its maximal shortening about 1 mm, when the trocanter turns over an angle of about 120°. The length of muscles *136* + *137* is about 4 mm, their maximal shortening about 0.5 mm.

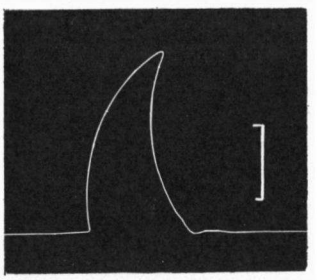


Fig. 4*). Kymographic record of one single twitch**) of the muscles *136* + *137*. Total duration of the twitch at 18–20°C about 0.16 seconds. S.w.***) Fast contraction and fast relaxation. These two muscles react together as a fast motor unit.

*) Each of the published myograms represents the characteristic type of a–sometimes considerable–number of similar records; naturally isolated phenomena were not considered.

**) When not indicated the duration of the single rectangular waves used for stimulation was 0.1 msec. Scale 1 cm.

***) Most of the myograms were recorded with a writer of about 22.5 mm in length. This is indicated as "short writer" = s.w. as distinct from l.w. = "long writer" of about 62.5 in length.

myogram from being interfered by movements of muscles not under consideration. The trunk serves only as a support. After inserting the writer the tendon between the *135* muscle group and the trochanter is cut. This is to preclude the muscles *135 d* and *e*, which are innervated also by nerve *5*, from taking part in the movements of the trochanter. Consequently, only the muscles *136* and *137* can act as extensors when the motor fibres in the distal part of nerve *5* are stimulated. The muscles *136* and *137* are supplied by nerve *5*, ramus *1 a* and ramus *1 b*, respectively. One common axon bifurcating into these rami innervates both muscles.

It could be predicted–according to this anatomical fact–that the pair of muscles behave as one motor-unit. Indeed we found a sharp threshold of excitation, followed by an all-or-none response which does not increase when the stimulus rises remarkably above threshold value.

The form of one single contraction in response to one single square pulse of 0.1 msec. duration is shown in Fig. 4. The curve of the twitch is fairly uniform in its ascending and descending part. The duration of the twitch at 18–20°C is about 0.16 sec., which is in accordance with data found by several authors in other insect muscles (cf. WIGGLESWORTH, 1950, pg. 100). The muscles react with distinct twitches up to about 6 cycles/sec.; they gradually fuse at higher frequencies and at about 35 cycles/sec. a smooth tetanus appears. Some summation occurs, the tetanic height being approximately 1.0–1.2 times that of the twitch (Fig. 5).

During tetanic contraction the muscle very soon fatigues. At continued stimulation with a low frequency the twitches can disappear almost completely within a few seconds (cf. Fig. 24). Not uncommonly a smooth tetanus is followed by a contracture.

The fast contraction and fast relaxation together lasting for about a sixth of a second; the inability to perform sustained work in continuous contraction, and in addition a small summation, are apparently the properties of a muscle of the fast type.

As the muscle is innervated by only one axon, it is not surprising that inhibition has never been observed in these muscles.

When nerve ramus *1 a* or *1 b* is cut alternately, contractions of either *137* or *136* are prevented and the other can contract independently. In that case one muscle of the pair gives a myogram which is com-

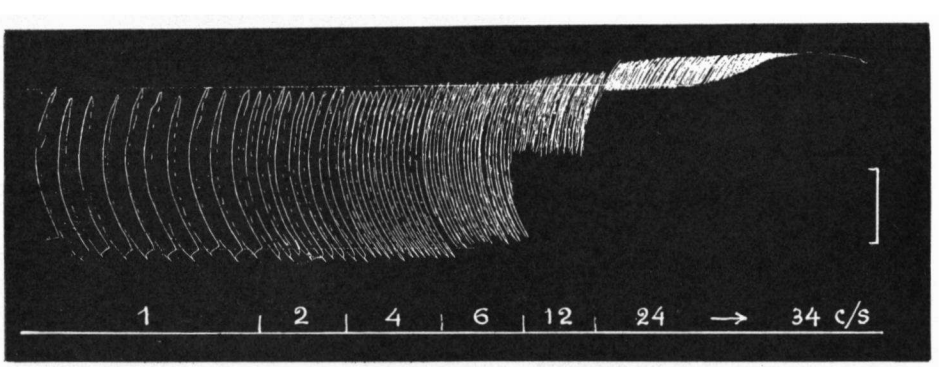


Fig. 5. Record of muscles *136* + *137* stimulated with increasing frequencies. Voltage unchanged; s.w. The stepwise reduction of the recorded movements and the rise of total contraction height are due to summation. This reduction must not be confused with the different contraction heights as seen, for instance, in the records of muscle *135 a*.

pletely similar to the other, but somewhat smaller compared with that of the complete preparation.

135. Main extensor of the trochanter, the most powerful muscle of the leg and mesothorax. It originates on several parts of mesothorax and coxa; its branches converge to a broad, spoon-shaped apodeme inserted with a tendon on the median apophysis of the trochanter.

135 a. Tergal branch. Origin on the antero-lateral part of the tergum.

135 c. Basalar muscle of the forewing. Origin on the anterior edge of the basalar plate.

The extensors *135 a* and *135 c* represent the strongest part in this group of muscles. They are innervated by nerve *4*, ramus *3*, which contains motor fibres only; *135 a* is supplied by three axons and *135 c* by one.

A neuromuscular preparation is made similarly to that described above. The tendon which connects the muscle with the trochanter is left intact. In some experiments the tendons of *136* and *137* were dissected but it did not appear necessary to do so in order to obtain a correct preparation. The electrodes were placed under ramus *3*, which supplies *135 a* and *c*. The rami *1* and *2* that supply other muscles, and then the base of nerve *4* near the ganglion, were dissected. The peripheral part of the nerve now hangs freely on the electrodes. As in most cases it was possible to recognize and cut smaller branches of ramus *3*, severance of *135 a* and *c* is also feasible.

It appears that muscle *135 c* behaves as a motor-unit of the fast type, since the several properties described for muscles *136* + *137* are quite similar.

As opposed to this muscle, stimuli of successively increasing voltage to the nerve branch of muscle *135 a* result in three different contraction heights (Fig. 6). In addition three distinct tetanic contractions could be observed, although this was rather difficult. The tetanus/twitch ratios of both muscles are of the same order of magnitude as is found in the muscles *136* and *137*, namely 1.0–1.2. This muscle also clearly belongs to the fast type.

The smallest nerve branches toward *135 a* which were still visible under dissection microscope always contained 3 axons. If all these branches but one are cut we find, on stimulation, three different contraction heights (Fig. 7). In accordance with the slight mass of the functioning part of the muscle, the twitches have a small height. This myogram is interesting as it can be compared with records to be discussed later on.

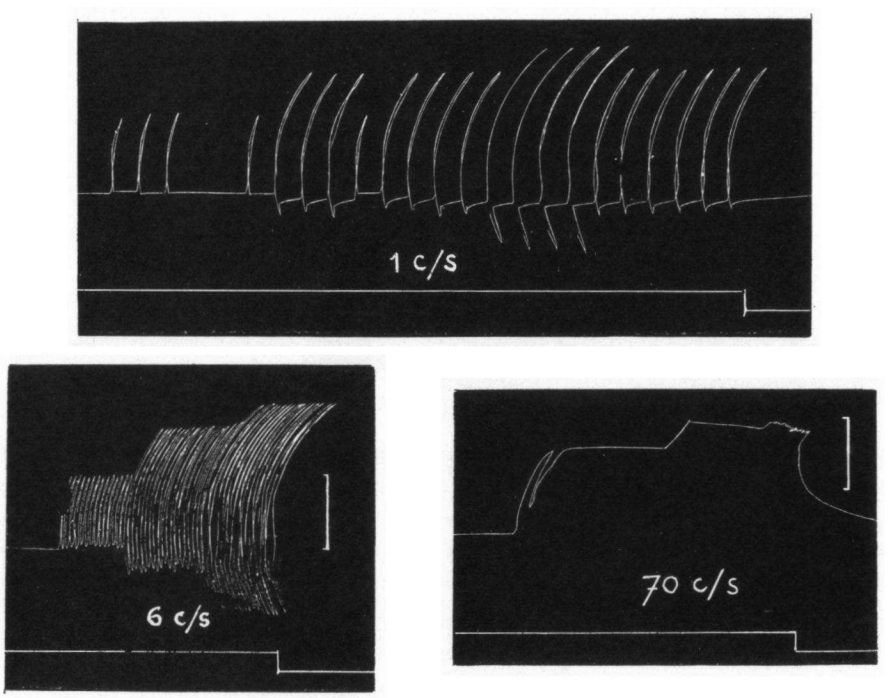


Fig. 6. Record of the fast muscle *135 a*. Stimulus frequencies are 1, 6 and 70 c/s. S.w. Steadily increasing voltage gives rise to three steps of increased contraction heights, which evidently correspond with the three motor fibres.

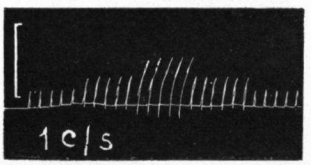


Fig. 7. Record of the contraction of a small part of muscle *135 a*, when stimulated through one small nerve branch still containing three nerve fibres. Same conditions as indicated in Fig. 6. The contractions and relaxations are fast; compare in this respect those of other contractions of about the same height e.g. in Fig. 16. S.w.

135 b. Sternal branch. Origin on a downward-bent flange of the sternal arm; it inserts on the common apodeme. It is a small, band-shaped muscle innervated by nerve *4*, ramus *2 b*, which supplies it with at least four axons.

The neuromuscular preparation is made again as described previously. The absolute shortening of this muscle is very small. To obtain a myogram with a height of contraction about as great as those of other muscles, it is necessary to insert a writer which has a lever about three times longer than that generally used. The electrodes are placed under ramus 2; the rami *1* and *3* of nerve *4*, running to the muscles *105* and *135 ac* respectively are then dissected; ramus *2* to muscle *135 b* is left intact. Finally, it is necessary to cut off the nearly invisible ramus *2 a*, containing only one axon which supplies the remotor muscle *132*. Contractions of this muscle may otherwise change the myogram out of all recognition.

The muscle *135 b* showed scarcely any response to stimuli of low frequencies, up to 25 cycles/sec. Only very small twitches occurred, which could seldom be recorded on the kymograph. Under the dissection-microscope, however, they could be observed very clearly. Stimulation with higher frequencies resulted in a considerable contraction of the slow type. When the frequency of stimuli was gradually increased the contraction height also increased up to a maximum at about 130 c/s (Fig. 8). When the muscle was stimulated continuously with a frequency of 130 c/s and at optimum intensity the slow contraction could last for as long as ten minutes before it exhibited signs of fatigue. The absence of abnormal contractures was checked by short interruptions of the stimulation. Every interruption during the course of the experiment was accompanied by a relaxation equal to that following a contraction of only a few seconds

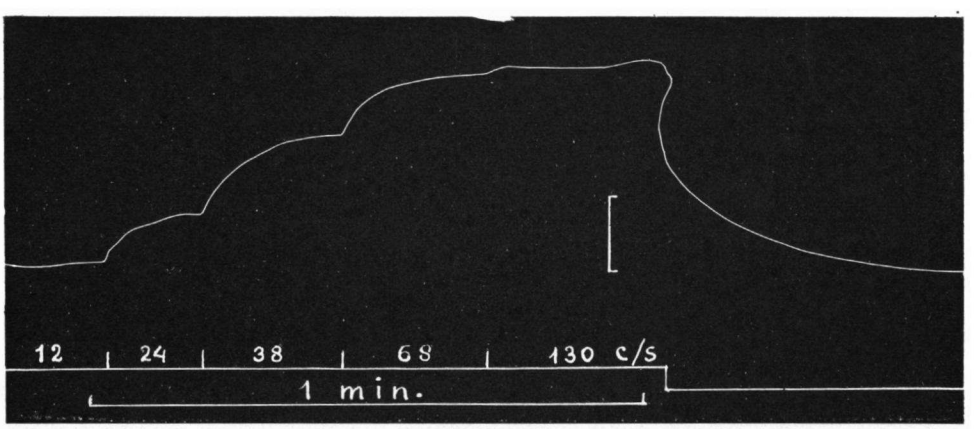


Fig. 8. Record of muscle *135 b* stimulated with stepwise increase of frequencies; voltage unchanged; l.w. Typical response of a slow type muscle.

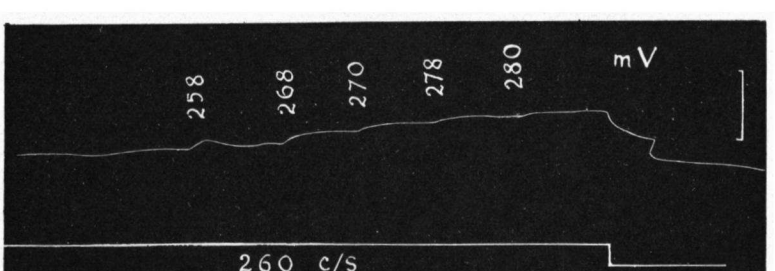


Fig. 9. Myogram of *135 b* stimulated with increasing voltages; frequency unchanged; l.w.

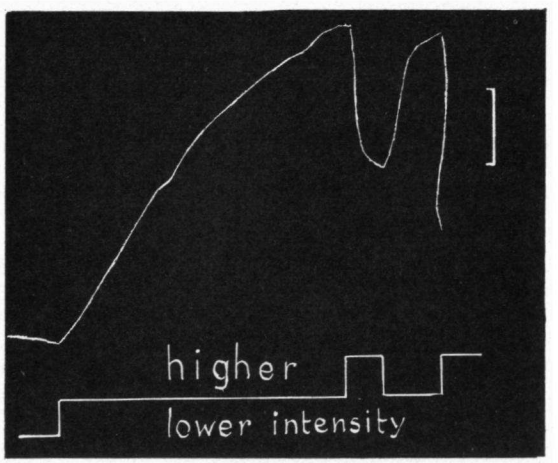


Fig. 10. Record of muscle *135 b*. After the initial response a further rise in voltage gives a sharply decreasing contraction height. Conversely, a drop in voltage gives a sharp increasing contraction height; l.w. The decrease in the contraction was probably caused by inhibition.

and which was therefore regarded as normal for this type of muscle.

On stimulation with a constant impulse frequency and increasing voltage, two phenomena could be observed which could be related to the innervation by a number of motor fibres. A series of increasing heights of contraction were sometimes noticed (Fig. 9).

In many preparations of *135 b* it was seen that an increase in the voltage of stimuli induced a rather sharp decrease in the height of contraction (Fig. 10). This phenomenon, which was always reversible, pointed to the existence of an inhibitory fibre in the supplying nerve. During the stimulation the frequency (e.g. 68 c/s) remained unchanged. Some measures were taken to prevent the current from lasting too long or its voltage from being too high, so causing polarization of the electrodes and damage to the nerve. The impulse duration was therefore fixed at 0.1 msec., whereas only the intensity of stimulation was changed over a range of about 300—400 mV. In our experiments the voltage differences necessary to reach the inhibition were seldom more than about 25 per cent above the threshold value. However, we are fairly sure that the phenomenon is not an artefact, though further evidence might be obtained by dissecting the nerve into separate axons, as was done by WIERSMA in Crustacea. It appeared impossible to do so in this object and other experiments, perhaps with the aid of intra-cellular micro-electrodes, may therefore be necessary before the problem can be solved.

135 d. Coxal branch. Origin on the coxal wall between those of *136* and *137*.

135 e. Coxal branch. Origin in the anterior part of the coxal rim, near the coxo-trochantinal articulation.

Both muscles insert again on the common apodeme. Here is a somewhat more complicated structure. The muscles are innervated by one common large axon, but over and above this, each of the muscles *135 d* and *135 e* is supplied by three smaller fibres. All

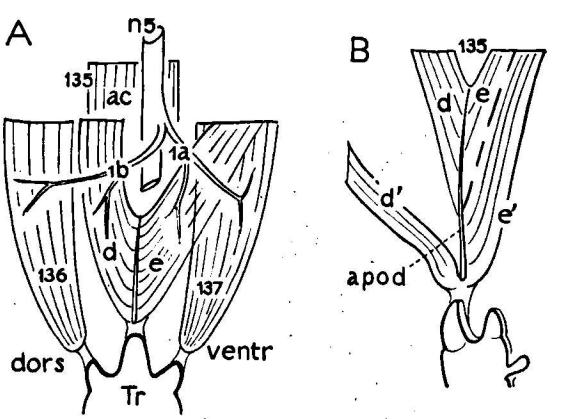


Fig. 11. A. Diagram of some coxal muscles, from medial in exploded view. B. Insertion of *135 d e* on the common apodeme in detail, seen from a more ventral side; *d'* is actually on the medial side and would have to be drawn beyond the plane of the figure.

Table 1. *Making of some neuromuscular preparations.*

Part of the nerve *5*		Part of the muscles		Expected and found contraction after stimulation of nerve *5*
Cut off	Intact	Cut off	Intact	
Ramus *1b*	*1a*	(*136* + *137*) dist.	*135* total	*135e*
1b	*1a*	(*136* + *137*) dist. + *135e*	*135* remainder	none
1a	*1b*	(*136* + *137*) dist.	*135* total	*135d*
1a	*1b*	(*136* + *137*) dist. + *135d*	*135* remainder	none

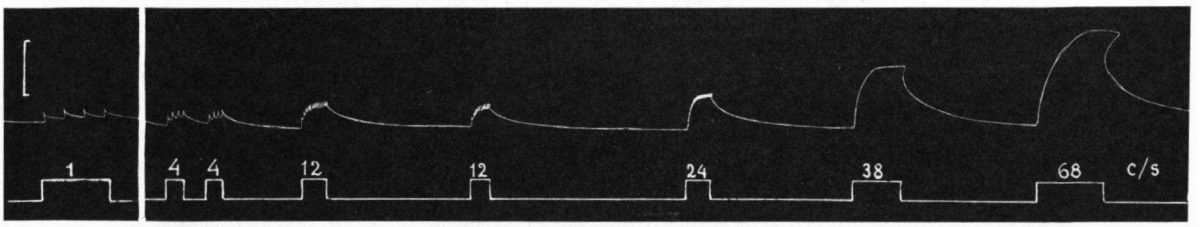


Fig. 12. Myogram of *135 d e*. Different frequencies; voltage unchanged; s.w. Summation is visible at lower frequencies.

of them follow the same way, running through the rami *1 a* and *1 b* of nerve *5*, as happens in *136* and *137*.

In order to illustrate the several possibilities in realizing neuromuscular preparations, the anatomical situation in some of the experiments is presented (Fig. 11 and Table 1).

The behaviour of the entire muscle group *135 de* at unchanged voltage and different frequencies is represented by the myogram in Fig. 12. At rather low frequencies a certain amount of summation already occurs. Together characteristic twitches of the fast system, the contraction rate and the relative indefatigability of the slow muscle type are recognizable in these contractions; this phenomenon was seen even more clearly in some flexors. In the record of muscle

ence of the number of functions with the four motor fibres found in the histological investigation.

In further analysis, with the aid of anatomical interference, it was nearly impossible to obtain myograms of the very thin parts of muscle left after dissection. However, many supplementary observations were made under the microscope which revealed a clear distinction between types of contraction. It was found that *135 d* and *135 e* are composed of fast portions called now *d'* and *e'* and slow ones *d* and *e*. These parts are well visible, especially in the original *135 d*; the part *135 d'* is an anatomical independent small muscle (Fig. 11 B) which is probably a motor unit.

FLEXORS

138. Anterior coxal flexor of the trochanter. Slen-

Fig. 13 A

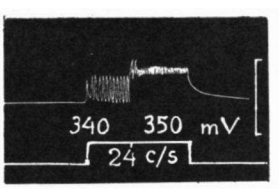


Fig. 13A. Record of muscles *135 d e* and in Fig. 13B of muscle *135 e*. Increasing voltages show several steps of increasing contraction heights. Frequencies unchanged; s.w.

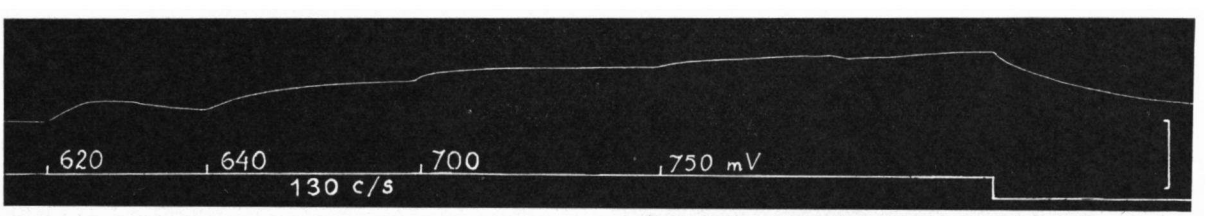


Fig. 13 B.

135 de (Fig. 13 A) the tonic component can easily be observed beside the twitches. The separate parts *135 d* and *135 e* behaved partly in the same manner.

As in muscle *135 b*, different heights of contraction were reached by stimulating the muscle *135 e* successively with different voltages. Fig. 13 B shows four levels of increasing contraction. In muscle *135 e* inhibition was probably recorded in some cases (Fig. 14); the same could not be observed with certainty in muscle *135 d*. Thus there was no close correspond-

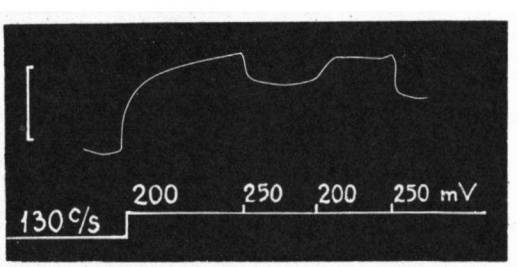


Fig. 14. Myogram of *135 e*. Rise in voltage gives decrease of the contraction height, and conversely; frequency unchanged; s.w. Inhibition probable.

der, weak muscle, originating on the anterior wall of the coxa.

139. Main coxal flexor of the trochanter. The branches *a, b* and *c* originate in the coxal rim and ridge which limit the meron.

140. Posterior coxal flexor of the trochanter. Rather small muscle with four branches (*a, b, c, d*) which originate on the posterior wall of the coxa.

The flexors converge to apodemes attached to the lateral edge of the trochanter.

in nerve *6B*. The arrangement of the fibres is rather complicated (DRESDEN and NIJENHUIS, 1958). In the *6B* group it is certain that no more than three axons enter any part of the muscle by way of the smallest nerve branches; consequently these axons are not the same in the different muscle-parts. An equal situation is found in the other flexor muscle group.

The complicated arrangement of fibres is expressed in the physiological behaviour of these muscles. The record of muscles *138* + *139c* (Fig. 15) shows a considerable summation of small twitches at the lower stimulus-frequencies and a further increment of the

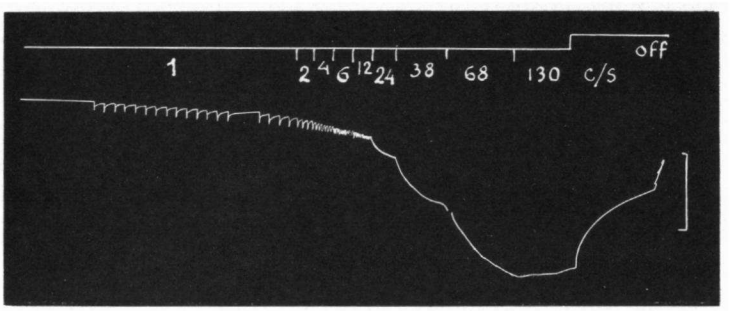


Fig. 15. Record of muscles *138* + *139 c* stimulated with increasing frequencies; voltage unchanged; s.w. Considerable summation.

Two groups of flexor muscles can be distinguished. The first group, *138* + *139c*, are innervated by nerve *3B*, and the second, *139ab* + *140abcd*, by nerve *6B*. The division of the muscle group *139* in two parts *c* and *ab* is in accordance with the movements observed after electrical stimulation of their nerves.

The supplying nerves of the two groups contain at least seven fibres in nerve *3B* and exactly seven fibres

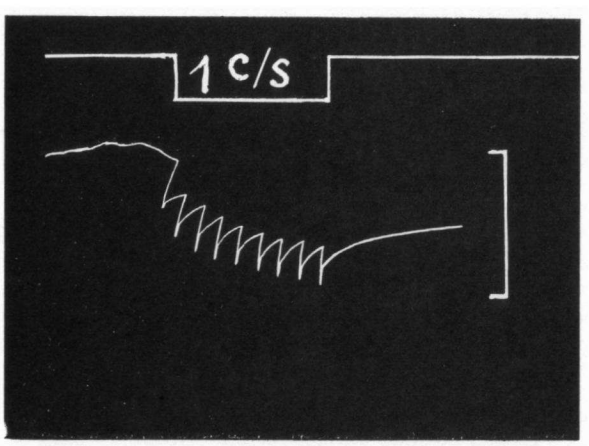


Fig. 16. „Cock's comb" myogram recorded from the muscles *138* + *139 c*; frequency and voltage unchanged; s.w. Steeply rising twitch and slow relaxation followed by remarkable summation. Record x 2.

smooth contraction at the higher frequencies.

Low frequency records (Fig. 16) have a very characteristic shape which we have called "cock's comb" myograms in our first description (BECHT and DRESDEN, 1956). Considering one contraction cycle of this "cock's comb" myogram in detail, a steeply rising part is seen belonging to the twitch, followed by a fairly slow relaxation curve. Next a twitch can be evoked again on the descending part of the curve. The same phenomenon was already seen in the muscles *135de*, but in a less pregnant form (Fig. 12).

Intensifying the stimuli by an increase in voltage or pulse duration enhanced the rate of summation or tone in contractions of the muscle groups *138* + *139c* and also of *135de*. This fact is observed very clearly when myograms Figs. 13A, 17B as well as 17A are compared with Fig. 7. Only the twitches of Fig. 7 became higher under the same conditions, but any sign of summation was completely absent.

After decreasing voltage of the stimuli to nerve *6B* of the muscle group *139ab* + *140* at least five different heights of fast contractions appeared (Fig. 18).

In records of the muscles *139ab* alone, four twitch heights (Fig. 19A) and at least three tetanic contractions could be recognized. The maximum tetanus twitch ratio here was about 1 to 1.2 (Fig. 19B).

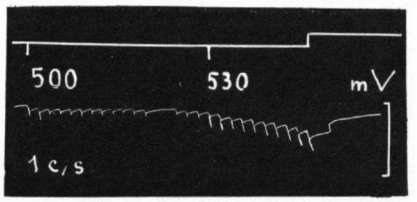


Fig. 17A. Record of muscles *138* + *139 c* stimulated with increasing voltages; frequency unchanged; s.w.

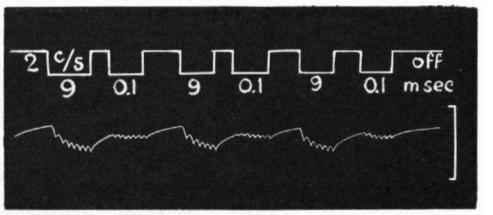


Fig. 17B. Record of muscles *138* + *139c*, two different stimulus intensities caused by changed impulse-durations; frequency and voltage unchanged; s.w.

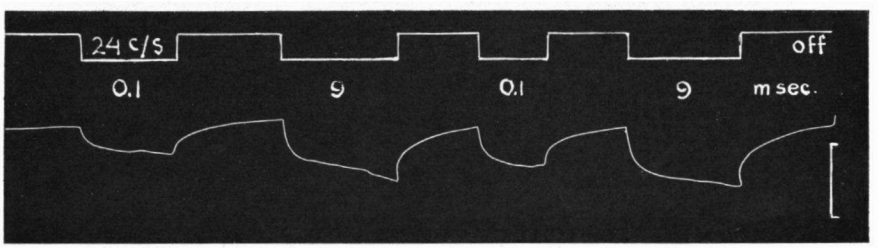


Fig. 17C. As in Fig. 17B, but at higher frequencies.

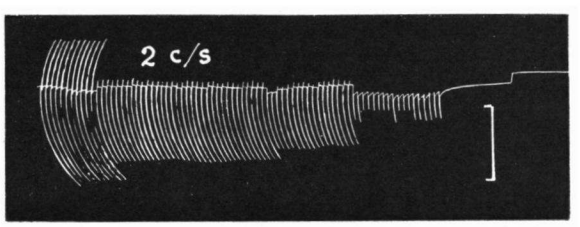


Fig. 18. Myogram of *139 ab* + *140* stimulated with decreasing voltage; frequency unchanged; s.w. Five steps of contraction heights, probably corresponding with an equal number of motor fibres.

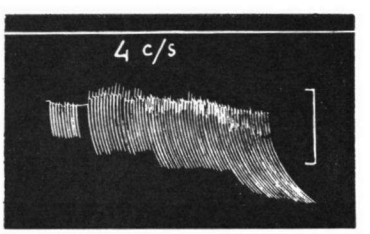


Fig. 19A. Record of muscles *139 ab*; increasing voltage; frequency unchanged; s.w.

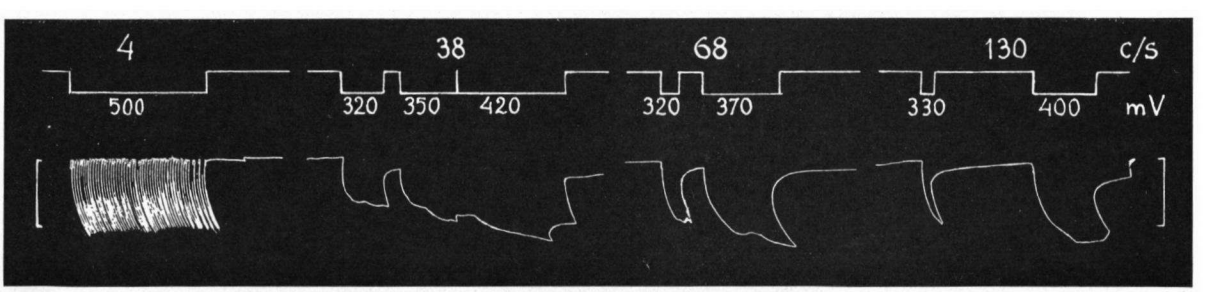


Fig. 19B. Parts of a record of one preparation of muscles *139 ab* stimulated with increasing voltage at different frequencies. The maximum contraction height at 4 c/s was reached at an intensity of 500 mV. This height may be compared with that of the highest tetani. Slight summation.

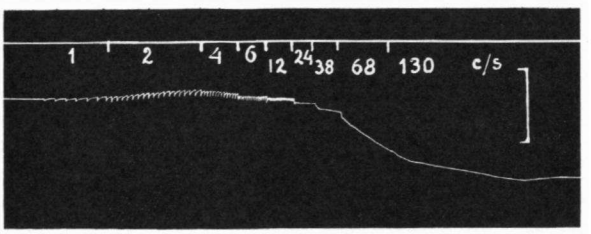


Fig. 20. Record of muscle *140*; increasing frequencies; voltage unchanged; s.w.

The muscle *140* showed, compared with *139*, much more summation at low stimulus frequencies and a considerable slow contraction at high frequencies (Fig. 20).

The part played by fast and slow systems in this type of contraction is unknown.

From these facts, found after extensive use of dissection technique, most of the fast system seemed to be located in muscle *139*, whereas *140* and, probably *138* exhibited a slower function. Nevertheless, it was difficult to obtain a clear and reliable picture, partly because of the mutual bias of the movements which took place in joined parts of the muscle. An attempt was then made to use ryanodine for further analysis.

3. Further analysis with ryanodine

Ryanodine

Ryanodine is an alkaloid derived from the stems of a woody tropical plant, *Ryania speciosa* Vahl, fam. Flacourtiaceae (ROGERS et al., 1948).

Although not uncommon as an insecticide inside or outside the U.S.A. (PEPPER and CARRUTH (1945); CHARLES (1954); STARR and CALSETTA (1955)), the alkaloid itself did not appear to be available. Ryania wood was eventually received through the kind intermediary of the Department of Local Government, British Guiana. The Institute of Organic Chemistry T.N.O. of the National Council for Applied Scientific Research in the Netherlands (= T.N.O.) at Utrecht, were kind enough to prepare the crude extract according to ROGERS' prescription (l.c.).

From this extract an alcoholic stock solution was obtained from which a dilution with saline can be prepared. The alcoholic concentration of the dilution was sufficiently low to exclude toxic actions.

Ryanodine symptoms

The common picture of external symptoms of poisoning after injection with 1–10 μg ryanodine per gram of bodyweight shows a quickly increasing slowing down of the movements, so that the insect–even after vigorous stimulation of e.g. the cerci–can move forward only with difficulty. This gradually changes into complete flaccid paralysis. Spasms, tremors or convulsions are absent. The symptoms of poisoning often develop so rapidly that the time taken for preparation prevents us from observing the course of the poisoning on the myogram. This requires administering the poison topically in the coxa, but only after all preparatory operations have been carried out. If the intoxication is to proceed within a reasonable time it is necessary to administer concentrations of 0.5×10^{-3}–1×10^{-5} for perfusion.

Action of ryanodine on muscle preparations

Neuromuscular preparations when stimulated by impulses with frequencies of, e.g., 1–2 c/s may, under normal circumstances produce twitches of invariable height for a fairly long time. Ryanodine acts in such a way that the contractions soon decrease and finally fade out. We were immediately struck by the fact that in the intoxicated leg, the fast muscles (*136, 137* and *135ac*) were more quickly paralyzed than those in which a slow component was clearly present (*135b*). This behaviour was reflected in the symptoms of the intact insect. Surprisingly, in the different flexors with their more or less pronounced "cock's comb" myogram, the fast component happened to disappear much sooner than the slow one during the progressing intoxication. This could be clearly observed on injecting 2–5 μg per insect or on local injection close to the muscle with a corresponding concentration. While the results of a considerable number of experiments all pointed in the same direction, the time required to block the function was noted in only of part of them. Some figures in table 2 may illustrate this.

In at least ten experiments with flexors a sufficient anatomical severance was obtained beforehand between the different muscles *138, 139,* and *140*. In these cases too, in which one muscle body was functioning, the fast component was paralyzed more quickly than the slow one. This could repeatedly be confirmed by microscopical observation of the muscle tissue.

In some experiments a deviation was found from the results described above. At the time that the fast contractions had already been blocked by ryanodine poisoning, a tonic contraction was brought about in response to *low* frequency stimulation. For the time being this fact stands alone.

4. Discussion

a. *Remark on the anatomy and the numbering*

The topographical relation between the leg muscles

Table 2. *Blocking action of ryanodine on the fast and slow contractions of neuromuscular preparations.*

Number of experiments	Average time in minutes necessary to block fast and slow functions respectively. Number between brackets indicates muscles used in experiment.	
12	Fast 14 (*136 + 137*)	Slow 21 (*135 de*) > 14 (*135b*, not further determined)
6	< 5 (*138 to 140*)	> 20 (*138 to 140*)
In one experiment even	1 (*138 + 139c*)	15 (*138 + 139c*)

and their nerves is complicated (see Figs. 3E and 3F).

It is unusual for the same functional unit to be innervated by two nerves. PRINGLE (1939) observed that two anatomically separated nerves *4* and *5* pass to different parts of the "functional unit of the extensor trochanteris" including the parts *135abc* (nerve *4*) and *135de + 136 + 137* (nerve *5*).

Towards the periphery the different parts of the group *135* are seen to insert on one common apodeme, which is connected with the trochanter. Because of this, the parts possess the same mechanical function and somehow form a unit.

CARBONELL (l.c.) for this reason, denominated them as "main depressor of the leg" and gave them one collective number. On the other hand, however, the remarkable distribution of nerves is emphasized even more by the fact that the parts *a b c d e* of the muscle group *135* originate in different places of coxa and thorax. Besides, they have dissimilar physiological properties, as was demonstrated in our experiments.

The position of the flexors is somewhat parallel. Here the complexity is increased by the manner of numbering. The muscles *138* up to *140* clearly form a topographical and functional unit in which two anatomically separate muscle groups can be recognized. In histological preparations the group *138 + 139c*, according to our observations, strongly create the impression of being one muscle. The other group includes *139ab + 140*.

At the side of the trochanter both groups insert on clearly distinguishable separate apodemes. Moreover, these two groups are innervated by the nerves *3B* and *6B* respectively.

In accordance with the anatomy and physiology, muscle *139c* could easily be numbered as a part of *138*, thus improving our insight into the structure.

b. *Remark on the technique*

Before proceeding to the physiology we shall first discuss our method of recording.

There are in principle two methods for studying the muscle contractions: isotonic and isometric. Both are approximations of the ideal suggested by their name.

The isotonic ideal is the most difficult to obtain as it seems that the mechanical tension in the muscle can hardly be kept constant. This is an unavoidable drawback. It would therefore seem that the ideal isotonic recording cannot be carried out, not even under normal physiological circumstances.

Assuming that the normal physiological circumstances represent the optimum at which the muscle is able to work, it will be wise first to try to realize this optimum in mainly qualitative recording experiments.

With the method applied, it is true, the load moment of the recording pen, and hence its inertia, is greater than that of the extremity replaced. In experiments with somewhat different load moments the deviations of records considered typical were so small, however, that important influence on the results need not be feared.

The isometric ideal seems more easily approachable. This method has been tried for a short time on our subject with the RCA 5734 miniature triode, a very sensitive electro-mechanical transducer. Since the reliability of the latter was unknown and the first method was far simpler, we did not continue with it.

c. *The localisation of fast and slow functions in the insect muscle*

It is an interesting question how far the fast and slow functions of the muscle possess a common contractile substrate. Its solution is also of importance if we want to get some idea of the structural and functional aspects of the neuromuscular transmission.

In his review WIERSMA (1957) writes: "In arthropods, on the other hand, all indications are that the same muscle fibers are involved in the various

contraction types, and that the type of innervation is much the same. In other phyla fast and slow contractions are sometimes definitely due to two different types of muscle fibers". This pronouncement should be considered an introductory generalisation, for in the same review the author gives a number of deviating examples that show that not always all fibres in one single crustacean muscle receive the same number of axons.

Nearly the same picture was found by HOYLE (1955) in the extensor tibiae of *Locusta*, but WILSON (l.c.) found two different muscle fibre types for the slow and fast contraction of the flexor tibiae of *Periplaneta*.

The most fascinating problem encountered in this case is how one muscle *fibre* is able to contract in various ways. We shall try to relate some of the results from our work to this problem.

To begin with, the problem of the contraction of the polyneuronally innervated *muscle* will be treated more in detail:

α. How many contractile systems in the muscles can be recognized and how do they differ from each other?

β. What are the criteria in localizing them in anatomical or histological structure-elements?

α. If we compare the myograms of the following muscles or muscle groups *135b*, *138 + 139c*, *140*, *135de*, *139ab* and *136*, *137* or *135ac* (Figs. 8, 15, 20, 12, 19A, 5, 6 respectively) we observe an increasing fast component and a decreasing slow one respectively.

As far as can be observed a number of muscles (*136*, *137*, *135ac*) do not even have a slow component; they can give fast contractions only. One contraction cycle takes about 1/6 sec., after which the muscle is again in its original state (Fig. 4). Although the twitches have completely fused at about 30 c/s, and the summation has then reached the maximum, summation is nevertheless slight, as many experiments demonstrated (tetanus/twitch ratio = 1.0–1.2) (Fig. 5).

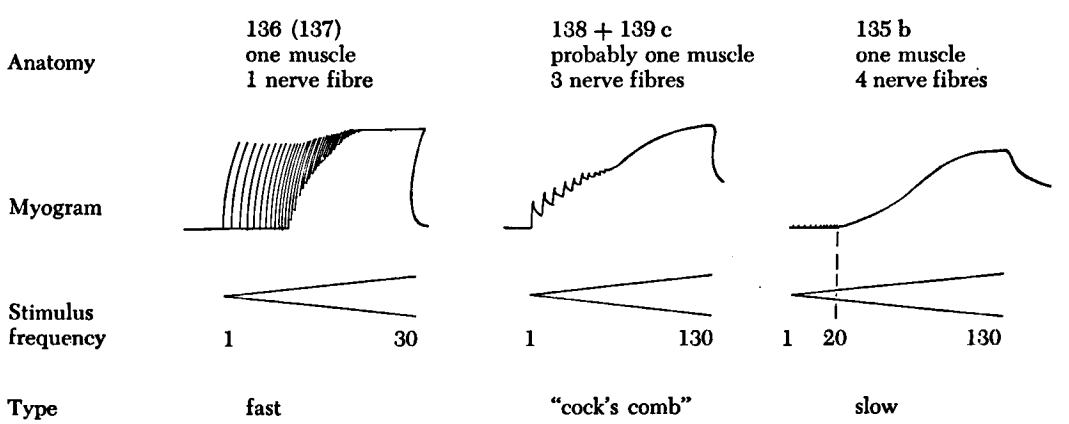
The muscles *136 + 137* and *135c* are units, whereas *135a* has three contraction heights. It is innervated by three nerve fibres which can be found in the finest nerve branches anatomically recognizable. From this one cannot conclude whether the principle of the polyneuronal innervation of the arthropods was maintained i.e. that every muscle fibre received three separate nerve fibres with which equal functions are cumulated, or whether each of the nerve fibres innervates a group of muscle fibres, hence this muscle is of the vertebrate type. In the meantime a histological investigation of SMIT (unpublished) shows that the last mentioned point holds.

In other muscles or muscle groups, such as *135de* (Fig. 12), *138 + 139c* (Fig. 16) or *140* (Fig. 20), fast contractions are also seen to occur, but the muscle takes a fairly long time before it is again in its original state–approximately 1 second. With stimulation frequencies between 24 and 38 c/s the contraction becomes smooth in character. The relaxation remains slow. The ratio of the height of the twitch to that of the fused contraction is 3–6. With a further increase in stimulation frequency up to, for instance, 130 c/s the height may increase by a factor of 3–5.

There is also a muscle type *135b*, with barely visible twitches. The fusion frequency is therefore difficult to indicate. With stimulation frequencies higher than 20 c/s, a slow smooth contraction takes place, followed by a slow relaxation. Both ratios just mentioned are estimated here to be in the order of some tens.

In some myograms, smooth contractions are seen to reach, stepwise, a higher level with increasing stimulation intensities, i.e. it seems as if several smooth

Fig. 21. *Some characteristics of muscle types.*



contractions are summated (Figs. 9 and 13 B). As a matter of course it is difficult to decide which is to be considered a tetanic or tonic contraction.

Sometimes it is clear that a fast as well as a slow contraction contribute to the entire shortening (Figs. 13 A, 17 A and 17 B).

A diagram giving some characteristics of the three important muscle types is reproduced above (Fig. 21).

It should be said, that by using anatomical expedients, we tried to obtain the largest possible severance between the components. Most of the muscle preparations seemed to consist of homogeneous pieces of tissue. A more refined analysis than reached so far by this means, has not yet appeared possible, and seems almost impossible bearing in mind the small size of the subject.

From the phenomenological data we arrive at the following conclusions. It is clear, that with *Periplaneta* in the anatomical unit that is usually called muscle nerve preparation, fast and slow systems can more or less be recognized in the myogram by their speeds of contraction as well as relaxation. The fast and slow components may occur separately or together (and in combination with inhibition, Figs. 10 and 14) and they seem to occur in different muscles in different amounts, they are mostly distinguished by their different stimulation thresholds, the order of which is not yet certain, but evidently correspond with as many nerve fibres. Considering the small summation of the fast system, it is assumed that strong summation is a typical characteristic when a separate slow contraction system is present. A survey of the main results of the physiological experiments is found at the end of this discussion.

Although the results obtained do not give a clear insight into the problem of polyneuronal innervation, they may form an excellent introduction to further investigation in that direction.

The answer to our first question is, therefore, that at least two contractile systems occur which differ in speed of contraction and of relaxation, in rate of summation and in stimulation threshold.

β. The next point in our discussion is the search for criteria in localizing the different contractile systems in anatomical or histological structure elements.

The first question under this heading is: how should the relaxation linked to the contractile mechanism be conceived.

Let us consider again in detail the contraction cycle of the type "cock's comb" myogram (in particular of *138 + 139 c*, Fig. 16). It consists of a steeply rising part belonging to the twitch, followed by a rather slow relaxation curve. A new twitch may arise on the slope of this curve. The higher the stimulation frequency, the more summation occurs, evidently favoured by the slow relaxation. Also facilitation would favour summation, but deciding from the decreasing height of the twitches this phenomenon is absent. At higher frequencies the behaviour also indicates the presence of the slow contraction type with slow relaxation. So we noticed in one muscle two contraction types, with one relaxation rate.

HOYLE (1955) observed two contraction types in *Locusta* with one relaxation rapidity and he is of opinion that this probably indicates the presence of one contraction substrate. This means the one substrate, *in casu* one muscle fibre or a smaller anatomic unit, must be able to perform different types of contraction. This opinion is supported by the histologically found cases of muscle fibres with a twin nerve supply and by the different types of intracellular action potentials derived from one single fibre.

In the case of *Periplaneta*, especially muscles of type *138 + 139 c*, however, the last-mentioned arguments for one contraction substrate are still lacking. Perhaps separate muscle fibres innervated differently consist of a similar material which should give them the same relaxation rate, but there is no further evidence for it.

Attempts may be made to approach the problem theoretically. Apart from inhibition there seem to be four factors that influence the form of the myogram: fast contraction, fast relaxation, slow contraction and slow relaxation. With the simplest combinations one finds at least four realizable possibilities, of which three perhaps occur in our preparations (Table 3).

We recognize the possibility to reduce the question of the localisation of the different functions, in par-

Table 3. *The occurence of different factors in several neuromuscular preparations.*

Muscle	Contraction		Relaxation	
	Fast	Slow	Fast	Slow
ext. tib. *Locusta* (HOYLE)	+	+	+	
e.g. 136	+		+	
138 + 139c	+	+		+
135b		+		+

ticular the relaxation, to the question of the degree of mechanical linkage between the different structural elements. We may think here of muscle fibres that are mutually different, or different fibrils in one fibre. We need more facts on the viscous and elastic properties before the argument of relaxation speed in the localisation of fast and slow functions can help us. Accurate mechanical myography (as carried out by BUCHTHAL, WEIS-FOGH and ROSENFALCK, 1957) and investigation into the finer structure of the tissue could provide us with further information.

In our endeavour to localize the fast and slow functions in the structure of the muscle we arrive at the following aspects to be discussed. The experiments with ryanodine showed a selective sensitivity of the fast and slow functions, from which it appeared that in the muscles of the cockroach, at least, two more or less independent systems may exist side by side, a quickly contracting system that paralyses more rapidly than a slowly contracting one.

In passing, it would be highly interesting to examine how an insect muscle with clearly polyneuronally innervated fibres, such as the extensor tibiae of *Locusta* (HOYLE, l.c.), would behave toxicologically to ryanodine.

We will discuss the way in which fast and slow contraction systems co-exist (although in general the existence of an in-between independent system need not *a priori* be rejected). With the following arguments we aim at correlating the function of the fast and slow systems to certain anatomical structure elements.

EDWARDS et al. (1948) studied the action of ryanodine on neuromuscular transmission in the cockroach. High dosages of this poison, for instance, by injecting 0.05 ml per insect of a solution of 10^{-4} in saline or perfusion into a leg cause paralysis within ten minutes. After that, the leg muscles can no longer be made to contract with electrical stimulation. Nevertheless the muscle action potential of the extensor tibiae remains unchanged for one hour under these circumstances. Also, the transmission in ganglia, and the activity of sense organs are unaffected. It is obvious that the functions of nerves are undisturbed. The oxygen consumption of insects and isolated legs can reach to ten times the normal. ATP (10^{-3}) increases the enhanced oxygen consumption and also emphasizes the paralysis. In the frog, paralysis is followed by irreversible rigor. Discussing the observations, the authors conclude that the poison acts on biochemical processes in the muscle fibre (phosphagen—ATP—actomyosin cycle).

If this conclusion is related to the supposed presence of two systems, there is good reason to seek (e.g. microscopically visible) structure differences in muscle fibres which can be correlated to the difference in functions. Linking up with our data this was provisionally investigated by SMIT (1958). According to SMIT it seems that in the interior of the differently functioning muscle fibres of *Periplaneta* the myofibrils are arranged dissimilarly. Moreover, the fibres of the slow muscles appear to be richer in lipoids and water and to be of greater reducing power than the fibres of the fast muscles. According to an unpublished report, sarcomeres of different length were found in the fibres mentioned which is in agreement with the data concerning some crustaceans provided by JASPER and PEZARD (l.c.). For the present, there is no reliable confirmation on these data and further investigation is desirable (EDWARDS et al., 1954). Also, a comparison with similar results from an examination of other animal groups (KRÜGER, l.c.; GEORGE, NAIK and SCARIA, 1958) may yield interesting details. We shall now sum up all structural parts in which the separate functions may be thought to be localized: (I) whole muscles or parts; (II) entire muscle fibres; (III) fibrils or other intracellular structures. We shall then marshal the arguments in favour of some localization.

The possibility (I) that different muscle functions could be localized in a fundamentally important way in muscles or in parts which can already be recognized with the usual anatomical means, is probably of insignificant importance to the problem as such a distinction could presumably be reduced to differences mentioned in (II) or (III).

The correlation of difference in functions with difference in arrangements of fibrils, in biochemical composition and possibly in sarcomere lengths, as found by SMIT (l.c.), points to a localisation of functions in the individual muscle fibres (II).

The occurrence of two different muscle action potentials in the membranes of different muscle fibres (WILSON, l.c.) has also been mentioned as an argument for localisation in different muscle fibres (II).

From the action of ryanodine it may be concluded that there are two separate contractile systems but their order still enables the possibilities (II) and (III).

It is clear that a somewhat general conclusion from these scanty data is still impossible, but several investigations on the cockroach point to the direction of a localisation of functions in separate muscle fibres. This conclusion would then be in contrast to the earlier view that arthropod muscle fibres should in general have a polyneuronal motor innervation.

In the meantime, it appears from WIERSMA's review (1957) that in other invertebrates too, the crustaceans included, numerous variations on the theme of polyneuronal innervation have been found, and that in spite of the far more detailed knowledge of the latter animal group, there is anything but a clear picture. In any event, it is certain that in the arthropods, at least, some sort of muscle fibres exists which can perform two types of contractions.

To explain this, HOYLE (1955) discussing on his work with the insect *Locusta* stated that the type of nerve ending is responsible for the amount of contraction of only one material in the muscle fibre, and that the amount of contraction is related to the rate of contraction.

The understanding of this statement is dependant on the definition of "amount of contraction". The physical dimension of amount of contraction might be amount of work; in an isotonic contraction it is the product of force and shortening. As long as we have no information on the difference between the relations of force and shortening at fast and slow contraction of the same material, it is unsatisfactory to explain characteristic velocities of contractions in terms of amount of contraction.

For this reason the emphasis in HOYLE's theory becomes directed in my opinion towards the diversity caused by the type of innervation. As a connection between sarcomere length (2—4 μ) and the interval of nerve endings (40—80μ) is apparently absent, the topographical aspect of the distribution of nerve endings along the muscle fibre seems to be unimportant as explanatory factor. It is not yet clear what the function of nerve endings may be in the control of the velocity of contraction. Perhaps different chemical transmittors affect the contraction rate.

With regard to structure investigation, considerable progress has fairly recently been made by HUXLEY and NIEDERGERKE (1954) and by HUXLEY and HANSON (1954). These authors made an exhaustive investigation into muscle fibres, under different circumstances, with the aid of interference, phase-contrast and electron microscopy. A fairly simple model of muscle contraction was developed, the unit of contraction being a sarcomere. It extends from a Z-line or KRAUSE's membrane, half-way through an I-band, to the following Z-line. Submicroscopic rods of myosin extend from one end of the A-band, through the H-zone, to the other end of the A-band and their length is unaltered by stretch, passive shortening or by contraction. From the Z-line, actin filaments extend through the I-band into the adjacent A-band leaving a blank H-zone. Thus, rodlets of myosin and actin filaments lie partly side by side in the A-band. The width of the I-band and in part of the H-zone was nearly completely dependent on the changes in length of the fibre during passive stretch and isotonic contraction, but it was not influenced during isometric contraction. This makes very attractive the hypothesis that during contraction the actin filaments are drawn into the A-bands between the rodlets of myosin.

"If a relative force between actin and myosin is generated at each of a series of points in the region of overlap in each sarcomere then the tension per filament should be proportional to the number of these points, and therefore to the width of this zone of overlap. This is in fair agreement with observation. In arthropod striated muscle, there is a wide range of sarcomere lengths *in situ*, and narrowness of striation appears to be correlated with high speed of contraction. This would be expected if the relative velocity between actin filaments and myosin rods in any one zone of overlap were the same for muscles of different sarcomere lengths, since the number of sarcomere shortening in series per unit length is inversely proportional to sarcomere length. On this basis it would also be expected that the muscle with longer sarcomeres would be capable of producing a greater tension, but we are not aware of any experimental results on this point."

In this theory the difference in contraction speed is myogen in character and is thus in contrast with the neurogene theory of HOYLE. Perhaps both are correct or one may conceive a compromise between the two. The latter, for instance, would require the presence of sarcomeres of different length in one polyneuronally innervated muscle fibre. Sarcomeres of different length should then have to be innervated by different nerve fibres, but there is still no indication in that direction.

This discussion has finally led to the conclusion that the solution of the problems, may be expected from a combined study of anatomy, histology, myography of mechanical and electrical properties and toxicology, and always on one subject, a condition no investigation has thus far fulfilled.

5. Survey of the principal results of physiological experiments [5])

The results have still a mainly qualitative character.

[5]) Preliminary results on the electrical activity recorded with micro-electrodes of single fibres of some of these muscles will be published by BECHT, HOYLE and USHERWOOD in due time.

Table 4.

Muscle	Innervated by	Myogram	Type
136 + 137	1 axon	1 twitch-height; fast relaxation	fast, all or none unit
135 c	1 axon	1 twitch height; fast relaxation	fast, all or none unit
135 a	3 axons	3 twitch-heights; fast relaxation	three combined fast units
135 b	at least 4 axons	very small fast twitches; some (?) heights at slow contraction; inhibition; slow relaxation	mixed mainly slow muscle
135 de	4 axons	"cock's comb"-myogram with 1 twitch-height; some (?) heights at slow contraction; slow relaxation; *135 e* inhibition	muscle with slow and fast parts
138 + 139 c	7 axons, distributed over groups of some axons per muscle part	"cock's comb"-myogram with 2 fairly small twitch-heights; slow contraction; slow relaxation	mixed muscle
139 ab	7 axons, distributed over numerous groups with maximal 3 axons per muscle part	4 fairly large twitch-heights	mixed, mainly fast muscle
140		small fast twitches; slow contraction	mixed, mainly slow muscle

There is often a striking agreement in the number of distinguishable physiological functions and the number of fibres found in earlier anatomical investigation (Table 4).

IV. THE MUSCLE TOXICOLOGY

A. THE LITERATURE ON THE TOXICOLOGY OF γ-HCH AND DDT

That part of the literature which is concerned with the neuromuscular system is discussed here.

The external symptoms of DDT-poisoning of insects consist of hyper-motor activity. In the cockroach, YEAGER and MUNSON (1945) observed changes in this activity which occurred after cutting certain nervous connexions; the operations were combined with administering nicotine that blocks the activity of the ganglia. On the ground of their observations these authors located the action of DDT in the peripheral nerves or in the neuromuscular junctions.

TOBIAS and KOLLROS (1946) applied essentially the same method to the cockroach. They localized dependently on the DDT concentration its action in the sensory or in the motor peripheral nerve structures.

WELSH and GORDON (1947) and GORDON and WELSH (1948) extensively examined the action of DDT and a number of DDT-analogues: for the most part they used the legs of Decapode crustaceans but they also made experiments with legs of cockroaches. Symptoms of poisoning are "trains" of impulses induced in the nerve, causing the muscle pertaining to it to contract. Such a "train" consists, as may be assumed to be known, of a fairly high frequency series of action potentials with nearly constant amplitude. On the basis of their experiments on the effect of DDT on the equilibrium of Ca, Mg and K ions, the authors are of opinion that the action of the poison is to be found in the surface of the motor axon. In this connection it is interesting to note that YAMASAKI and ISHII (1957) advocated the hypothesis that the first and most important site of the DDT action is the neuron-soma. However, it is beyond the scope of our investigation to deal further with this problem.

ROEDER and WEIANT (1948) injected cockroaches with a suspension of more than 500 p.p.m. DDT. In the course of the poisoning the efferent nerve of the flexor tibiae was stimulated with single electrical impulses; trains of muscle action potentials could be recorded. Thus, it has been made plausible that

DDT is active in the motor system, in particular its nervous part, but according to the last mentioned authors this is not the only and not the most important site which should be looked for especially in the campaniform sensilla.

Much of the literature on the possible action of DDT has been discussed extensively by DRESDEN (1949). In his own experiments on the nerve muscle preparations of the frog and the cockroach he found no toxic effect of DDT, judging from the values of stimulus threshold and from the contractions caused by electrical stimulation. Depending on the dosage used, spontaneous contractions were observed in a limited number of amputated legs of the cockroach, as described by YEAGER and MUNSON (l.c.) and ROEDER (1946).

FRITSCH (1952) and FRITSCH and KRUPP (1952) compared the influence of γ-HCH and DDT on nerve muscle preparations of *Dytiscus marginalis*. The preparations consisted of strips of the abdominal body wall containing muscles and nerves but no central ganglia. A negative temperature coefficient was found. Supported by this fact FRITSCH in the first paper, still assumed the possibility of a DDT action on the muscle itself, as a negative temperature coefficient seems to appear in a number of other muscle poisons.

Also, FRITSCH considered peripheral ganglia in the muscle system as described by DOMENJOZ (1944) as a possible site of action.

Since FRITSCH and KRUPP demonstrated the same temperature effect for the isolated nerve cord, the first assumption loses much of its value. The second assumption was rejected by them on the ground of inactivity of nicotine.

In other experiments they started from separated abdomina. The nerve cord and its ganglia were freely exposed in a small watchglass spatially separated from the rest of the preparation. The nerve cord was then poisoned with suspensions of γ-HCH and DDT in concentrations of 10^{-6}–10^{-4}. In both cases frequent contractions and increase of tonus occurred, which were recorded kymographically. After extirpation of the ganglia all motor activity disappeared, while the muscle tonus decreased. The preparation was then used for investigating the action on the periphery. The poison was injected into the cavity of the abdomen, in which case γ-HCH (up to 5.10^{-5}) showed no action, whereas DDT (2.10^{-6}) evoked very quick, rhythmical contractions.

ERDMANN (1952) examined the action of curare on the same abdominal preparation of *Dytiscus*. Effects could be observed on the chronaxies of ganglion, nerve and muscle and on the contractions after indirect and direct stimulation of the muscles. Owing to the lack of adequate means to fix or to determine the site of action in the subject, the results must be considered unsatisfactory.

This last group of authors did not take into account the considerable disadvantage that hardly anything is known of the normal physiological properties of the preparations used. Because of the position of those muscles in the body it may be expected that a differentiation in functions, as in locomotory muscles would not exist. The object mentioned would therefore fall short when compared with one that does enable this differentiation.

It is interesting to notice how the anatomical and physiological data of HOYLE (1955 a, b) soon afterwards became the starting point for the toxicological investigation below.

P. ANNE HARLOW (1958) extensively described the action of a number of drugs on the neuromuscular system of *Locusta*. Two main types of preparation were made, both using the hind leg. The first was a nerve muscle preparation in which the motor nerves of an isolated leg were stimulated electrically and the kick recorded mechanically on a smoked drum in the usual way. (The second one, although it was interesting but not of importance to us, made use of the reflex via the metathoracic ganglion by which the leg was retracted after stimulation of the tarsus by heat or touch). The drugs investigated were some cholinesters and anti-cholinesterases including organophosphorus insecticides; atropine; nicotine; tubocurarine and decamethonium; adrenalin; DDT and γ-HCH. The chemicals were formulated in a solution or suspension in saline buffered to pH 7 and the fluid perfused through the isolated femur at a rate of generally 0.25–0.5 ml per minute. Of all the substances tested only DDT affected the stimulated nerve muscle preparation at concentrations lower than 10^{-3} M (= 1000 p.p.m.). Gamma-HCH and tubocurarine in the same concentration had no effect. Nicotine and eserine blocked the nerve muscle preparation reversibly only at rather high concentration (10^{-2} M). Acetylcholine (10^{-5}–10^{-1} M) had an uncommon action; sometimes the muscle gave a large twitch or went into a brief tetanus which relaxed gradually, while the response to electrical stimulation continued unaltered. The response to acetylcholine was not affected by eserine or atropine; the anticholinesterases produced no effect. It was concluded that there is no evidence for a cholinergic mechanism at the neuromuscular function.

BETTINI et al. (1958) considered it necessary to

find a simple and rapid method for inducing contractions on an isolated insect muscle in contact with different substances (halogenated acetic acid) in an aerated bath. The electrical stimulation of the muscle *135ac* of *Periplaneta* was "direct". According to the authors none of the methods of PRINGLE (l.c.), FRITSCH and KRUPP (l.c.), DRESDEN (1956) or HARLOW (l.c.) possessed the advantage of a rapid substitution of the solution in which the muscle was immersed, as is normally applied with vertebrate muscle preparations. On the other hand, they introduced the earlier mentioned disadvantage that in a muscle of complex structure such as the one used the precise effect of stimulation and poisoning can hardly be checked.

Another aspect that may generally play a part in toxicological experiments is, according to MERRILL, SAVIT and TOBIAS (1946) "the possible exhaustion of metabolic substrates due to the great hypermotor activity following poisoning". In cockroaches poisoned by DDT they found "a marked depletion (about 90%) of total body glycogen and glucose. There was also evidence of increased pyruvate and fat utilization".

Other investigators made a number of parallel experiments concerning other physiologically important substances, but these yielded less satisfactory results.

Since the authors mentioned found that "the changes in carbohydrate content can be prevented by prolonged anaesthesia with CO_2 or cyclopropane", they concluded that these changes "are apparently a function of the motor hyperactivitiy and not an effect of the toxic agent *per se*". Naturally the knowledge of this relation is of importance in our examination of muscles.

Finally WINTERINGHAM and BARNES (1955) "reviewing on a comparative basis the physiological and relevant biochemical action of some selected halogen containing insecticides on both mammals and insects" conclude "that the acute symptomatology of DDT poisoning is associated with disturbances in the peripheral rather than the central nerve system, while in the case of γ-HCH the reverse may be true".

Concluding from the literature on the toxicology of the neuromuscular systems mentioned so far, we see that nearly all of these studies are still at a stage of a description and localization of the poison action and then mainly with that of DDT and only for a small part that of HCH. I think we may underline in this connection the last authors' opinion that "there is a need for considerably more work in this potentially important field of drug action."

B. TOXICOLOGICAL EXPERIMENTS ON MUSCLES

1. General data and symptoms

For our toxicological work we chose the fast motor unit *136 + 137* and the slow muscle *135b* from the nerve muscle preparations described in the section on physiology. These represent two available principal types of physiological behaviour, of which it may be expected that they would be suitable for investigation into possible toxicological effects. A limited number of experiments were performed with the flexor group *139ab + 140*.

The extent of accessibility and its easy preparation are, as a matter of course, considerations of a practical nature of importance in deciding whether the preparations chosen are also suitable for routine work. A preparation of the fast motor unit, as well as one of the slow muscle can, with some training, be made within 10 to 15 minutes and may survive for many hours.

The externally visible symptoms which in *Schistocerca* are caused by γ-HCH, were described and arranged in tabular form by PASQUIER (1947) (cited by BROWN, 1951, pg. 319). In a number of points there is resemblance with the poisoning symptoms in *Periplaneta*; his arrangement, however, appeared to be unworkable. According to this example a new list of symptoms in *Periplaneta* was made (Table 5). A diagram has also been added (Fig. 22).

When estimating the poisoning symptoms of DDT with *Periplaneta*, use can be made of the relevant description by DRESDEN (1949).

All conclusions following below were derived from toxicological experiments carried out with different dosages of poison.

2. Results of experiments with γ-HCH

From the nature of the experimental method it easily follows what symptoms can be distinguished. The behaviour of the fast nerve muscle preparation *136 + 137* when poisoned with γ-HCH was estimated by the following features: (a) excitability, (b) the shape of the myogram, in particular the height and regularity of the twitches.

a. *Excitability of 136 + 137*

From the start certain scattered values were observed of electrical stimulus threshold; these were

Table 5.

The succession of symptoms shown by cockroach, *Periplaneta americana*, when poisoned by γ-HCH:

Phase 1. Slight restlessness mainly developed in antennae and appendages of the head.

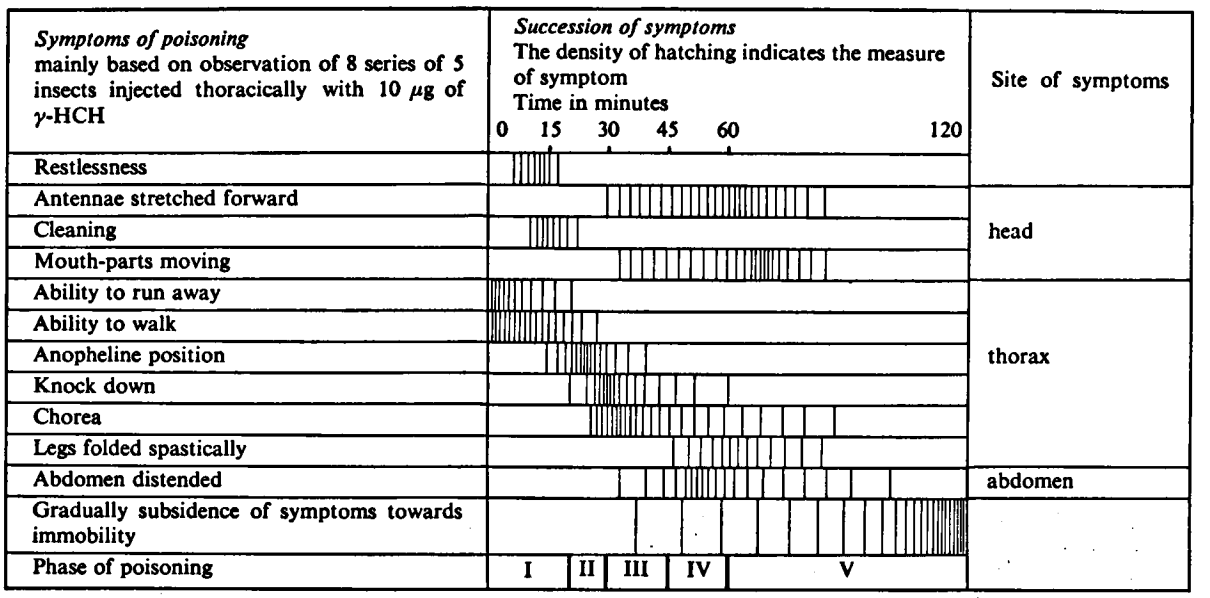
Phase 2. The body is raised, often to a position characteristic of anophelines; this is apparently due to spasms in the extensors of the legs. The insect tries to clean the legs with its mouth. The ability to run or to walk decreases; sometimes the wings make flight movements. The co-ordination of behaviour becomes disturbed, for instance, the forelegs try to walk, the hindlegs cling to the foothold.

Phase 3. The insect falls on its side or back. It is unable to walk ("knock-down"). Violent unco-ordinated spasms in the mostly extended legs appear and disappear at random (chorea).

Phase 4. The abdomen is distended; regurgitating probably caused by internal tensions, sometimes haemolymphe is pressed out. Legs folded spastically, mouth-parts move regularly, antennae often stretched forward.

Phase 5. Gradually subsidence of the symptoms towards weak tremors of the legs and other appendages, finally resulting in complete immobility.

Fig. 22



attributed to the different places of the electrodes with regard to the excitable tissue. In order to obtain some insight into this scattering, thresholds were measured in greatly different situations by indirect stimulation and in positions of direct stimulation (note: in fact indirect too; see ROEDER and WEIANT, 1950). The experiments were carried out with preparations which, as has been stated, could be made fairly uniformly.

The position of the pair of electrodes was varied from the visible part of the nerve, the exposed surface of the muscle, to the inner part of the muscle; the mutual distance between the electrodes was also varied. Duration of impulse and frequency of the stimuli were kept constant. Considering the apparently irreversible nature of the poisoning, the best chance of discovering a toxicological change is after a relatively long period of poisoning. For this reason we investigated four preparations at the advanced stage, five of HCH poisoning and eight controls. A typical experiment yielded the following results (Table 6):

The results of all cases examined can be summarized as follows:

The shape of the myogram appeared to be unaffected by the position of the electrodes.

On the same site in one preparation a certain variation in stimulation threshold could successively be observed.

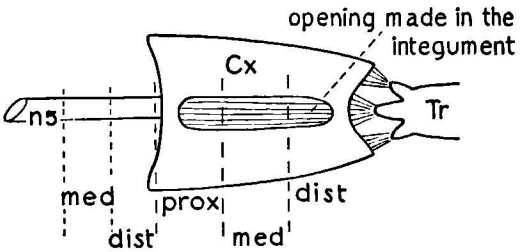


Table 6. *Variations in stimulation thresholds at different positions of electrodes.*

Distance between the electrodes	Position of electrodes on the nerve	Threshold	Position of electrodes on the muscle	Threshold
ca. 2.5 mm	med. superficial	0.82 V	med. superficial	0.7 V
	med. superficial	0.76 V	med. superficial	0.84 V
	dist. superficial	0.75 V	med. penetrated	0.6 V
ca. 0.5 mm	med. superficial	0.95 V	med. superficial	0.54 V
	med. superficial	0.98 V	med. superficial	0.55 V
	dist. superficial	0.86 V	med. penetrated	0.56 V

No definite correlation could be found between corresponding sites in the different preparations and the stimulus threshold found there. These results allow the following conclusions: because of the variation and the lacking correlation the values of stimulus threshold cannot be used in charaterizing our experiments.

Although the position of the electrodes is evidently not a factor that can have substantial influence on the experimental results we aimed at uniformity in making our preparations.

b. *The form of the myogram of 136 + 137*

a. With normal preparations *small differences* were always observed in the heights of the twitches; with poisoned preparations these differences were generally more pronounced. The irregular distribution of these fluctuations in the myogram, however, led us to assume that they were mainly caused by imperfections in the recording technique.

As a result of these experiments it must be assumed that the height is hardly or not at all influenced by poisoning with γ-HCH. Thus the heights of the

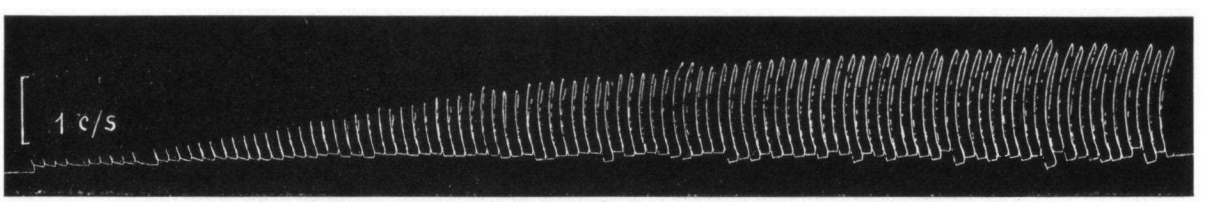


Fig. 23. Record of muscles *136 + 137*; frequency and voltage unchanged; s.w. Increasing height of contractions after fatigue may be caused by enhanced circulation following movement.

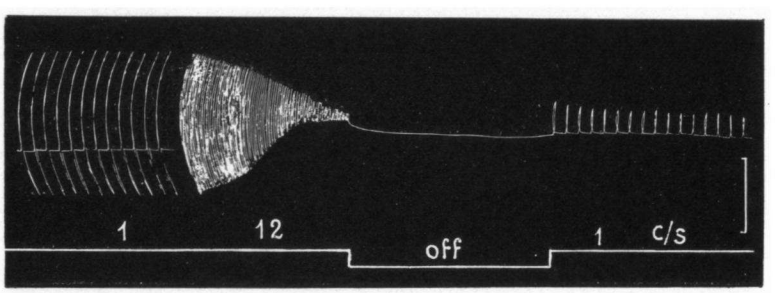


Fig. 24. Record of muscles *136 + 137*, stimulated with frequencies of 1 and 12 c/s to demonstrate fatigue; s.w.

twitches in the myogram are not indicative of this poisoning.

β. A steadily *increasing size* of the twitches when stimulated with a low frequency (e.g. 1 c/s) of constant voltage and impulse duration was fairly often observed at apparently arbitrary moments (Fig. 23). This phenomenon, resembling facilitation, occurred not only with poisoned preparations but also with non-poisoned ones, if the muscle under examination was fatigued. It is evident that this is not a specific characteristic of poisoning either.

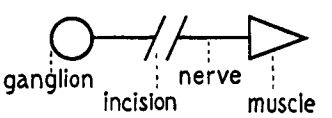
(It should be noted that a phenomenon of the same shape, described chiefly for the vertebrate heart muscle, has been known for more than 70 years by the name "staircase of BOWDITCH").

γ. With a normal preparation stimulated under uniform conditions the time was measured for the contractions to reduce to zero. The reciprocal of the time is a measure for the *fatigue* of the muscle. A stimulation frequency of 12 c/s was chosen to induce the fatigue (Fig. 24); the voltage was adjusted just above the stimulus threshold. A symptom of the

Table 7.

Number of insects	Treatment and type of preparation	Not excitable	Very fatiguable
17	preliminary experiments > 5 hours after injection of 10–15 μg, mainly phase 5	8	–
12	increasing times of poisoning after injection of 5, 10 or 15 μg, successive phases	2	7
8	normal preparations	0	1
9	injected with 10 μg, phase 4–5	1	3
6	injected with 20 μg, phase 5	5 1	– –
5	blank injections	0	0
7	injected with 15 μg, phase 4	0	1

LD 50 = about 5 μg



occurring fatigue is the small height of the next low frequency twitches.

If in a poisoned insect the muscle had remained attached to the ganglion, it appeared that at about stage 3–4 of the poisoning the muscle could hardly or sometimes not at all be stimulated into contraction. After some time, generally at the end of the stage 4 or at stage 5, some recovery usually took place. The succession of these phenomena resembling fatigue and their recovery was rather well correlated with the succession of stages and hence with the externally visible changes in the motility of the insect.

The problem is whether the decreasing function of the neuromuscular preparation is caused by a direct action of the poison on any site in the system or by ordinary fatigue of the muscles due to hypermotor activity. A second point is the question of the origin of the hypermotor activity.

Considering the results of MERRILL et al. (1946) on the relation between hypermotor activity and exhaustion of substrate, in our subject it is not improbable that exhaustion in its turn will be the direct cause of the fatigue of the separate muscles.

In this connection the following remark is apposite: The injection fluid always contained the polysaccharide gum arabic. It is not certain, but it is conceivable, that this substance could play a part in the carbohydrate metabolism, and thus be of influence on the fatigue of the muscle. However, a number of experiments showed that the fatigue appeared to be unaffected by the three to five-fold amount of gum arabic as present in the normal emulsions.

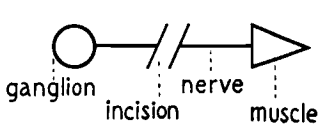
A number of experiments were carried out in which the efferent nerve to the muscle was cut before the poisoning symptoms set in. This was done in the following manner: The injected poison was left to circulate for five to ten minutes. Next, under narcosis of CO_2, the intact insect was pinned up in such a fashion that the efferent nerve (*n* 5) to the muscle could be cut by a simple superficial incision. The

Table 8.

number of insects	treatment and type of preparation	not excitable	„abnormalities"	remaining number	responce* to low frequency stimulation				response to high frequency stimulation			
					minute twitches	tonic contraction	absent	unknown	tonic contraction			unknown
									normal	medium	weak	
21	normal preparations and blank injections	1	1	19	15	2	4	0	10	4	4	1
8	injected with 5–10 µg, phase 1–4	0	0	8	8	1	0	0	1	6	1	0
21	injected with 10–20 µg, mainly phase 4	5	1	15	2	0	13	0	1	5	9	0
18	injected with 20 µg, mainly phase 5	2	1	15	6	6	5	4	9	3	3	0

LD 50 = about 5 µg

* one preparation can give different responses



opening in the integument was made small and usually it closed quickly by coagulation of haemolymphe. It may be assumed that the circulation of haemolymphe is hardly disturbed. The insect is further kept under normal conditions and the development of symptoms is awaited. During the whole course of the poisoning the muscle appeared to respond normally to electrical stimulation, that is as far as was noticeable, the muscle appeared to be unaffected (Table 7). In connection to this observation it is taken for granted that the hypermotor activity is caused by an abnormally increased stream of motor impulses from the ganglion.

Referring to (α), mention should be made again of the small abnormality. It is difficult to describe, since strict criteria are lacking. When the efferent nerve was cut before the poisoning had a detectable effect (and hence fatigue was impossible), a few more irregularities were found in the myogram than is normally the case. Perhaps this represents a slight, direct poison action via the haemolymphe, or there is an indirect action of metabolic products from the whole insect, either normal, as a result of the hypermobility, or abnormal under the influence of the poisoning itself.

Nerve muscle preparation 135b

Special toxicological experiments on the excitability of the slow nerve muscle preparation *135b* were not performed, since the same difficulties could be expected as with the fast muscle.

No particulars could be found in the shape of the myogram of the poisoned muscle *135b*.

The examination of the behaviour of this nerve muscle preparation was concerned only with the fatigue during poisoned and non-poisoned condition. In accordance with what was already known of the normal physiological behaviour of the muscle *135b*, this behaviour was now estimated from the results of low and high frequency stimulation.

The small twitches following on low frequency stimulation appeared to diminish more quickly, evidently because of fatigue, than did the far stronger tonic contraction caused by high frequency stimulation (see Table 8). Here, too, we observed the striking, usually fairly clear recovery to the normal condition of the muscle that occurred in the paralytic stage of poisoning.

The general picture of the changes in the peripheral nerve muscle preparation, when poisoned with γ-HCH, can be described as follows: In a first series of experiments in which the efferent nerve was connected with the ganglion, there appeared to be an increasing "block". Later on, however, it appeared that cutting the efferent nerve before injecting the poison yielded a fairly normal myogram of the muscles. This result is most clearly expressed in the fast motor unit *136 + 137*, but also in the less fatigueable slow muscle *135b*.

3. Results of experiments with DDT

A limited number of experiments with DDT were carried out; dosages of 30–50 μg per insect were injected (LD 50 = about 25 μg). As far as the fatigue is concerned the results obtained differ little from those with γ-HCH experiments. That is, if, as with the γ-HCH experiments, the nerve is cut to prevent the muscle from exhaustion by a presumed abundance of motor impulses, the myograms of different muscles show no abnormalities. This indicates a certain insusceptibility of the neuromuscular system to DDT.

A remarkable difference in the case of poisoning with DDT, however, is the contractions the leg muscles can show after the nerves have been cut, or after the whole leg has been amputated. These contractions may develop without any perceptible influence and they occur more frequently according to the increased dosage of poison. This phenomenon has already been described by several authors (see e.g. DRESDEN, 1949) but its action has never been further localized.

In our experiments we found that the flexors trochanteris show this phenomenon more often than

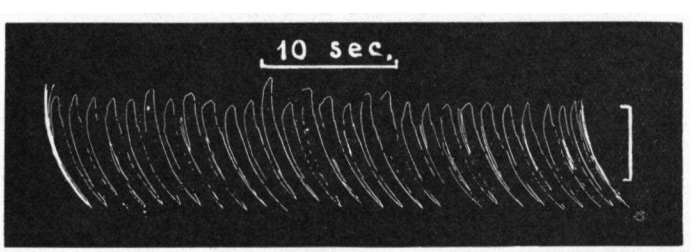


Fig. 25. Record of spontaneous contractions of the flexors trochanteris 1.5 hours after injection of 40 μg DDT; ganglion removed; s.w.

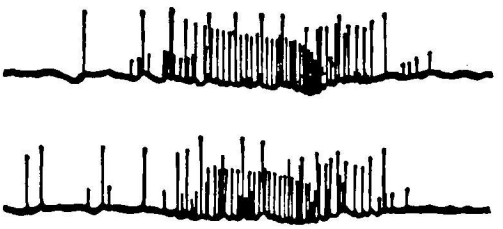


Fig. 26. Electrical activity recorded from the nerve *6B* of the same preparation as in Fig. 25. Volleys appeared synchronously with muscle-contractions. The volleys are nearly identically shaped.

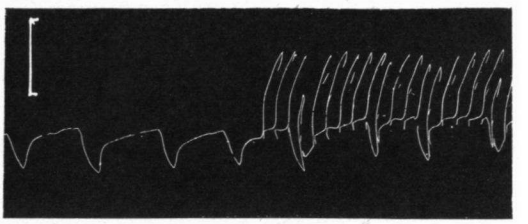


Fig. 27. Myogram of both flexors and extensors three hours after the injection of 40 μg DDT; ganglion removed; s.w. Type of preparation the same as used for Fig. 25. Frequency of the spontaneous flexor contractions is much lower; nerve 5 was stimulated by 1 c/s making the antagonistic muscles *136* + *137* contract.

the extensors. Moreover, in a number of cases the activity was very regular, a condition that greatly favours a closer investigation.

On the ground of a possible analogy with the known action of DDT on the motor axons of crustaceans (WELSH and GORDON, 1947), attempts were made to localize the phenomenon by recording accompanying action potentials, if any, in the motor fibres.

Nerve *3B* was entirely unsuitable for our purpose, as the often strongly spontaneous activity of the sense organs is also recorded and the latter makes the record too complicated. According to DRESDEN and NIJENHUIS (1958), nerve *6B* contains (only two) sensory fibres originating from some hair sensilla on the meron. These, however, never showed any spontaneous activity. It was even extremely difficult to record a stimulus response of these sense organs. So this condition is far more favourable to the experiment meant.

Fig. 25 is a myogram of a leg showing regular spontaneous responses under the influence of DDT poisoning. The leg was completely severed from the central nervous system, and records were made also of nerve *6B* in which volleys of action potentials appeared synchronously with the spontaneous contractions of the flexors (Fig. 26). Movements forced by contractions of the extensors after electric stimulation of nerve 5 had no effect on (1) the regularity of the spontaneous contractions (Fig. 27). The resulting myogram is well recognizable as the mechanical superposition of the two combined forms of contraction; nothing is seen of another interaction between the forced and the spontaneous movement. (2) The volleys of action potentials also continued to appear entirely independent of the extensor contractions. (3) Mechanical stimulation of the sensilla on the meron remained without result.

Another phenomenon was incidentally revealed. The electrical impulses (1 c/s, 0.1 msec.) in nerve 5 sometimes caused tetani in the extensors *136* + *137* instead of the twitches which would normally arise (Fig. 28). It must be ascribed to trains of action potentials which are evoked in the stimulated motor fibre, apparently by the action of the poison.

4. Discussion

The results of the experiments with forced movements demonstrated that spontaneous muscle fibre contractions, possibly also caused under the influence of the DDT-poisoning, cannot stimulate the sense organs on the meron and thus indirectly evoke action potentials in nerve *6B*.

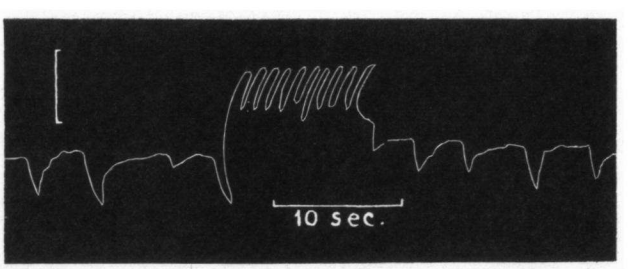


Fig. 28. Myogram 1.5 hours after injection of 40 μg DDT. Object and conditions of experiment the same as used for Fig. 27. Tetanic contractions of the muscles *136* and *137* appear after stimuli of 1 c/s and 0.1 msec duration.

It is in general highly improbable that an electrical activity of the muscle fibres, if any, is conducted via the myoneural junctions again to the nerve.

The only possible sites of origin of action potentials remain the nerve itself or the myoneural junctions which, unstabilized through the action of DDT, would then show spontaneous action potentials. Such a spontaneously occurring action potential transmitted in a distal direction causes a muscle contraction, while the same action potential transmitted in a proximal direction is electrically recorded.

There is a striking uniformity of the volleys in amplitude as well as in frequency. This uniformity is possibly brought about because the excitability in the axons has been abnormally increased and ephaptic transfer becomes possible. Because of the constant anatomical relations a uniform pattern of action potentials might be the result. However, this assumption introduces the possibility that in addition to the motor fibres the more susceptible sensory fibres might also be the source of the first activity. This last assumption seems to be incorrect because of the high concentrations of DDT which are absolutely necessary to bring about the phenomenon in question; the toxicological action of DDT on the sensory systems takes place at much lower concentrations.

The conclusion may therefore be drawn that, in accordance with the indications from earlier observations, the axons or myoneural junctions of the motor system are fairly certainly affected by high concentrations of DDT, whereas the muscle fibres are not.

The results obtained from experiments with γ-HCH can be interpreted as follows:

A specific action of γ-HCH on isolated nerve muscle preparations has not been observed, or if so to a very small extent; fatigue occurs when the muscles are connected with the central nervous system. Although there is no direct supporting evidence, it may be concluded that a more-or-less strong fatigue of muscles in γ-HCH poisoned insects is caused by the apparent abnormal abundance of motor impulses which from the central nervous system can reach the muscle via the nerve. The fatigue demonstrated by our preparations can be related to the similar observation of exhaustion of substrate in the case of hypermotor activity found by MERRILL et al. (l.c.). The fairly total fatigue of nerve muscle preparations at about the third stage of poisoning and the slight recovery during the paralytic stage indicate that the mean frequency of efferent impulses passes a maximum at about the third stage, and decreases thereafter. This means that the recovery itself evidently points to a change in the activity of the central nervous system; the small extent of recovery noticeable in the periphery indicates the slight reversibility of metabolic processes, that is, exhaustion of substrate or possible accumulation of metabolic products.

It should further be realized that it is generally assumed that insect muscles can be stimulated only via nerve tissue (ROEDER and WEIANT, 1950). In other words, to effect a contraction in response to a stimulus, at least an intact axon, a myoneural junction and a muscle fibre are needed. This means that on the grounds of the normal myograms obtained with poisoned and isolated preparations, it may be assumed that neither muscle fibre, myoneural junction, nor motor axon are appreciably influenced.

Everything indicates that the decreasing mobility of the insect at about the third and fourth stages of γ-HCH poisoning is due to fatigue and exhaustion of the muscles. The recovery of nerve muscle preparations in the final stage is an event that is apparently favoured by paralysis of the central nervous system.

V. SUMMARY

1. Extensors and flexors trochanteris of the second thoracic leg of *Periplaneta americana* were investigated physiologically and toxicologically.
2. The movements of the separate muscles were recorded with the aid of a special myographic technique.
3. Nerve muscle preparations of a completely fast and a nearly completely slow function type could be studied in this way. Some muscles represent a form in which both function types occur, probably mixed. When analysed, ryanodine appeared to be a valuable expedient. In some types of nerve muscle preparations inhibition could be demonstrated.
4. Linking up with what is known, it is reasonably certain that the action of high dosages of DDT actually takes place on motor axons or myoneural junctions and not on the muscle fibre itself. Not all of the different nerve muscle preparations seemed to be of the same sensitivity to this poison.
5. γ-HCH appears to have a very slight influence on the function of the different types of isolated nerve muscle preparations. However, because of the intense motor activity the muscles become greatly fatigued.

VI. SAMENVATTING

1. Een van de centrale problemen van de toegepaste entomologie is de betrekking tussen giftige werking en chemische structuur van insecticiden. Een onderdeel van dit probleem is de bestudering van veranderingen in physiologische functies, die optreden onder invloed van deze stoffen.

2. Van gehalogeneerde koolwaterstoffen zoals DDT (Dichloor Diphenyl Trichloorethaan) en γ-HCH (Hexachloor Cyclo Hexaan) wordt gezegd dat ze werken op het spier-, en zenuwstelsel. Het onderwerp van ons onderzoek was de werkingswijze van deze stoffen op insectenspieren.

3. De spieren waren de hoofdzakelijk in de coxa gelegen extensors en flexors trochanteris van de tweede thoracale poot van de amerikaanse kakkerlak, *Periplaneta americana* (L.). Ze werden eerst physiologisch en later toxicologisch onderzocht.

4. Dit onderzoek bleef in beide gevallen beperkt tot de registratie van de contracties van afzonderlijke spieren, die via hun zenuw door electrische blokspanningen werden geprikkeld.

5. Het onderzoek was mogelijk doordat over uitvoerige microscopisch-anatomische gegevens aangaande de spieren en hun innervatie kon worden beschikt.

6. Er komen niet minder dan vijftien afzonderlijke spieren in de slechts enkele tientallen milligrammen wegende coxa voor. De kleinheid van de objecten stelt bijzondere eisen aan de registratie. Voor de registratie werd daarom gebruik gemaakt van een speciaal bij het object passende techniek (zie fig. 1).

7. Spierzenuwpreparaten van een geheel snel (fig. 4, 5 en 6) en een vrijwel geheel langzaam contractietype (fig. 8) werden gevonden. Sommige spieren vertegenwoordigen een vorm waarin beide contractietypen vermoedelijk gemengd naast elkaar voorkomen (fig. 15). Bij de analyse daarvan bleek het alkaloid ryanodine een waardevol hulpmiddel.

8. Perifere remming van spiercontracties bij Arthropoden is een verschijnsel dat voor zover bekend algemeen voorkomt bij Crustaceeen, maar vrijwel niet bij Insecten. Het kon in enkele typen van de hier onderzochte insectenspieren met grote waarschijnlijkheid worden aangetoond (fig. 10 en 14).

9. In de discussie werd vooral aandacht besteed aan het probleem van de innervatie van één spiervezel door een aantal verschillend functionerende zenuwvezels, z.g. polyneuronale innervatie. Dit houdt in dat één spiervezel in staat moet zijn verschillende typen van contractie uit te voeren. Een duidelijk inzicht daarover bestaat nog niet.

Uit de discussie blijkt verder dat de tot voor kort nog gangbare opvatting over het voorkomen van uitsluitend één type van polyneuronale innervatie in een enkele spier van een Arthropood gewijzigd dient te worden. Er kunnen namelijk in één spier spiervezels voorkomen die op verschillende manieren worden geïnnerveerd.

10. Experimenteel werd gevonden dat hoge doses DDT een werking uitoefenen op motorische axonen of op myoneurale verbindingen.

De verschillende typen van spierzenuwpreparaten schijnen niet alle even gevoelig voor dit vergif te zijn.

11. Gamma-HCH scheen oorspronkelijk een verlammende invloed te hebben op de functie van verschillende typen van spierzenuwpreparaten. Doorsnijding van de nerveuze verbindingen tussen het centrale zenuwstelsel en de spier, mits uitgevoerd vóór het vergift zijn werking kan ontplooien, sluit deze invloed van HCH-vergiftiging uit.

Geconcludeerd werd dat de hevige motorische activiteit van met γ-HCH vergiftigde dieren, kennelijk wordt veroorzaakt door een overvloed van motorische impulsen uit het centrale zenuwstelsel. De frequentie van de motorische impulsen bereikt zijn hoogtepunt omstreeks het derde stadium van vergiftiging (zie tabel 7 en 8). De spieren raken daardoor zo uitgeput dat ze verlamd schijnen, maar in een later vergiftigingsstadium treedt weer enig herstel op ten gevolge van de afnemende stroom van motorische impulsen.

12. Een soortgelijk verloop van verschijnselen, zij het in minder uitgesproken vorm, kon zich voordoen bij door DDT vergiftigde dieren.

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