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**A COMPARATIVE STUDY OF METABOLIC EFFECTS
OF THE CORPUS ALLATUM IN TWO ADULT
COLEOPTERA, IN RELATION TO DIAPAUSE**

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1. INTRODUCTION

The endocrine factors in insects are known to play an important rôle in postembryonic development and reproduction. Many investigations almost exclusively deal with the endocrine control of postembryonic development. The literature dealing with humoral functions in the imaginal stage, including those active in reproduction, is much less voluminous and somewhat controversial as well (DE WILDE, 1964).

Since the part played by the incretory activity of the corpora allata of insects was first demonstrated by WIGGLESWORTH (1936), much attention has been paid to the many-sided effects of their secretory product, mostly referred to as the 'juvenile hormone' or 'neotenine' (WIGGLESWORTH, 1940). This hormone induces the immature insect to retain larval characters as long as it is present in the blood in sufficiently high concentrations. At the end of larval life, the corpora allata cease to secrete this hormone and the insect undergoes a metamorphosis. The glands usually enter a new secretory phase needed to deposit proteid yolk during oögenesis (HIGHNAM et al., 1963; HIGHNAM, 1964; SLÁMA, 1964 b). The hormone produced has been termed gonadotropic hormone (VOGT, 1940 a; THOMSEN, 1942; JOLY, 1945).

It is known that in many insect species the larval corpora allata can induce egg maturation when implanted into allatectomized adult females, and that adult corpora allata can inhibit adult differentiation when implanted into allatectomized larvae (PFLUGFELDER, 1940; VOGT, 1943; PFEIFFER, 1945 a; SCHARRER, 1946; BODENSTEIN, 1947; WIGGLESWORTH, 1948; ENGELMANN and LÜSCHER, 1957). These results suggest that the juvenile hormone and the gonadotropic hormone are identical. This has recently been borne out by the fact that substances with juvenile hormone activity like farnesol (WIGGLESWORTH, 1961), farnesyl methyl ether (WIGGLESWORTH, 1963) and cecropia-extract (WILLIAMS, 1956; CHEN et al., 1962) have the juvenile as well as the gonadotropic effects. However, a 'multiple hormone' hypothesis has in some cases gained acceptance (BODENSTEIN, 1954; LÜSCHER and SPRINGHETTI, 1960; LÜSCHER, 1960; SÄGESSER, 1960).

The juvenile hormone is apparently neither species-specific nor even order-specific in its action. This follows from the transplantation experiments of PIEPHO (1950), NOVÁK (1951, a,b) and WIGGLESWORTH (1952, 1954) who showed that corpora allata exerted juvenilizing effects even when exchanged between species as distantly related as *Calliphora erythrocephala* (Diptera) and *Rhodnius prolixus* (Hemiptera). Furthermore, the corpora allata do not seem to be sex-specific since gland implants from male as well as female donors may furnish the hormone necessary for the maturation of eggs (PFEIFFER, 1940; THOMSEN, 1942, VOGT, 1940 b; WIGGLESWORTH, 1936).

1. 1. FUNCTIONS OF THE CORPUS ALLATUM IN ADULT INSECTS

1.1.1. *Effect on ovarian activity*

The control of yolk formation and egg maturation by the corpus allatum was first demonstrated by WIGGLESWORTH (1936) in *Rhodnius* and has subsequently been confirmed in a large number of species of different orders: *Melanoplus* (PFEIFFER, 1936, 1939, 1940), *Leucophaea* (SCHARRER, 1946; LÜSCHER and ENGELMANN, 1955; ENGELMANN and LÜSCHER, 1956 a,b; SCHARRER and VON HARNACK, 1958), *Periplaneta* (BODENSTEIN, 1953), *Locusta* (STRICH-HALBWACHS, 1957; JOLY, 1960), *Diptoptera* (ENGELMANN, 1959), *Schistocerca* (HIGHNAM et al., 1963), *Anisolabis* (OZEKI, 1949), *Oncopeltus* (JOHANSSON, 1954, 1958), *Pyrrhocoris* (SLÁMA, 1964 b), *Dytiscus* (JOLY, 1945), *Leptinotarsa* (DE WILDE and DE BOER, 1961), *Calliphora* (THOMSEN, 1940, 1942), *Phormia* (ORR, 1964a), *Drosophila* (VOGT, 1940 a,b, 1943; BODENSTEIN, 1947), *Anopheles* (DETINOVA, 1945), *Aedes* (GILLETT, 1956), *Culex* (CLEMENTS, 1956; LARSEN, 1958). In many of these insects the impaired yolk formation in allatectomized adult females may be restored by implantation of corpora allata.

Some species of Phasmida and Lepidoptera are exceptions to the above-mentioned rule: *Dixippus* (PFLUGFELDER, 1937), *Sipyloidea* (POSSOMPÈS, 1958), *Bombyx* (BOUNHIOL, 1938, FUKUDA, 1944). Moreover, LEA (1963) has shown that allatectomy has a varied effect on egg development within a single genus, *Aedes*, and even within one species. Other complications arise when the extirpation takes place in the juvenile stages. Thus, THOMSEN (1952) found that *Calliphora* females allatectomized in the adult stage were incapable of oögenesis, while POSSOMPÈS (1949, 1956) found that adult *Calliphora* females obtained from allatectomized larvae formed normal eggs. Likewise, FUKUDA (1944) showed that young allatectomized lepidopterous larvae moulted to the pupal and after that to the adult stage (with omission of one or more larval instars) eventually developing fully functional gonads. He assumed, in this case, the importance of the prothoracic gland hormone in the absence of the juvenile hormone for gonad maturation.

1.1.2. *Effect on accessory sex glands and their secretions*

Data in the literature are divergent about the effect of the corpora allata on the accessory sex glands.

1.1.2.1. Females

In females of *Melanoplus*, the secretory activity of the epithelial lining of the oviduct, corresponding to the accessory sex glands of other insects, depends on the presence of the corpora allata (PFEIFFER, 1939). In the female cockroach, accessory glands (collateral glands) are involved in the secretion of the protein-containing material used in the formation of the cockroach oötheca. SCHARRER (1946) found that the secretory activity of these glands was controlled by the corpora allata, since implantation of corpora allata into allatectomized females of *Leucophaea* restored the ability of the accessory sex glands to pro-

duce normal amounts of secretory material. Similar results were obtained with *Periplaneta* by BODENSTEIN and SPRAGUE (1959), again with *Leucophaea* by ENGELMANN (1957), and with both *Blattella germanica* and *Pycnoscelus surinamensis* by ROTH and STAY (1959). The same was found for the female accessory sex glands in *Calliphora* (THOMSEN, 1942).

In contrast to the situation in the former insects, allatectomy has no effect on the female accessory sex glands of *Lucilia* and *Sarcophaga* (DAY, 1943).

1.1.2.2. Males

In 1936 WIGGLESWORTH found that the accessory glands of male *Rhodnius* did not secrete when the corpora allata had been removed. In *Calliphora* (THOMSEN, 1942) the male accessory glands also appear to be to some extent under the hormonal control of the corpus allatum. However, the male accessory glands of *Lucilia*, *Sarcophaga* (DAY, 1943) and *Leucophaea* (SCHARRER, 1946) were not affected by allatectomy.

1.1.3. Effect on mating behaviour

Apparently, in some insects at least mating depends on the presence and activity of the corpora allata. Allatectomy either impairs the ability of the female to perceive the male odour, essential in their courting (as found by ENGELMANN, 1960a with *Leucophaea*), or it inhibits female pheromone secretion (as demonstrated by BARTH, 1962 with *Byrsotria*).

In adult females of *Melanoplus* (PFEIFFER, 1936), *Diploptera* (ENGELMANN, 1960b) and *Galleria* (RÖLLER et al., 1963), however, allatectomy does not affect mating behaviour.

In male insects, the corpora allata mostly do not seem to interfere with their mating behaviour. Nevertheless, LOHER (1960) demonstrated that the corpora allata are essential in the sexual behaviour of the gregarious phase of adult *Schistocerca*. In this case, production of a pheromone by the mature male which stimulates maturation and responsive behaviour in the recipient locusts is apparently under the control of the corpus allatum which, in turn, may be activated by the pheromone *per se*. Similarly STAAL (1961) considered the yellow colour, produced only under crowded conditions, in *Locusta* males to be caused by the influence of a possible pheromone on the corpus allatum.

1.1.4. Effect on metabolic rate

1.1.4.1. Fat metabolism

The presence of the corpora allata tends to reduce the storage of fat in the fat bodies of most insects. In adult females, when yolk is being deposited in the ripening eggs, the corpora allata also facilitate the uptake of neutral fat as triglycerides by the ovary (ORR, 1964b). This takes place in all insects when the reproductive cycle is started after the adult moult. However, in adult insects which show no gonadotrophic cycles, this relationship seems to be absent (WIGGLESWORTH, 1964).

A detailed study of the production and transport of fat in the grasshopper *Melanoplus* has shown that the corpora allata cause a change in the metabolism in the adult females. This change was manifested from an early phase of fatty acids increase during the first 15 days of the adult stage to a later phase afterwards when the fat stores are depleted and some of the fat is passed into the egg yolk.

In allatectomized females with ovaries left intact or removed, the fatty acid content continues to rise at the same rate as that observed during the early phase. In castrated insects, the usual depletion of the fatty acids follows, showing that the ovary is not involved in the above-mentioned mobilization (PFEIFFER, 1945b).

Similar results were obtained by ORR (1964 b) in *Phormia* and this led to the conclusion that the hormone from the corpus allatum acts directly on the fat body and nowhere else; removal of the gland, in any case, leads primarily to lipid deposition in the fat body. Likewise, VOGT (1949) working on *Drosophila* and BODENSTEIN (1953) on *Periplaneta* have shown that allatectomy leads to an increase in fat storage.

1.1.4.2. Protein synthesis

Many workers have stated that the corpus allatum is involved in the induction of protein synthesis in several insects (DAY, 1943 in *Lucilia* and *Sarcophaga*; VOGT, 1949 in *Drosophila*; THOMSEN, 1952 in *Calliphora*; L'HÉLIAS, 1953 in *Dixippus*; BODENSTEIN, 1953; WANG and DIXON, 1960 in *Periplaneta*). WIGGLESWORTH (1964), however, suggested that this effect may well be the result of a feed-back from the ovary upon the neurosecretory system, since HIGHNAM et al. (1963) assert that protein synthesis in *Schistocerca* is controlled by the neurosecretory system during oöcyte development, whereas the corpora allata secrete a gonadotropic hormone facilitating protein uptake by the growing oöcyte. Similarly, the experiments of THOMSEN and MØLLER (1959, 1963) on *Calliphora* favour the idea that the cerebral neurosecretory cells are involved in protein synthesis. The possibility remains, however, that the corpora allata affect protein synthesis indirectly by the activation of the neurosecretory cells (LEA and THOMSEN, 1962; HILL, 1963). HILL (1963) pointed out that the decrease in oxygen consumption observed in *Schistocerca* after allatectomy may be caused by the resulting cessation of protein synthesis and not to any direct effect of the corpus allatum hormone.

Anyhow, whereas the part of the corpus allatum hormone in protein synthesis seems to be well established, the actual mechanism by which it exerts its effect is still provoking a great deal of dispute.

1.1.4.3. Oxygen consumption

The oxygen consumption of female *Calliphora* drops about 24% after allatectomy (THOMSEN, 1949); an effect not caused by lack of development in the ovaries in the absence of the corpus allatum hormone, since ovariectomized

insects have a normal oxygen consumption (THOMSEN and HAMBURGER, 1955). Similar reduction in oxygen uptake was reported in *Leptinotarsa* (DE WILDE and STEGWEE, 1958), in *Leucophaea* (SÄGESSER, 1960), and in *Locusta* (ROUSSEL, 1963). All these authors favour the view that the corpus allatum hormone has a direct and general stimulating effect on the respiratory metabolism. Moreover, DE WILDE and STEGWEE (1958) and STEGWEE (1960) inferred that this effect is achieved at the sub-cellular level, perhaps between succinate and cytochrome c. CLARKE and BALDWIN (1960) also suggested that the site of action of the corpus allatum hormone on respiratory metabolism lies perhaps within the citric acid cycle.

Moreover, STEGWEE (1960) reported that the oxidative phosphorylation, in sarcosomal preparations obtained from the thoracic muscles of diapausing *Leptinotarsa*, was stimulated by the addition of rather low concentrations of the cecropia-extract.

However, negative results have been reported in *Dixippus* (PFLUGFELDER, 1958; NEUGEBAUER, 1961), egg maturation in the stick insect being independent of the corpora allata. PFLUGFELDER's earlier view (1952) assumed an indirect effect of the corpus allatum hormone on respiratory metabolism by the activation of the ovarian growth. This view was confirmed later for *Pyrrhocoris* by NOVÁK et al. (1959), and SLÁMA (1964 a).

1.1.5. *The rôle in adult diapause*

DE WILDE and DE BOER (1961) were the first to demonstrate the endocrine origin of diapause in adult insects. They showed that in the adult Colorado beetle the complete syndrome of diapause is produced after allatectomy. Reproduction comes to a standstill, the oxygen consumption is lowered to as little as 20% of the normal value (DE WILDE and STEGWEE, 1958) and behaviour changes from feeding to burying. For this reason, these authors described diapause in *Leptinotarsa* as 'pseudo-allatectomy'. However, whereas allatectomy-diapause may be easily reversed by implanting 2-4 active corpora allata, this does not affect normal-(short-day) diapause. They concluded therefore that diapause in this insect is not merely a syndrome of corpus allatum hormone deficiency.

The corpus allatum of the aquatic beetle *Dytiscus* shows a cyclic activity during the reproductive season; its hormone is released about every twelve days. There is a periodical oösortion of the terminal oöcyte when the hormone titre is low, similar as in diapause (JOLY, 1945). DE WILDE (1964) suggested that diapause in this insect is different from that in *Leptinotarsa*. Whereas the corpus allatum is completely inactive during diapause in *Leptinotarsa*, it shows some activity in *Dytiscus*, though at its minimum level which is also observed in the oösortion period in the active insect.

In *Pyrrhocoris*, SLÁMA (1964a) related the imaginal diapause to the endocrine deficiency of the cardiacum-allatum complex and not only to that of the corpus allatum.

1.2. BASIC PROBLEMS OF THE MULTIPLE ACTION OF THE CORPUS ALLATUM

The endocrine control by the corpus allatum on a multitude of processes may either be caused by correlated effects of one and the same hormone or to the effects of several coexisting corpus allatum hormones. Our present knowledge does not allow a decision between these two possibilities. This lack of knowledge is manifested in two conflicting points of view:

- a. The corpus allatum produces a 'gonadotropic hormone' which is similar to that in vertebrates, acting specifically on the ovary and consequently affecting over-all metabolism.
- b. The corpus allatum furnishes a 'metabolic hormone' directly affecting over-all metabolism.

The more specific term 'gonadotropic hormone' has been used by various authors (VOGT, 1940 a; THOMSEN, 1942; JOLY, 1945). At the same time, others (PFLUGFELDER, 1939; DAY, 1943) have suggested that the various known actions of the corpora allata may be explained by the postulation of a hormone which function is the control of certain metabolic processes. This interpretation is much supported by PFEIFFER's (1945 b) furnishing experimental evidence of the existence of a 'metabolic hormone' in *Melanoplus*. Subsequently, the influence of the corpus allatum hormone on the metabolism was quantitatively proved by the work of THOMSEN (1949). She studied the influence of allatectomy on the oxygen consumption in adult *Calliphora* flies and found that allatectomy leads to a decrease in oxygen consumption by about 24 %, whereas the implantation of extra three active corpora allata brings about an increase of only 19 %. Similar results were reported by PFLUGFELDER (1952) on the basis of measurements of the oxygen consumption in *Dixippus*. However, in a detailed discussion on this matter, PFLUGFELDER (1958) concluded that there is no convincing evidence for such a 'metabolic hormone'. Recently, HIGHNAM et al. (1963) and HIGHNAM (1964), working on adult *Schistocerca*, favoured the conception of the corpus allatum hormone being gonadotropic in its action. Likewise, the experiments of NOVÁK et al. (1959) and SLÁMA (1964a, b) on *Pyrhocoris* suggested that the corpus allatum hormone is a 'gonadotropic' one.

Whilst there is no doubt that the corpus allatum hormone has a gonadotropic action, several problems are still to be solved. If the corpus allatum contains more than one hormone and if any hormone from the corpus allatum directly affects metabolism are matters needing detailed research. Anyhow, the control of egg maturation by the corpus allatum hormone(s) clearly is part of a very complex system (see discussion by TELFER, 1965).

1.3. THE ENDOCRINE HIERARCHY CONTROLLING OVARIAN FUNCTIONS

As yet, three organs may be considered to be involved in the endocrine interplay controlling the activity of the female reproductive system. These organs are: the brain, the corpus allatum, and the ovary itself.

In the previous sections the rôle of the corpus allatum in reproduction was reviewed and the target systems of its secretion in this respect. Many studies

have centered on the regulation of the secretory activity of the adult corpus allatum and its integration into the internal and external *milieus* (for details, see DE WILDE, 1961; DOANE, 1962). This integration is mediated through the brain by a bi-partite neuro-hormonal control (SCHARRER, 1958). Moreover, the corpus allatum must be kept informed of the physiological state of the ovaries (SCHARRER, 1958), but whether or not some sort of humoral feed-back mechanism is involved remains open to dispute. However, according to VAN DER KLOOT (1962), there is no evidence for the secretion of an ovarian hormone in any insect.

WIGGLESWORTH (1964), in his comprehensive survey, tried to explain most of the so-called 'metabolic-effects' of the corpus allatum hormone on the basis of a homeostatic regulation. However, due to the findings of PREIFFER (1945b) in *Melanoplus* and of THOMSEN and HAMBURGER (1955) in *Calliphora* that the corpus allatum affects the storage metabolism even in the absence of the ovaries, he concluded that both processes (direct metabolic action and feed-back effects) may be operating simultaneously and that their relative importance may vary in different insects.

1.4. TOPIC OF THE PRESENT STUDY

Because of the controversial data, reviewed in the preceding pages, on the action of the corpus allatum hormone in the control of egg-maturation in different insect species studied so far, it seems difficult to outline a single unifying concept. Allatectomy in the adult female *Leptinotarsa* results in the complete syndrome of diapause (DE WILDE and DE BOER, 1961), whereas in other adult insects, lacking the 'diapause mechanism' its only effect, if any, is on reproduction and some aspects of metabolism.

Our attention therefore, was focused on the problem if this 'diapause mechanism' merely consists of an 'off-switch' of the corpus allatum or if it is also located in the peripheral tissues. In other words: do the tissues of an insect with a 'diapause mechanism' react differently to low titre of the corpus allatum hormone than those in non-diapausing adult insects? Secondly, we were interested to know if the metabolic effects of the corpus allatum hormone are merely caused by its effect on a primary target organ, e.g. the ovary, or if there also is a more general effect on other metabolic centres.

The intention of this study was mainly to compare the effect of allatectomy on respiratory rate and respiratory quotient (R.Q.) to that of castration in two adult coleopterans: the Colorado potato beetle (*Leptinotarsa decemlineata* Say) which has a 'diapause mechanism' and the yellow mealworm (*Tenebrio molitor* L.) in which this mechanism is absent.

2. MATERIALS AND METHODS

2.1. COLORADO POTATO BEETLES

2.1.1. *Rearing under standardized conditions*

All test animals were kept at 25°C on potato foliage. Active and operated beetles received a daily photoperiod of 18 hours with white fluorescent tubes controlled by a switch-clock. Diapausing beetles were obtained from cultures receiving a 10-hours' photoperiod. Experimental insects were reared separately in glass crystallizers of 6 cm diameter.

2.1.2. *Allatectomy*

The corpora allata were removed by the method described by DE WILDE and DE BOER (1961). This method left the corpora cardiaca intact. Sham-operations were carried out in a similar way to allatectomy except that the corpora allata were left untouched.

2.1.3. *Implantation*

The allatectomized beetle was narcotized by exposure to the vapour of diethyl ether for about four minutes. It was subsequently fixed in a wax basin and submerged in Ringer's physiological saline 'Ephrussi and Beadle modification'. Six corpus allatum-cardiacum complexes from active ovipositing females were implanted by very finely ground watchmaker's forceps through a small puncture pierced dorso-laterally in the intersegmental membrane between the metathorax and the first abdominal segment. Sham-operations were done in a similar way to implantations, except that the insect was supplied with a piece of cervical muscle corresponding in size to the implanted glands.

2.1.4. *Castration*

A newly emerged beetle was etherized, fixed with the dorsal side upwards in a wax basin and submerged in physiological saline. Wings and elytra were stretched sidewise. A slit was cut in each side of the abdomen just above the ovary at the first and the second abdominal segments or above the testes at the first and the second abdominal segments. Then, the ovaries or testes were pulled out by very fine forceps and very cautiously detached from both the tracheoles and the Malpighian tubules. The slits were sealed with paraffin wax. In these operations the accessory sex glands of either sex were left intact. Sham-operated insects were obtained by the same treatment except that their gonads were left undisturbed.

2.2. *TENEBRIO MOLITOR*

2.2.1. *Rearing under uniform conditions*

All test insects were kept in separate small dishes covered with moist cloth at 25°C in a dark room and were supplied with rusk.

2.2.2. *Allatectomy*

This operation was carried out exactly as in Colorado beetles except that it was performed on the second day after emergence to avoid the high mortality observed when younger insects were operated. It is interesting to mention that pure allatectomy, i.e. only removing the corpora allata, leaving the corpora cardiaca intact, is much easier in these insects than removing the post-cerebral complexes, quite the reverse of the situation in the Colorado beetle. In order to extirpate the post-cerebral complex in *Tenebrio*, it was imperative to bend the tips of the forceps into a very fine hook.

2.2.3. *Castration*

This was performed in a similar way to that in Colorado beetles; only here the slits were made at the second and the third abdominal segments in females and at the third and the fourth ones in males.

2.3. RESPIRATION MEASUREMENTS

Oxygen consumption and carbon dioxide output of all experimental animals were manometrically determined by the Barcroft differential respirometer (UMBREIT, BURRIS and STAUFFER, 1957). The animals were starved before every determination. The required time of starvation was 24 hrs for *Leptinotarsa* and 1 hr for *Tenebrio*. The insects were immobilized in small cylindrical perforated metal gauze cages closed with a plug of cotton-wool. This treatment was applied to obtain a rate of metabolism approaching the basal level. The difference in volume between the oxygen consumption and the carbon dioxide output during a period of one hour was at first determined without adding KOH-solution. Oxygen consumption of the same insects during one hour was subsequently determined after providing the central-well of both the reaction and compensation vessels of each manometer with a strip of filter paper soaked in 0.2 ml of 20% KOH-solution. During each experiment, the manometers were kept in a thermostatically controlled water-bath at 25°C ± 0.01. A period of 15 minutes was allowed for equilibration after which the stopcocks were closed and the manometer values were recorded at 10 minutes' intervals. Each individual insect was weighed directly after the experiment.

Thus, oxygen consumption read by the direct change in pressure and carbon dioxide output was calculated according to the equation:

$$\text{CO}_2 = \text{O}_2 - (\text{O}_2 - \text{CO}_2)$$

where, (O₂ — CO₂) is the difference between the O₂-consumption and the CO₂-output as obtained by the first part of the experiment. The exact volume of the

gases was expressed in $\mu\text{l.}$ per gram of wet weight per hour taking the flask constants into account.

The above procedure is only allowed if the changes in respiratory rate are negligible during each period of measurement. As shown in the tables I and II, the constancy of the basal metabolic rate sufficiently meets this requirement.

Because of necessity to measure long-term changes in respiration rate, the determinations were made on single insects over periods of 20 days for Colorado

TABLE I. Respiration rate of 7-days'-old adult ♀ *Leptinotarsa decemlineata* of two prolonged experimental periods. There was an $\frac{1}{2}$ hour's interval between the two measuring-periods

Time in minutes Insect no.	Manometer readings in mm									
	1	2	3	4	5	7	8	9	10	
0-10	24	14	15	11	32	17	21	20	18	
10-20	19	10	21	10	28	19	21	18	16	
20-30	19	11	24	8	30	17	19	21	18	
30-40	18	11	23	9	29	18	20	21	16	
40-50	18	11	22	9	28	16	18	22	15	
50-60	20	10	21	9	27	20	19	21	20	
0-10	21	12	23	10	29	20	21	19	18	
10-20	20	12	21	9	31	17	20	22	19	
20-30	18	10	22	10	28	16	21	18	16	
30-40	19	11	22	10	29	18	18	20	16	
40-50	18	10	21	9	30	17	19	20	18	
50-60	19	11	22	11	32	17	19	21	16	

TABLE II. Respiration rate of 4-days'-old adult ♀ *Tenebrio molitor* of two prolonged experimental periods. There was an $\frac{1}{2}$ hour's interval between the two measuring-periods

Time in minutes Insect no.	Manometer readings in mm									
	1	2	3	4	5	7	8	9	10	
0-10	9	7	7	14	12	9	10	14	9	
10-20	8	6	8	12	11	8	9	13	8	
20-30	9	5	7	11	12	9	8	14	9	
30-40	10	6	8	12	11	8	9	15	7	
40-50	9	7	9	10	11	8	8	17	8	
50-60	10	6	10	11	11	8	9	16	7	
0-10	8	8	7	11	10	10	9	14	7	
10-20	10	7	7	11	11	9	9	14	8	
20-30	10	6	8	12	12	8	10	15	7	
30-40	9	6	8	12	11	10	9	15	7	
40-50	10	6	7	11	11	8	10	18	7	
50-60	8	6	9	11	12	8	8	16	8	

beetles and at least 16 days for *Tenebrio molitor*. The data of the experimental insects which died during these test periods were not taken into consideration. To avoid possible differences in the metabolic rate between different batches of insects reared at different times, special precautions were taken to assure that the groups of operated and sham-operated insects to be compared were always obtained from the same batch.

Whereas, the experiments with *Tenebrio* were carried out every other day, those with *Leptinotarsa* could only be carried out every four days. Preliminary experiments done every other day with the intact long-day *Leptinotarsa* resulted in a very low respiration rate similar to that of normal-diapausing or allatectomized insects. This may be due to a partial starvation since these insects, as has already been mentioned, were starved for 24 hrs to obtain the basal metabolic rate and they could not resume their feeding activities because of the experiments carried out every other day. This could be inferred from table III.

TABLE III. O_2 -consumption of intact long-day *Leptinotarsa decemlineata*¹

Age in days	Males ²	Females ³
	$\mu l O_2/g/hr$	$\mu l O_2/g/hr$
0	1368.2 \pm 208.4 (S.E.)	1186.1 \pm 214.5 (S.E.)
2	1118.3 \pm 198.1	983.7 \pm 203.2
4	416.2 \pm 103.9	410.4 \pm 31.1
6	527.7 \pm 75.8	449.3 \pm 109.6
8	643.5 \pm 93.3	481.9 \pm 65.6
10	630.0 \pm 124.2	526.1 \pm 27.3
12	634.3 \pm 181.7	559.5 \pm 64.5
14	626.1 \pm 168.9	646.6 \pm 60.5
16	654.1 \pm 262.8	680.1 \pm 55.0
20	740.8 \pm 156.4	761.1 \pm 79.9
24	781.3 \pm 349.1	753.7 \pm 87.8

¹ Investigations were carried out every other day.

² Mean of 5 individuals.

³ Mean of 9 individuals.

2.4. COMPARATIVE STUDIES ON NORMAL-DIAPAUSING AND ALLATECTOMY-DIAPAUSING ADULT FEMALE COLORADO BEETLES

2.4.1. *Experimental insects*

The insects used were normal-(short-day) diapausing as well as allatectomy-diapausing female Colorado beetles. All short-day treated insects usually enter into diapause within 7-14 days from the day of emergence, whereas 20-30% of allatectomized long-day ones go into diapause within 2-3 weeks (DE WILDE and DE BOER, 1961).

In our investigations the allatectomized insects which entered diapause within this period were used only. Because of unknown conditions, some of the insects after the allatectomy laid eggs and/or resumed feeding activities. These

insects were rejected. At the same time it was observed that under short-day treatment, a number of beetles failed to enter diapause. Both phenomena may have been caused by the selection of a strain with decreased incidence of diapause by our breeding procedure in which diapausing beetles were always excluded from reproduction (DE WILDE, personal communication).

All experimental insects of either diapausing group were obtained from the same batch.

2.4.2. *Quantitative determinations of some body constituents (fat, glycogen, trehalose)*

2.4.2.1. Chemicals

Reagent grade D (+)-trehalose hydrate and glycogen (shell-fish) were obtained from 'Sigma' Chemical Co., Missouri, U.S.A.

2.4.2.2. Experimental procedure

The estimations were carried out by the procedure outlined by BADE and WYATT (1961) with some modifications. These consisted of prolonging the heating period for colour development from 15 minutes to 30, the dissolution of glycogen obtained from a single insect into 5 ml of H₂O instead of 50 ml, and the dissolution of trehalose obtained from a single individual into 5 ml of H₂O instead of 0.5 ml.

Each determination was checked for the recovery of glycogen or trehalose. This recovery, ranging between 70–80% of pure materials, was taken into account in representing the final data.

Only 4 experimental insects were available to be used for each age-group in either diapausing state.

2.4.3. *Measurements of the Respiratory Quotients (R.Q.)*

Because of the short supply of experimental insects, according to the restrictions outlined (see section 2.4.1.), these determinations were carried out on two different groups of insects than those used for biochemical estimations (under section 2.4.2.), one group of allatectomy-diapausing insects, the other of normal diapausing insects. However, they were taken from the same batch used for the biochemical investigations. Moreover, the same insects from each group were used successively for the whole test period. The data of the experimental insects laying eggs and/or resuming feeding activities were discarded.

2.5. STUDIES ON FERTILITY OF MALE COLORADO BEETLES

2.5.1. *Observations on allatectomized insects*

Each allatectomized male in this group was kept with a virgin female in a separate dish. As a control, a normal male of the same age was retained in contact with a virgin female. All females under observation were examined every day for the number of eggs laid.

2.5.2. Observations on normal-diapausing insects

Each male was supplied during the illumination period of the short-day treatment with a virgin long-day female. To keep these females in active condition, they were daily transferred to long-day conditions and only returned to the males during the next short-day illumination period.

3. EXPERIMENTAL RESULTS

3.1. FLUCTUATIONS IN RESPIRATORY INTENSITIES AND THEIR INTERPRETATION

It was noticed that the respiratory intensities as a rule fluctuated in the course of time in all groups of both insect species studied. These fluctuations were less in short-day treated beetles and, especially, in diapausing ones.

In trials to investigate the actual cause of these fluctuations, determinations were carried out on animals normally fed from their imaginal emergence up to 21 days later at fixed times twice a day, once in the early morning and again in the late afternoon. Oviposition was recorded for females over these determination periods.

As our daily measurements of respiratory intensities were obtained from readings made in the morning and afternoon, we were interested to learn at what time of day respiration varied most. Comparison of morning and afternoon curves showed no difference (fig. 1).

Secondly, the possibility was investigated if the fluctuations were caused by fluctuations in body weights. This was not confirmed in the tests (fig. 3 and 7).

Thirdly, the hypothesis was tested whether respiration fluctuations followed gonotrophic cycles or rhythms in oviposition. No such correlation could be shown. In males of both species studied and in females without any oviposition (fig. 8) the respiratory fluctuations still manifested themselves.

It was suspected therefore that the insects, in spite of the anticipated immobilization inside the metal gauze tubes, were still struggling, and might be responsible for the fluctuations observed in the respiratory curves. This may be related to the observation that *Leptinotarsa*, as many other beetles, shows the phenomenon of catalepsy, i.e. feigning death whenever it is disturbed. A phenomenon which is most obvious in young beetles (DE WILDE, personal communication) and is perhaps responsible for the lack of respiratory fluctuations early in adult life (see fig. 3).

Observations performed under the binocular microscope confirmed this idea. Both sexes, in *Leptinotarsa* as well as in *Tenebrio* kept in the metal gauze tubes showed different degrees of mobility from resting to struggling. Freshly emerged *Leptinotarsa* usually showed a resting behaviour.

3.2. 'RESTING' AND 'STRUGGLING' RESPIRATION

To study the actual effects on the respiratory rate of different surgical operations, it is imperative to find the most basal metabolic rate.

The analysis of the results obtained, presented in the previous section, reveals that the basal metabolic rate 'the resting respiration, is generally concealed by 'the struggling respiration' which may easily give rise to misleading conclusions. Practically, it is almost impossible to secure a resting condition in the insects

inside the metal gauze tubes without a tendency to struggle. Attempts have been made to find the respiration data of the resting condition from the original data. Therefore, the data of both insects studied, excepting those of the first four days in *Leptinotarsa*, have been classed in the frequency polygon of the respiratory intensities presented in fig. 2. The figure shows that the respiratory intensities in *Leptinotarsa* are dispersed according to a normal frequency distribution throughout the period of observation, whereas in *Tenebrio* the frequency distribution is

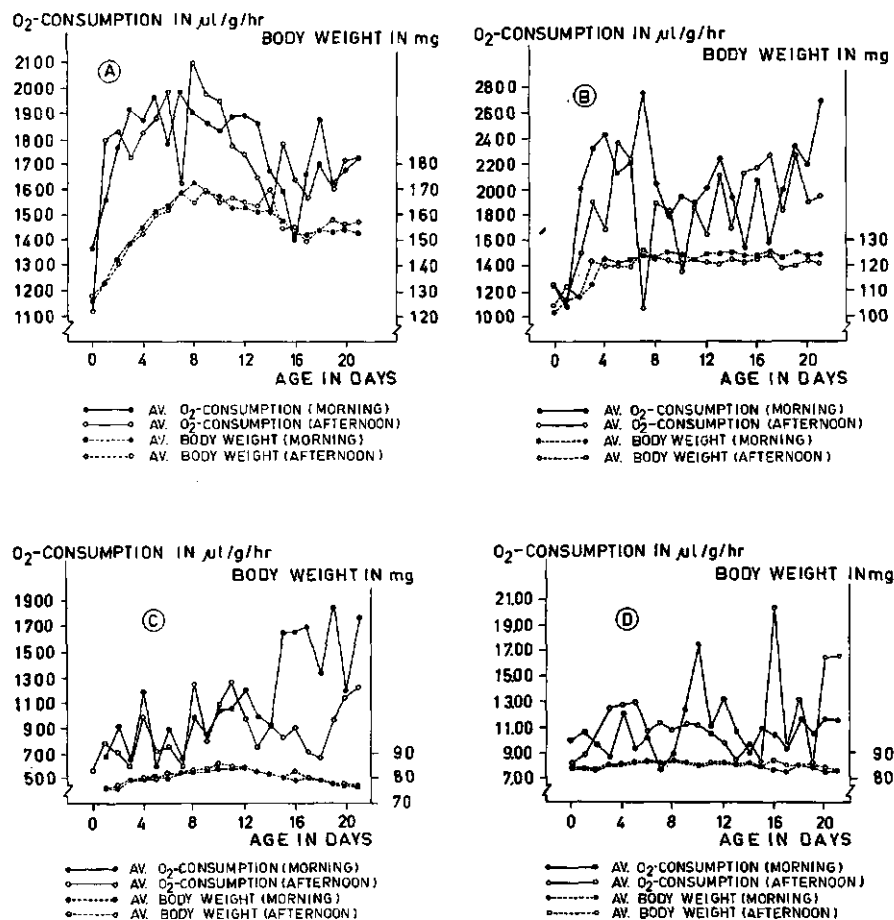


FIG. 1. Oxygen consumption and fresh body weight of intact normally fed beetles in the morning and in the afternoon. A, average curves derived from seven long-day *Leptinotarsa decemlineata* females. B, average curves derived from five long-day *Leptinotarsa decemlineata* males. C, average curves derived from six un-mated *Tenebrio molitor* females. D, average curves derived from ten un-mated *Tenebrio molitor* males.

distinctly skew. The graph also indicates that arbitrary $1000 \mu\text{l O}_2/\text{g/hr}$ and $1100 \mu\text{l O}_2/\text{g/hr}$ may be considered a maximum level of resting respiration of *Tenebrio* females and males respectively, while higher values are considered to belong to the struggling respiration. Considering the normal frequency distribution in the respiratory intensities of *Leptinotarsa*, however, the lowest values of oxygen consumption over the whole test period, apart from those of the first four days and irrespective of the insects' age, are assumed to approximate those of the resting respiration, whereas the highest values approximate the rates of the struggling respiration.

3.3. ARE THE RESPIRATORY QUOTIENTS INFLUENCED BY THE STRUGGLING RESPIRATION?

To test this possibility, the mean respiratory quotients obtained from the original respiration data of the intact long-day *Leptinotarsa* (excluding

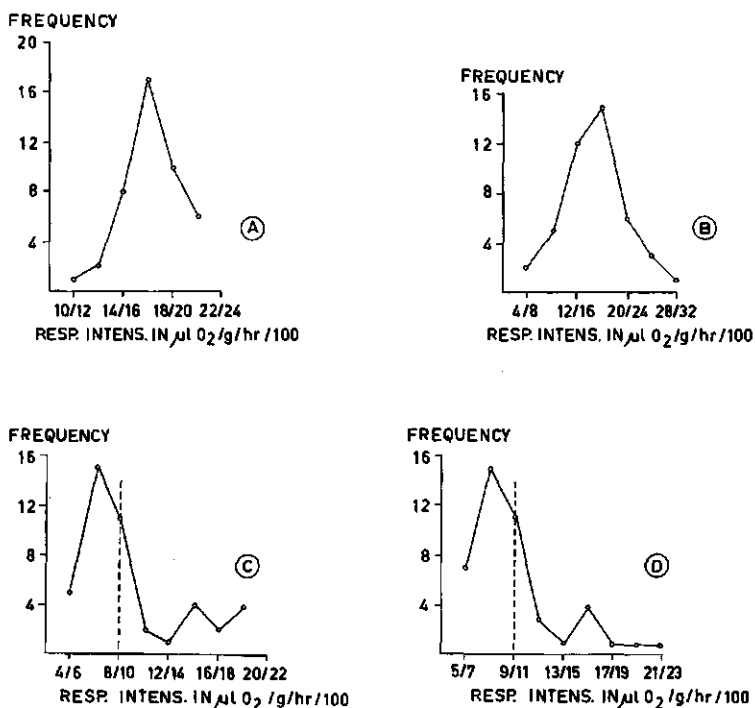


FIG. 2. Frequency polygon of respiratory intensities. A, in intact long-day normally fed *Leptinotarsa decemlineata* females. B, in intact long-day normally fed *Leptinotarsa decemlineata* males. C, in intact normally fed, un-mated, ovipositing *Tenebrio molitor* females. D, in intact normally fed, un-mated *Tenebrio molitor* males.

the data of the first four days) as well as those of *Tenebrio* were compared to the mean respiratory quotients of the resting respiration. The *t*-test was applied since it was recognised that the respiratory quotients of all tested groups followed the normal frequency distribution. This comparison, as obvious from table IV, has led to the conclusion that there is no significant difference between the two groups of values. This means that the values of the respiratory quotients in the original data are not influenced by including the respiratory quotients of struggling animals.

TABLE IV. Effect of 'struggling' respiration on the respiratory quotients of the intact insects

	R.Q. \pm S.E. (original data)	R.Q. \pm S.E. (resting data)	Comparison of means
Long-day ♂ <i>Leptinotarsa</i>	0.770 \pm 0.026 (n = 21)	0.817 \pm 0.037 (7 individuals)	Not significant
Long-day ♀ <i>Leptinotarsa</i>	0.716 \pm 0.044 (n = 24)	0.668 \pm 0.065 (6 individuals)	Not significant
♂ <i>Tenebrio</i>	0.840 \pm 0.033 (n = 45)	0.847 \pm 0.064 (n = 21)	Not significant
♀ <i>Tenebrio</i>	0.918 \pm 0.037 (n = 45)	0.904 \pm 0.098 (n = 13)	Not significant

3.4. RESPIRATORY ACTIVITY IN *LEPTINOTARSA DECEMLINEATA*

3.4.1. *O₂*-consumption and R.Q. of the intact insects

Fig. 3 shows that the average respiratory curves of the intact long-day insects normally fed, males or females, closely follow the average fresh body weight curves throughout the determination period. However, the respiratory curves fluctuate irregularly around the general trend, whereas the fresh body weights follow a rather smooth curve. Both sexes during the first three days of adult life exhibited a rapid increase in the oxygen consumption as well as in fresh body weights.

In males, this increase in oxygen consumption or body weights stabilized to a rather constant level for the rest of the test period.

In female insects, however, the oxygen consumption after attaining the maximum value of this initial rapid increase remained almost constant until they were 10 days old, after which it gradually decreased. As summarized in table V, the average resting oxygen consumption during this period of decrease is found to be only significantly lower at the 10 %-level than that in the preceding period. Moreover, the lowest values for oxygen consumption were located almost exclusively between 11–21 days of age, whereas the highest values were between 5–10 days. A comparison of the ratios of the struggling with the resting respiration of both periods shows that the ratios are significantly different, indicating

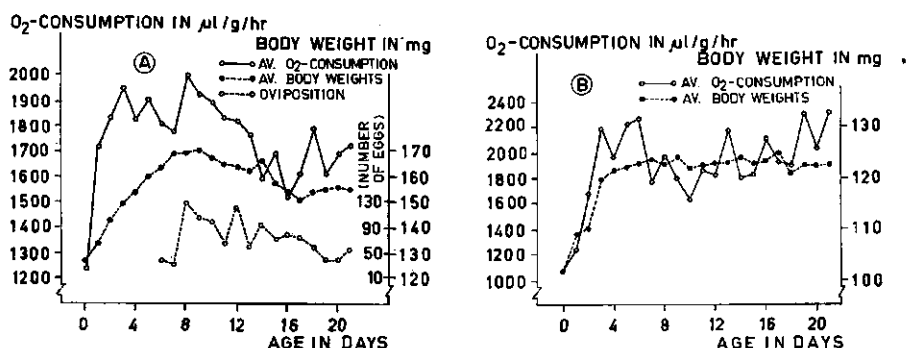


FIG. 3. Oxygen consumption and fresh body weight of intact long-day normally fed *Leptinotarsa decemlineata* (presented as a mean of morning and afternoon readings). A, average curves derived from seven females. The oviposition curve is demonstrated. B, average curves derived from five males.

that the struggling respiration is independent of the resting respiration. However, their levels are almost parallel throughout the period of observation. The body weight curve in females, after attaining the maximum value of the initial rapid increase, remained almost the same until about 10 days of age, after which it decreased gradually. This decrease in body weights was found to be significant as shown in table VI.

Leptinotarsa females seem to exhibit a gonotrophic cycle repeated about every three weeks, the oviposition decreases gradually till the end of this period. During the cycle studied, the oögenesis is apparently a continuous process that, however, slows down gradually during the reproductive cycle. This can be deduced from table VI, which shows that the average decrease in fresh body weights determined during the period from 11–21 days (8.3 mg) is less than the weight of the average deposited eggs during the same period (37.3 mg). The average weight of a single egg is 0.55 mg. After this initial rapid increase, presumably the body weight curve closely follows the oögenesis rate. From tables V and VI it can be deduced that the resting oxygen consumption and oögenesis are almost parallel throughout the gonotrophic cycle.

The average resting oxygen consumption of the intact fed insects of either sex was found not to be significantly different from the corresponding value of the animals starved for 24 hours. This can be seen in table VII. Nevertheless, feeding statistically proved to increase the struggling oxygen consumption in males only.

Table VIII shows that there is no sexual difference in *Leptinotarsa* as far as the resting respiration is concerned, since the oxygen consumption of the one is not significantly different from that of the other.

Table IX clearly shows that the respiratory quotients of the beetles starved for 24 hours, males or females, are mostly of a value significantly below unity.

On the other hand, the average curves of the short-day insects of either sex,

TABLE V. O_2 -consumption over the test period of the intact normally fed long-day ♀ *Leptinotarsa decemlineata*¹

	$\mu l O_2/g/hr$ \pm S.E.	Ratio of resting struggling (5-10 days)	Ratio of resting struggling (11-21 days)	$\mu l O_2/g/hr$ \pm S.E. (11-21 days)	Comparison of means
Resting respiration	1377.9 \pm 72.3	1:1.716	1:1.921	1113.1 \pm 106.4	P < 0.10
Struggling respiration	2364.2 \pm 100.1			2138.1 \pm 113.5	Not significant
Comparison of ratios		P < 0.05			

¹ Average of 7 individuals.

TABLE VI. Change in the fresh body weight of the intact normally fed ♀ *Leptinotarsa decemlineata* over the test period

	5-10 days	10-21 days	Comparison of means
Average fresh body weight in mg (7 individuals)	166.3	158.0	P < 0.01 ¹

¹ The Wilcoxon-test (distribution free) has been applied because the frequency-distribution of the observations need not be assumed to be normal.

TABLE VII. Effect of feeding on the respiratory activity of *Leptinotarsa decemlineata*

	Fed (5 ♂ & 7 ♀)	24 hours'-starved (7 ♂ & 6 ♀)	Comparison of means	
♂	Resting respiration ($\mu l O_2/g/hr \pm S.E.$)	863.7 \pm 166.7	920.0 \pm 189.0	Not significant
	Struggling respiration ($\mu l O_2/g/hr \pm S.E.$)	3151.2 \pm 188.7	2302.0 \pm 152.0	P < 0.01
♀	Resting respiration ($\mu l O_2/g/hr \pm S.E.$)	1113.1 \pm 106.4	1073.0 \pm 135.9	Not significant
	Struggling respiration ($\mu l O_2/g/hr \pm S.E.$)	2364.2 \pm 100.1	2145.5 \pm 182.2	Not significant

TABLE VIII. Sexual difference in O₂-consumption of *Leptinotarsa decemlineata*

		♂ (5 fed & 7 24 hours'-starved)	♀ (7 fed & 6 24 hours'-starved)	Comparison of means
Fed	Resting respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	863.7 \pm 166.7	1113.1 \pm 106.4	Not significant
	Struggling respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	3151.2 \pm 188.7	2364.2 \pm 100.1	P < 0.01
24 hours'- starved	Resting respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	920.0 \pm 189.0	1073.0 \pm 135.9	Not significant
	Struggling respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	2302.0 \pm 152.0	2145.5 \pm 182.2	Not significant

TABLE IX. R.Q. of the intact long-day *Leptinotarsa decemlineata*¹

Age in days	♂ (7 individuals)	♀ (6 individuals)
0	0.838 \pm 0.067 (S.E.)	0.901 \pm 0.023 ² (S.E.)
4	0.834 \pm 0.060 ²	
8		0.753 \pm 0.033 ²
12	0.851 \pm 0.043 ²	0.634 \pm 0.040 ²
16	0.703 \pm 0.056 ²	0.743 \pm 0.067 ²
20		
24	0.757 \pm 0.033 ²	0.732 \pm 0.040 ²

¹ 24 hours'-starved insects² Significantly below unity at P = 0.05

presented in fig. 4, show that these exhibited an increase in the oxygen consumption at the beginning of adult life followed by a continuous decrease probably correlated to the onset of the diapause condition. Meanwhile, they did not show those fluctuations characteristic of the long-day ones. Female beetles of this group did not oviposit. Regarding the fresh body weight curve, it runs parallel to that of the oxygen consumption in males, and after the initial increase it runs at a rather constant level in females (see fig. 4).

Table X indicates that the respiratory quotients of the short-day insects older than 10 days are usually above unity but this difference is significant only in a limited number of observations. This rise in R.Q. is more explicit at the later ages when some insects are supposed to be in a complete diapause.

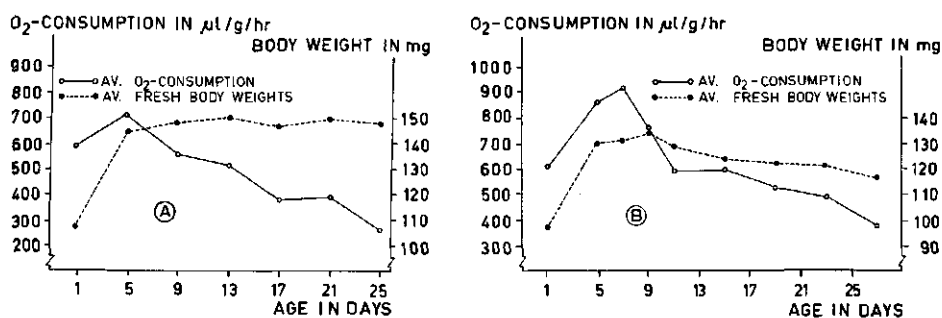


FIG. 4. Oxygen consumption and fresh body weight of short-day *Leptinotarsa decemlineata*. A, average curves derived from eight females. B, average curves derived from seven males.

TABLE X. R.Q. of the intact short-day *Leptinotarsa decemlineata*¹

Age in days	♂ (7 individuals)	♀ (8 individuals)
1		1.012 \pm 0.054 (S.E.)
5		0.837 \pm 0.034 ²
7	0.956 \pm 0.053 (S.E.)	
9	0.993 \pm 0.042	0.985 \pm 0.059
11	1.009 \pm 0.084	
13		1.322 \pm 0.042 ³
17		1.000 \pm 0.050
19	1.248 \pm 0.115	
21		1.167 \pm 0.093
23	1.298 \pm 0.077 ³	
25		1.365 \pm 0.143 ³
27	1.052 \pm 0.053	

¹ 24 hours'-starved insects

² Significantly below unity at $P = 0.05$

³ Significantly above unity at $P = 0.05$

3.4.2. Effect of allatectomy on O_2 -consumption and R.Q.

Only eight males, out of a great number of operated ones, survived the test period. The data obtained from six of these survivors are illustrated in fig. 5. The other two were excluded when it was found that the extirpation of the glands was incomplete as checked microscopically after the experiments.

Like the sham allatectomates, the allatectomates show the fluctuations described earlier for the intact insects (section 3.4.1.). The curve shows that allatectomy resulted in a reduction in the oxygen consumption, whereas it induced an increase in the fresh body weights. Table XI shows that the average ratio of the fresh body weights of the allatectomates of all age groups over the period from 8 to 24 days to the average fresh body weight on the fourth day (taken as

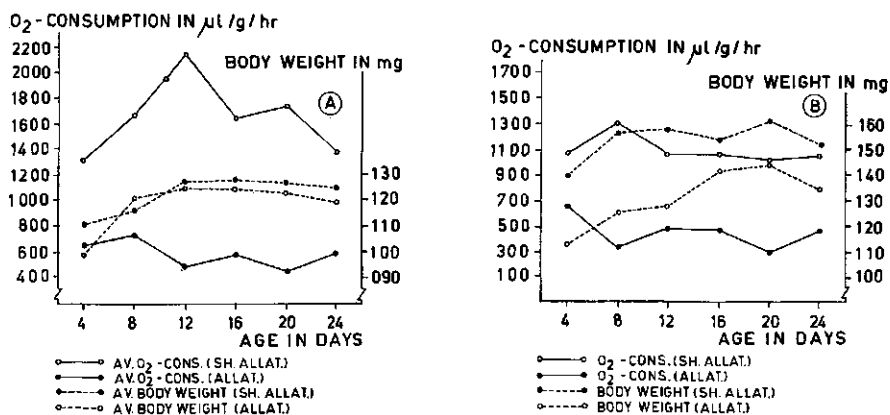


FIG. 5. Oxygen consumption and fresh body weight after allatectomy in *Leptinotarsa decemlineata*. A, average curves derived from six and nine male allatectomates and sham allatectomates respectively. B, average curves derived from six and nine female allatectomates and sham allatectomates respectively.

an initial average weight) is significantly higher than its corresponding average ratio in the sham allatectomates. Because this increase in weight may be the result of the accumulation of inert fat (see section 3.7.5.), the average resting oxygen consumption of the allatectomates could be higher than expressed per gramme of weight of the entire animal. However, this difference was found to be insignificant. From the table it is evident that allatectomy in males led to a significant reduction of 67.3% ($1025.2 \pm 216.7 \mu\text{l O}_2/\text{g/hr}$ and $335.0 \pm 114.3 \mu\text{l O}_2/\text{g/hr}$ for sham allatectomates and allatectomates respectively) in the resting O_2 -consumption. However, the reduction in the struggling respiration is estimated at only 56.2% ($2300.7 \pm 206.0 \mu\text{l O}_2/\text{g/hr}$ and $1007.3 \pm 127.4 \mu\text{l O}_2/\text{g/hr}$ for the sham allatectomates and allatectomates respectively). This latter reduction was found to be significantly lower than the former reduction ($P < 0.001$)¹. Allatectomy, in some cases, also resulted in respiratory quotients significantly above unity, especially at later ages when some allatectomates are supposed to be in a complete diapause. This can be found in table XII.

Only seven females survived the operation and lived for several months after the test period ended. Out of these, only one insect laid a few eggs and was thereupon discarded. Microscopical investigations confirmed the complete excision of the glands from the remaining six insects.

Fig. 5 shows that the female allatectomates reduced their oxygen uptake in comparison with the sham allatectomates, but both groups still exhibited the fluctuations characteristic of the intact insects (see section 3.4.1.). Like the male allatectomates, the fresh body weights of the female allatectomates increased

¹ See footnote on page 38

significantly as compared to those of the sham allatectomates (see table XI). Again, the difference in the resting as well as in the struggling oxygen consumption proved not to be significant. The results indicate that allatectomy in females reduced the resting oxygen consumption by as much as 77% (from $612.0 \pm 74.9 \mu\text{l/g/hr}$ for the sham allatectomates to $140.7 \pm 37.7 \mu\text{l O}_2/\text{g/hr}$ for the allatectomates), whereas the struggling oxygen consumption by only 52.1% ($1565.9 \pm 149.6 \mu\text{l O}_2/\text{g/hr}$ and $749.9 \pm 208.0 \mu\text{l O}_2/\text{g/hr}$ for sham allatectomates and allatectomates respectively). The latter reduction was found to be significantly lower than the former one ($P < 0.001$)¹. Table XII also shows that the respiratory quotients of the allatectomates are mostly of a value significantly above unity whereas those of the sham allatectomates are often below unity. Moreover, allatectomy led to a significant rise in R.Q. at all ages studied.

3.4.3. *Effect of castration on O₂-consumption and R.Q.*

After the microscopical check, only eight males out of ten survivors, were used for the data incorporated in fig. 6. From the figure, it seems that testictomates decrease their oxygen consumption throughout the whole determination period as compared to the sham testictomates. The castrates as well as the sham castrates still showed the fluctuations observed for the intact insects. However, table XIII clearly shows that the average of resting as well as struggling oxygen consumption of the testictomates were not significantly different from those of the sham testictomates. It may also be inferred from this table that castration brought about a significant increase in the average fresh body weights (the average weight on the fourth day was taken as the initial value), although this increase does not significantly affect the oxygen consumption if it is figuratively assumed to be caused by accumulated inert materials. In contrast to allatectomy, castration did not result in a rise of R.Q. to a value above unity, but in some cases it significantly decreases the respiratory quotients as compared to those of the sham castrates. Moreover, the respiratory quotients of either group are often significantly below unity. This can be observed from table XII.

Out of eleven survivors, only seven females were utilized for the data presented. The other four were found to be ovipositing due to incomplete ovariectomy as microscopically verified. Fig. 6 demonstrates that castration in these females may induce a distinct reduction in the oxygen consumption, but only till the age of 12 days after which, in contrast, a remarkable increase could be observed as compared to the operated controls. However, table XIV indicates that castration did not significantly affect the mean oxygen consumption of the whole test period, whereas the analysis of the effect over the period proved that only the increase shown in the figure is significant (as demonstrated in table XV). Castration also induced a significant increase in the fresh body weights (average weight on the fourth day was the initial value). But, this increase in weights has no significant effect on the oxygen consumption if it is

¹ See footnote on page 38

TABLE XI. Effect of allatectomy on the O₂-consumption and the fresh body weight of *Leptinotarsa decemlineata*¹

	Allatectomates (6 ♂ & 6 ♀)	Comparison of means	Sham allatectomates (9 ♂ & 9 ♀)	Comparison of means
♂				
Fresh body weights (mean of weight ratios ± S.E.) ²	1.245 ± 0.012 (n = 5)		1.128 ± 0.020 (n = 5)	P < 0.01
Resting respiration (μl/g/hr ± S.E.)	303.7 ± 104.0	Not significant	1025.2 ± 217.0	P < 0.01
Actual resting respiration (μl/g/hr ± S.E.)	335.0 ± 114.3		1025.2 ± 217.0	P < 0.05
Struggling respiration (μl/g/hr ± S.E.)	913.2 ± 116.0	Not significant	1300.7 ± 206.0	P < 0.001
Actual struggling respiration (μl/g/hr ± S.E.)	1007.3 ± 127.9		2300.7 ± 206.0	P < 0.001
♀				
Fresh body weights (mean of weight ratios ± S.E.) ²	1.197 ± 0.033 (n = 5)		1.125 ± 0.011 (n = 5)	P < 0.10
Resting respiration (μl/g/hr ± S.E.)	132.4 ± 53.5	Not significant	612.0 ± 74.9	P < 0.01
Actual resting respiration (μl/g/hr ± S.E.)	140.7 ± 37.7		612.0 ± 74.9	P < 0.01
Struggling respiration (μl/g/hr ± S.E.)	705.5 ± 195.7	Not significant	1565.9 ± 149.6	P < 0.01
Actual struggling respiration (μl/g/hr ± S.E.)	749.9 ± 208.0		1565.9 ± 149.6	P < 0.01

¹ 24 hours'-starved insects.² For details, see text.

TABLE XII. Effect of surgical interferences on R.Q. of *Lepinotarsa decemlineata*¹

Age in days	Allatectomates (6 ♂ & 6 ♀)	Sham allatectomates (9 ♂ & 9 ♀)	Comparison of means	Castrates (8 ♂ & 7 ♀)	Sham castrates (9 ♂ & 9 ♀)	Comparison of means
4	0.885 ± 0.070(S.E.)	0.760 ± 0.068 ² (S.E.)	Not significant	0.700 ± 0.039 ² (S.E.)	1.012 ± 0.134(S.E.)	Not significant
8	0.936 ± 0.057	0.851 ± 0.071	"	0.731 ± 0.047 ²	0.850 ± 0.064 ²	"
12	1.269 ± 0.095 ³	0.873 ± 0.052 ²	P < 0.01	0.644 ± 0.058 ²	0.848 ± 0.037 ²	P < 0.01
16	1.452 ± 0.059 ³	1.002 ± 0.094	P < 0.01	0.797 ± 0.048 ²	0.815 ± 0.053 ²	Not significant
20	1.138 ± 0.104	0.784 ± 0.048 ²	P < 0.01	0.989 ± 0.034	0.860 ± 0.043 ²	P < 0.05
24	1.707 ± 0.156 ³	1.128 ± 0.063	P < 0.01	0.669 ± 0.048 ²	1.021 ± 0.050	P < 0.01
4	1.492 ± 0.203(S.E.)	0.742 ± 0.041 ² (S.E.)	P < 0.01	1.196 ± 0.144(S.E.)	1.118 ± 0.175(S.E.)	Not significant
8	1.543 ± 0.150 ²	0.837 ± 0.037 ²	P < 0.01	0.888 ± 0.068	0.771 ± 0.043 ²	"
12	1.468 ± 0.177 ³	0.905 ± 0.124	P < 0.05	0.764 ± 0.089 ²	0.772 ± 0.053 ²	"
16	1.200 ± 0.107	0.824 ± 0.062 ²	P < 0.05	0.836 ± 0.034 ²	0.863 ± 0.026 ²	"
20	1.143 ± 0.082	0.758 ± 0.033 ²	P < 0.01	0.903 ± 0.023 ²	0.942 ± 0.046	"
24	1.699 ± 0.208 ³	0.933 ± 0.080	P < 0.01	0.757 ± 0.054 ²	0.979 ± 0.091	"

¹ 24 hours'-starved insects.² Significantly below unity at P = 0.05³ Significantly above unity at P = 0.05

figuratively assumed to be caused by accumulation of inert metabolites (see table XIV). Table XII shows that castration has no significant effect on the respiratory quotients. Meanwhile, in contrast to allatectomy, it does not lead to a rise in R.Q. to a value above unity. Instead, both the ovariectomates and the sham ovariectomates mostly show respiratory quotients of a value significantly below unity. It is noteworthy to mention that the ratios of the struggling to the resting respiration of the ovariectomates in the two distinct periods, determined by the different levels of respiration (see table XV) vary significantly indicating that the struggling respiration is absolutely independent of the resting respiration like in intact insects (section 3.4.1.).

3.5. RESPIRATORY ACTIVITY IN *TENEBRIO MOLITOR*

3.5.1. O_2 -consumption and R.Q. of the intact insects

Either the oxygen consumption curve or the fresh body weight curve of both sexes exhibited a rather constant level from emergence as adult up to the end of the test period. Nevertheless, only the oxygen consumption curves showed the irregular fluctuations shown by the long-day *Leptinotarsa*. These fluctuations are reduced by the correction based on the resting respiration as shown in fig. 7.

Table XVI indicates that the resting oxygen consumption of normally fed *Tenebrio* of either sex is not significantly different from that of animals starved for one hour. However, feeding proved to induce a slight increase in the struggling oxygen consumption in females only.

As in *Leptinotarsa*, there is no sexual difference in *Tenebrio* as far as the resting respiration is concerned. This is demonstrated in table XVII which, however, shows that 1 hour of starvation induced a significant sexual difference.

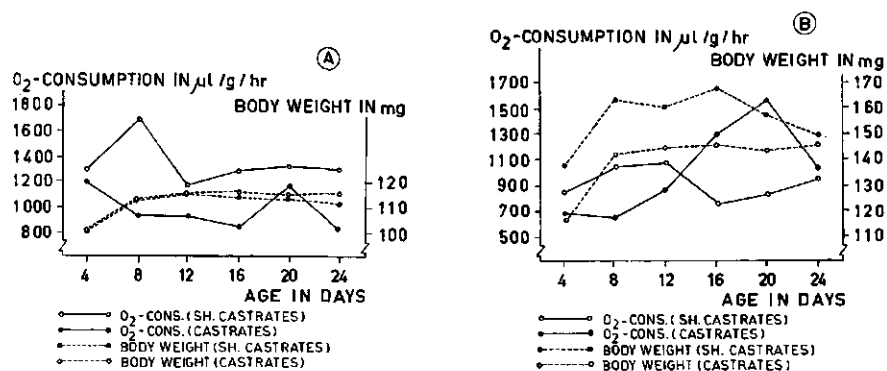


FIG. 6. Oxygen consumption and fresh body weight after castration in *Leptinotarsa decemlineata*. A, average curves derived from eight and nine male testictomates and sham testictomates respectively. B, average curves derived from seven and nine female ovariectomates and sham ovariectomates respectively.

TABLE XIII. Effect of castration on the O_2 -consumption and the fresh body weight of ♂ *Leptinotarsa decemlineata*¹

	Testictomates (8 individuals)	Comparison of means	Sham testictomates (9 individuals)	Comparison of means
Fresh body weights (mean of weight ratios \pm S.E.) ²	1.144 \pm 0.006 (n = 5)		1.120 \pm 0.007 (n = 5)	P < 0.05
Resting respiration (μ l/g/hr \pm S.E.)	539.0 \pm 95.5	Not significant	861.1 \pm 212.9	Not significant
Actual resting respiration (μ l/g/hr \pm S.E.)	551.4 \pm 97.7		861.1 \pm 212.9	Not significant
Struggling respiration (μ l/g/hr \pm S.E.)	1566.5 \pm 133.5	Not significant	1925.4 \pm 324.9	Not significant
Actual struggling respiration (μ l/g/hr \pm S.E.)	1602.5 \pm 136.6		1925.4 \pm 324.9	Not significant

¹ 24 hours*-starved insect² For details, see text.

TABLE XIV. Effect of castration on the O₂-consumption and the fresh body weight of ♀ *Leptinotarsa decemlineata* throughout test period¹

	Ovariectomates (7 individuals)	Comparison of means	Sham ovariectomates (9 individuals)	Comparison of means
Fresh body weights (mean of weight ratios \pm S.E.) ²	1.255 \pm 0.008 (n = 5)		1.160 \pm 0.022 (n = 5)	P < 0.01
Resting respiration (μ l/g/hr \pm S.E.)	568.7 \pm 73.1		528.9 \pm 92.1	Not significant
Actual resting respiration (μ l/g/hr \pm S.E.)	614.8 \pm 79.0		528.9 \pm 92.1	Not significant
Struggling respiration (μ l/g/hr \pm S.E.)	1589.2 \pm 180.8		1320.2 \pm 123.8	Not signification
Actual struggling respiration (μ l/g/hr \pm S.E.)	1717.9 \pm 195.4		1320.2 \pm 123.8	Not significant

¹ 24 hours'-starved insects² For details, see text.

TABLE XV. Analysis over the test period of the effect of castration on the O₂-consumption of ♀ *Leptinotarsa decemlineata*¹

	Ovariectomates (7 individuals)	Sham ovariectomates (9 individuals)	Comparison of means
Resting respiration ($\mu\text{l/g/hr} \pm \text{S.E.}$, between 8-12 days)	576.6 \pm 72.9	751.7 \pm 128.5	Not significant P < 0.05
Resting respiration ($\mu\text{l/g/hr} \pm \text{S.E.}$, between 16-24 days)	866.2 \pm 66.3	635.3 \pm 82.1	
Struggling respiration ($\mu\text{l/g/hr} \pm \text{S.E.}$, between 8-12 days)	924.8 \pm 87.4	1184.0 \pm 156.1	Not significant P < 0.05
Struggling respiration ($\mu\text{l/g/hr} \pm \text{S.E.}$, between 16-24 days)	1589.2 \pm 180.8	1142.6 \pm 68.8	
Ratio of resting/struggling (between 8-12 days)	1:1.604		
Ratio of resting/struggling (between 16-24 days)	1:1.835		
Comparison of ratios	P < 0.05		

¹ 24 hours'-starved insects.

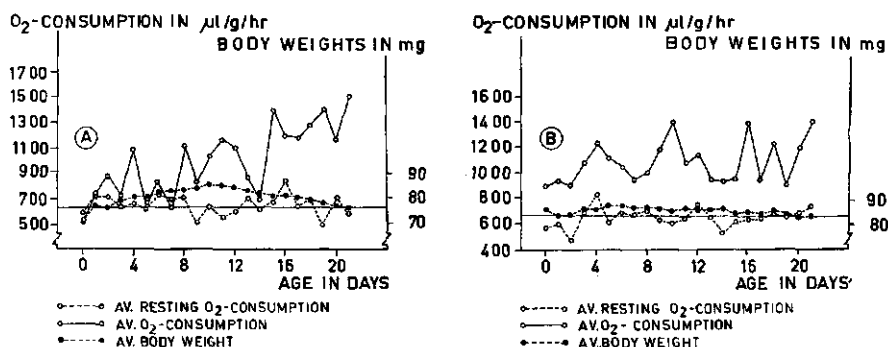


FIG. 7. Oxygen consumption and fresh body weight of intact normally fed, un-mated *Tenebrio molitor* (presented as a mean of morning and afternoon readings). A, average curves derived from six ovipositing females. B, average curves derived from ten males. (Notice the resting oxygen consumption curves after correction, for details see text).

TABLE XVI. Effect of feeding on the respiratory activity of un-mated *Tenebrio molitor*

	Fed (10 ♂ & 6 ♀)	1 hour-starved (9 ♂ & 9 ♀)	Comparison of means
♂			
Resting respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	664.8 ± 44.7 (n = 250)	768.8 ± 117.0 (n = 44)	Not significant
Struggling respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	1825.2 ± 61.7 (n = 145)	1848.8 ± 75.0 (n = 46)	Not significant
♀ ¹			
Resting respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	631.8 ± 16.0 (n = 150)	675.9 ± 38.0 (n = 33)	Not significant
Struggling respiration ($\mu\text{l O}_2/\text{g/hr} \pm \text{S.E.}$)	1712.1 ± 69.7 (n = 82)	1546.1 ± 58.9 (n = 55)	P < 0.10

¹ Ovipositing females.

Table XVIII shows that the respiratory quotients are often significantly below unity, especially in males.

During the preparation of the manuscript a paper was presented by MORDUE (1965a). This author concluded that both crowding and mating in *Tenebrio* females have a stimulatory effect on oögenesis and also prevent the resorption of the terminal oöcytes as histologically revealed. He also stated that the isolated virgin females never oviposit, whereas the mated ones oviposit. Throughout the study we have generally used isolated virgins. It was, therefore, very interesting to determine to what extent mating would affect the respiratory activity in *Tenebrio*. The data obtained, summarized in table XIX, indicate that there is no significant effect of mating in both sexes on either the resting or the struggling

TABLE XVII. Sexual difference in O₂-consumption of un-mated *Tenebrio molitor*

	♂ ¹ (10 fed & 9 1 hour-starved)	♀ ¹ (6 fed & 9 1 hour-starved)	Comparison of means
Fed			
Resting respiration ($\mu\text{l}/\text{O}_2/\text{g}/\text{hr} \pm \text{S.E.}$)	664.8 \pm 44.7 (n = 250)	631.8 \pm 16.0 (n = 150)	Not significant
Struggling respiration ($\mu\text{l O}_2/\text{g}/\text{hr} \pm \text{S.E.}$)	1825.2 \pm 61.7 (n = 145)	1712.1 \pm 69.7 (n = 82)	Not significant
1 hour-starved			
Resting respiration ($\mu\text{l O}_2/\text{g}/\text{hr} \pm \text{S.E.}$)	768.8 \pm 117.0 (n = 44)	675.9 \pm 38.0 (n = 33)	Not significant
Struggling respiration ($\mu\text{l O}_2/\text{g}/\text{hr} \pm \text{S.E.}$)	1848.8 \pm 75.0 (n = 46)	1546.1 \pm 58.9 (n = 55)	P < 0.01

¹ Ovipositing females.

TABLE XVIII. R.Q. of intact *Tenebrio molitor*¹

Age in days	♂ (9 individuals)	♀ (9 individuals)
2	0.740 ± 0.037 ² (S.E.)	0.822 ± 0.026 ² (S.E.)
8	0.975 ± 0.064	0.922 ± 0.041
12	0.905 ± 0.031 ²	0.986 ± 0.055
16	0.774 ± 0.032 ²	0.953 ± 0.070
20	0.806 ± 0.031 ²	0.905 ± 0.044

¹ 1 hour-starved.² Significantly below unity at P = 0.05.TABLE XIX Effect of mating on the respiratory activity of *Tenebrio molitor*¹

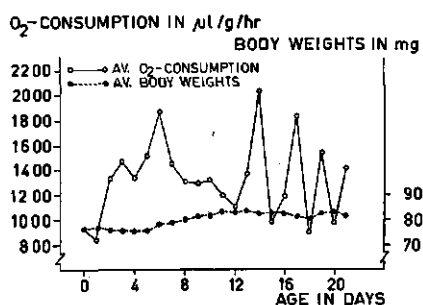
	Mated (9 ♂ & 5 ♀)	Un-mated (10 ♂ & 6 ♀)	Comparison of means
♂ Resting respiration (μl O ₂ /g/hr ± S.E.)	667.4 ± 13.9 (n = 201)	664.8 ± 44.7 (n = 250)	Not significant
♂ Struggling respiration (μl O ₂ /g/hr ± S.E.)	1779.2 ± 67.1 (n = 133)	1825.2 ± 61.7 (n = 145)	Not significant
♀ Resting respiration (μl O ₂ /g/hr ± S.E.)	679.7 ± 27.0 (n = 67)	631.8 ± 16.0 (n = 150)	Not significant
♀ Struggling respiration (μl O ₂ /g/hr ± S.E.)	1658.5 ± 54.4 (n = 105)	1712.1 ± 69.7 (n = 82)	Not significant

¹ Normally fed insects.² Ovipositing females.TABLE XX. Effect of mating on oviposition in *Tenebrio molitor*¹

Age in days	Mated									Un-mated								
10	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
11	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
13	-	1	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-
14	-	2	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
16	5	-	3	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-
18	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-
19	-	-	-	-	1	-	-	-	-	-	-	1	-	-	1	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Total number of deposited eggs	6	3	4	1	2	0	0	0	0	8	3	6	1	1	1	0		

¹ Normally fed insects.

FIG. 8. Oxygen consumption and fresh body weight of intact normally fed, mated, non-ovipositing *Tenebrio molitor* females. Average curves derived from four individuals.



oxygen consumption. Moreover, although the rate of oviposition in our experiments was generally low, mating did not appear to influence oviposition (see table XX). From table XXI, it is evident that the resting oxygen consumption of the ovipositing females does not significantly differ from that of the non-ovipositing ones. However, non-oviposition proved to significantly increase the struggling respiration of the insects. This can also be noticed from fig. 8 in which the respiratory fluctuations are extremely high.

TABLE XXI. Effect of oviposition on the respiratory activity of ♀ *Tenebrio molitor*¹

	Ovipositing (5 individuals)	Non-ovipositing (4 individuals)	Comparison of means
Resting respiration (µl O ₂ /g/hr ± S.E.)	679.7 ± 27.0 (n = 67)	640.9 ± 30.3 (n = 65)	Not significant
Struggling respiration (µl O ₂ /g/hr ± S.E.)	1658.5 ± 54.4 (n = 105)	1919.9 ± 79.0 (n = 84)	P < 0.01

¹ Normally fed and mated females.

3.5.2. Effect of allatectomy on O₂-consumption and R.Q.

Fig. 9 illustrates the original data obtained. These original data of allatectomates or sham allatectomates of both sexes still show the fluctuations of the respiratory intensities characteristic of the intact insects. Table XXII reveals that allatectomy has no significant effect in either sex on both the resting and the struggling oxygen consumption as well as on the fresh body weight. Allatectomy neither resulted in a comparable rise of the R.Q. to a value above unity as in allatectomized *Leptinotarsa* (see table XXIII). The respiratory quotients, however, are mostly of a value significantly below unity, especially in the sham allatectomates. Moreover, allatectomy seems to have no significant effect on the respiratory quotient of both sexes.

TABLE XXII. Effect of allatectomy on the O₂ consumption and the fresh body weight of *Tenebrio molitor*¹

	Allatectomates (7 ♂ & 5 ♀)	Sham allatectomates (9 ♂ & 9 ♀)	Comparison of means
♂			
Fresh body weights (mean of weight ratios ± S.E.) ²	1.020 ± 0.018 (n = 8)	1.054 ± 0.024 (n = 8)	Not significant
Resting respiration (μl/g/hr ± S.E.)	735.8 ± 42.6 (n = 38)	785.6 ± 31.9 (n = 32)	Not significant
Struggling respiration (μl/g/hr ± S.E.)	1412.0 ± 59.9 (n = 18)	1498.3 ± 52.0 (n = 40)	Not significant
♀			
Fresh body weights (mean of weight ratios ± S.E.) ²	1.112 ± 0.021 (n = 8)	1.079 ± 0.019 (n = 8)	Not significant
Resting respiration (μl/g/hr ± S.E.)	557.9 ± 118.0 (n = 27)	744.8 ± 179.0 (n = 18)	Not significant
Struggling respiration (μl/g/hr ± S.E.)	1488.1 ± 100.3 (n = 17)	1507.3 ± 54.7 (n = 51)	Not significant

¹ 1 hour-starved insects² For details, see text.

TABLE XXIII. Effect of surgical interferences on R.Q. of *Tenebrio molitor*¹

Age in days	Allatectomates (7 ♂ & 5 ♀)	Sham allatectomates (9 ♂ & 9 ♀)	Comparison of means	Castrates (5 ♀)	Intact control (9 ♀)	Comparison of means
♂						
4		0.743 ± 0.040 ² (S.E.)				
8	0.831 ± 0.073(S.E.)					
12	0.925 ± 0.079	0.886 ± 0.059	Not significant			
16	0.977 ± 0.082	0.827 ± 0.050 ²	Not significant			
20	0.925 ± 0.037					
22		0.731 ± 0.062 ²				
♀						
4	0.900 ± 0.042(S.E.)	0.784 ± 0.052 ² (S.E.)	Not significant	0.911 ± 0.059	0.889 ± 0.071 ²	Not significant
8		0.747 ± 0.064 ²		0.995 ± 0.055	0.858 ± 0.035 ^{2,3}	"
12	0.765 ± 0.072 ²	0.709 ± 0.053 ²	"	0.835 ± 0.040 ²	0.986 ± 0.055	"
16	0.882 ± 0.101	0.927 ± 0.040	"	0.877 ± 0.096	0.953 ± 0.070	"
20	0.907 ± 0.086			0.902 ± 0.038	0.905 ± 0.044	"
22		0.843 ± 0.064 ²				"

¹ 1 hour-starved insects.² R.Q. of sham overiectomates (5 individuals).³ Significantly below unity at P = 0.05.

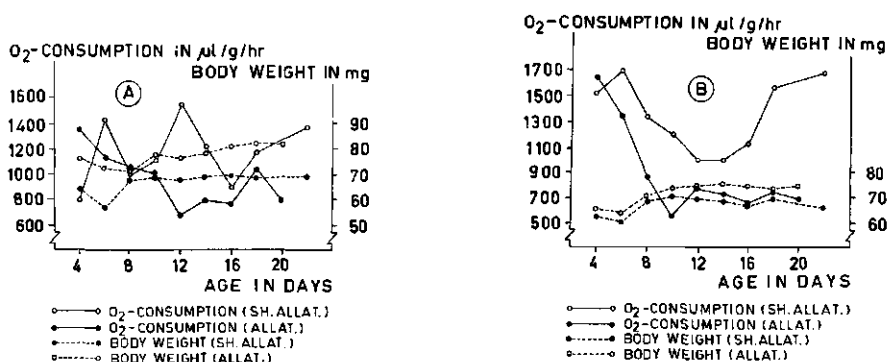


FIG. 9. Oxygen consumption and fresh body weight after allatectomy in *Tenebrio molitor*. A, average curves derived from seven and nine male allatectomates and sham allatectomates respectively. B, average curves derived from five and nine female allatectomates and sham allatectomates respectively.

3.5.3. Effect of castration on O₂-consumption and R.Q.

Probably because of the exorbitant sensitivity of the males to this operation, most of the castrated individuals did not survive longer than four days. A few of them, however, did live for a longer time, but they nevertheless died before

TABLE XXIV. Effect of castration on the O₂-consumption and fresh body weight of ♂ *Tenebrio molitor*^{1,2}

	Testectomates (one insect)	Sham testectomates (7 insects)	Comparison of means
Fresh body weights (mean of weight ratios ± S.E.) ²	1.192 ± 0.023 (n = 8)	1.250 ± 0.010 (n = 7)	P < 0.05
Resting respiration (µl/g/hr ± S.E.)	937.0 ± 18.9 (n = 8)	891.0 ± 49.3 (n = 15)	Not significant
Struggling respiration (µl/g/hr ± S.E.)	1310.6 ± 177.3 (n = 2)	1561.8 ± 48.6 (n = 44)	Not significant

¹ 1 hour-starved insects

² For details, see text.

the experiment ended. Only one insect survived for about one month. The data summarized in table XXIV could by no means prove that castration has any effect on either the resting or struggling oxygen consumption. Definite conclusions, however, cannot be obtained from just one test animal. The table also demonstrates that the fresh body weight of this male castrate decreases significantly (weight on the fourth day was taken as initial value).

Fig. 10.

Oxygen consumption and fresh body weight after castration in *Tenebrio molitor* females. Average curves derived from five ovariectomates and five sham ovariectomates.

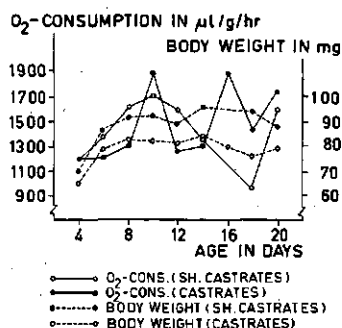


TABLE XXV. Effect of castration on the O_2 -consumption and the fresh body weight of ♀ *Tenebrio molitor*^{1,2}

	Ovariectomates (5 individuals)	Sham ovariectomates (5 individuals)	Comparison of means
Fresh body weights (mean of weight ratios \pm S.E.) ²	1.234 ± 0.014 (n = 8)	1.294 ± 0.019 (n = 7)	$P < 0.05$
Resting respiration ($\mu\text{l/g/hr} \pm$ S.E.)	860.6 ± 50.1 (n = 11)	781.0 ± 48.6 (n = 10)	Not significant
Struggling respiration ($\mu\text{l/g/hr} \pm$ S.E.)	1675.7 ± 107.7 (n = 31)	1710.6 ± 154.1 (n = 20)	Not significant

¹ 1 hour-starved insects.

² For details, see text.

Castration in females, shown in table XXV and plotted in fig. 10, has no significant effect on either the resting or struggling respiration, but a significantly reducing effect on the fresh body weights (taking the average weight on the fourth day as initial value).

3.6. SUMMARY OF THE EXPERIMENTAL RESULTS 3.1.-3.5.

The foregoing results indicate that all the insect groups of differently treated batches of both species studied either intact or operated, except those of the short-day *Leptinotarsa*, exhibited eerrhythmical fluctuations as far as oxygen consumption is concerned. Most interesting was to find that the type of change in the respiration intensities in the course of the determination period was remarkably different in the two species. Binocular observations elucidated how fully dependent these fluctuations in the oxygen consumption are on the struggling of the insects inside the metal gauze cages. Since it is practically impossible to avoid this struggling inside the metal gauze tubes, efforts have been made to

find the respiration values belonging to the most complete resting condition in the original data obtained. These original data proved to be entangled with the struggling respiration values which concealed the true resting respiration value needed to study the effects of the surgical interferences. These efforts are presented in sections 3.1. and 3.2. A test has been made to see whether the R.Q. calculated from the original data would be significantly different from that obtained only from the resting respiration. The results (see section 3.3.) reveal that there is no significant difference and the respiratory quotients may be considered not to be affected by the struggling of the insects.

By applying the above-mentioned approaches to the original data, we came to the following conclusions:

1. The struggling respiration is certainly independent of the resting respiration but their curves run parallel as is clear from the data presented for both intact and castrated *Leptinotarsa* females.
2. *Leptinotarsa* females seem to have a gonotrophic cycle repeated about every three weeks and oögenesis is most probably a continuous process, although the rate decreases in the course of the cycle. Moreover, the results show that the resting oxygen consumption is appreciably influenced by the process of oögenesis.
3. In intact long-day *Leptinotarsa*, of either sex, feeding proved to have no significant influence on the oxygen consumption as far as the resting respiration is concerned.
4. The respiratory quotients of the short-day *Leptinotarsa*, of either sex, are in some cases significantly above unity. Those of the long-day insects are usually significantly below unity.
5. In *Leptinotarsa*, allatectomy results in a significant reduction of the resting oxygen consumption estimated at 67.3% and 77% in males and females respectively¹. However, the struggling oxygen consumption was only reduced by allatectomy by 56.2% in males and by 52.1% in females². By contrast, allatectomy led to a significant increase in the fresh body weights, although this increase did not significantly affect the respiration. Allatectomy also resulted in a rise of R.Q. to a value significantly above unity in both sexes.
6. In either sex of *Leptinotarsa*, castration proved to have no significant effect on either the average resting or struggling oxygen consumption or the R.Q. Moreover, it has a significantly stimulating effect on the oxygen consumption of aging females and induces a significant weight increase in both sexes.
7. Table XXVI shows that this increase in the fresh body weights resulting from castration is not significantly different from that resulting from allatectomy in females. On the other hand, allatectomy in males induced a significantly higher increase in fresh body weights than castration.

¹ These percentages were found not to be significantly different at 5% probability. The χ^2 -test has been applied.

² These percentages of reduction in the struggling respiration were found to be significantly less than their corresponding percentage of reduction in the resting respiration at $P < 0.001$ in both males and females. The χ^2 -test has been applied.

TABLE XXVI. Effect of surgical interferences on the fresh body weight (mean of weight ratios)¹ of *Leptinotarsa decemlineata*²

	Castrates	Allatectomates	Comparison of means
♂	1.144 ± 0.006 (S.E.) (n = 5)	1.245 ± 0.012 (S.E.) (n = 5)	P < 0.001
♀	1.255 ± 0.008 (S.E.) (n = 5)	1.197 ± 0.033 (S.E.) (n = 5)	Not significant
Comparison of means	P < 0.001	Not significant	

¹ For details, see text.

² 24 hours'-starved insects.

8. Whereas the increase obtained in the fresh body weight by allatectomy in *Leptinotarsa* did not show any significant sexual difference, castration induced a significantly higher increase in females than in males (see table XXVI).
9. The resting respiratory rate in either sex of *Tenebrio* shows a constant level running parallel to the fresh body weight rate from the imaginal emergence until the end of the experiment.
10. Feeding has no significant effect on the resting respiration in either sex of *Tenebrio*.
11. Mating in *Tenebrio* does not have a significant effect on either the resting oxygen consumption in both sexes or the oviposition in females.
12. Intact non-ovipositing *Tenebrio* females do not show a significant difference in the resting oxygen consumption compared to the ovipositing ones.
13. *Tenebrio* female allatectomates do not show a significant difference with the intact non-ovipositing females concerning the resting oxygen consumption. However, their struggling respiration is significantly lower than that of the non-ovipositing insects (see table XXVII).

TABLE XXVII. Difference in respiratory activity between allatectomates and non-ovipositing ♀ *Tenebrio molitor*

	Allatectomates ¹ (5 individuals)	Non-ovipositing ² (4 individuals)	Comparison of means
Resting respiration (μl O ₂ /g/hr ± S.E.)	557.9 ± 118.0 (n = 27)	640.9 ± 30.3 (n = 65)	Not significant
Struggling respiration (μl O ₂ /g/hr ± S.E.)	1488.1 ± 100.3 (n = 17)	1919.9 ± 79.0 (n = 84)	P < 0.01

¹ 1 hour-starved and un-mated insects.

² Normally fed and mated insects.

14. Allatectomy nor castration in either sex of *Tenebrio* proved to have a significant effect on the resting oxygen consumption. Neither of these operations seems to affect the fresh body weight of males, but castration induces a significant decrease in females only.

3.7. THE 'METABOLIC' EFFECT OF THE CORPUS ALLATUM HORMONE IN FEMALE *LEPTINOTARSA DECEMLINEATA*

3.7.1. *R.Q. of insects in diapause induced by allatectomy*

Eighty insects were allatectomized and sixty-four survived the operation. Of these survivors, twenty insects entered diapause after 2-3 weeks. These diapausing insects were divided into two groups of ten. One group was kept under unchanged conditions during the respiration determination, the other group was kept under the same conditions to be subjected afterwards to a sham-implantation. Out of the non-implanted insects, two resumed feeding activities and one of these two laid a few eggs. The respiration data obtained by these two insects were rejected therefore. The R.Q. of the remaining eight allatectomy-diapausing females, summarized in table XXVIII, were found to be significantly above unity throughout the period studied.

3.7.2. *R.Q. of normal-diapausing insects*

From a group of short-day treated insects (all entering diapause within 1-2 weeks after emergence, and none resuming feeding activities or ovipositing) six were used for the respiration measurements. These six insects showed respiratory quotients of a value significantly above unity throughout the test period shown in table XXVIII.

3.7.3. *Effect of implantation of active corpus allatum-cardiacum complexes on the O_2 -consumption and the R.Q. of allatectomy-diapausing insects*

Each individual of the eight allatectomy-diapausing females used in the experiments in section 3.7.1. was implanted with six active corpus allatum-cardiacum complexes (obtained from active ovipositing females) on the sixth day after the onset of diapause. This operation was carried out by the method outlined in section 2.1.3. Only five insects survived the operation.

Sham-implanted females were obtained from the allatectomy-diapausing group, kept for this purpose as mentioned in section 3.7.1. Out of these ten insects, three resumed feeding activities and one laid eggs. These three individuals were excluded. Every one of the remaining seven insects was supplied with a piece of a cervical muscle corresponding in size to the implanted glands on the sixth day after the onset of diapause. Two of these sham-implanted insects died leaving five individuals.

In these investigations, both implanted and sham-implanted beetles were deprived of food and only supplied with moist filter paper to avoid the possible interference of the food with the metabolites already present.

Table XXVIII shows that the implanted insects did not change their oxygen

consumption significantly after implantation and a difference with the sham-implanted insects was neither observed. However, the R.Q. obtained on the fourth day after implantation was found to be significantly below unity, whereas the R.Q. of the sham-implanted insects was significantly above unity.

3.7.4. *Effect of transferring the normal-diapausing insects to long-day conditions on their O_2 -consumption and R.Q.*

The same insects used for the data presented in section 3.7.2. were transferred from short-day conditions to long-day ones on the sixth day after the onset of diapause and were provided under the new conditions (as the implanted group) with moist filter papers only. Control was provided by another group of six insects from the same culture continuously under short-day conditions. The data recorded in table XXVIII show that the transferred insects significantly increased their oxygen consumption compared to that of the controls. Nevertheless, they show a significant decrease in comparison with their oxygen consumption before transfer. The transferred insects show a R.Q. significantly below unity on the fourth day after the transfer, whereas the controls show a R.Q. significantly above unity.

3.7.5. *Body constituents in allatectomy- and normal-diapausing insects*

Because of the limited supply of material under the restrictions and conditions outlined (see section 2.4.1.), there was an insufficient number of replicates in these studies. This led to results with rather high values in the standard error of the means (see table XXIX). However, the data presented indicate that the rôle of the corpus allatum hormone might be to promote the oxidative break-down of fat to account for the energy needed by the activated insects. On the other hand, in the absence of the corpus allatum hormone by the surgical removal of the gland or its spontaneous inhibition during nor mal-diapause, the fat content appeared to be relatively higher.

3.7.6. *Summary and concluding remarks on the experimental results 3.7.*

The preceding results reveal that the physiological and biochemical states of both normal-diapausing and allatectomy-diapausing groups seem to be similar. Neither of them is significantly different, because the average oxygen consumption of normal-diapausing is $433.3 \pm 27.8 \mu\text{l } O_2/\text{g/hr}$ and that of allatectomy-diapausing $438.1 \pm 32.9 \mu\text{l } O_2/\text{g/hr}$. This may be inferred from fig. 11.

Moreover, in either case, the respiratory intensities did not show those irregular fluctuations characteristic of the long-day insects. It seems possible (see fig. 11) that the normal-diapausing insects continue to decrease their oxygen consumption more rapidly than the allatectomy-diapausing ones. The former group exhibited an oxygen consumption significantly lower than the sham-implanted insects ($222.9 \pm 16.2 \mu\text{l } O_2/\text{g/hr}$ and $445.3 \pm 27.0 \mu\text{l } O_2/\text{g/hr}$ respectively). There is no significant difference between allatectomy-diapausing insects and the sham-implanted ones ($438.1 \pm 32.9 \mu\text{l } O_2/\text{g/hr}$ and $445.3 \pm 27.0 \mu\text{l } O_2/\text{g/hr}$ respectively), whereas there is a highly significant difference between the normal-

TABLE XXVIII. Effect on the O_2 -consumption and R.Q. of allatectomy- and short-day induced diapauses and their termination in ♀ *Leptinotarsa decemlineata*¹

	Allatectomy-diapausing insects		Normal-diapausing insects	
	Experimental insects	Controls	Experimental insects	Controls
Days after the on-set of diapause	$O_2(\mu\text{l/g/hr} \pm \text{S.E.})$ R.Q. \pm S.E.	$O_2(\mu\text{l/g/hr} \pm \text{S.E.})$ R.Q. \pm S.E.	$O_2(\mu\text{l/g/hr} \pm \text{S.E.})$ R.Q. \pm S.E.	$O_2(\mu\text{l/g/hr} \pm \text{S.E.})$ R.Q. \pm S.E.
1	335.0 \pm 41.7	1.321 \pm 0.046 ²	509.9 \pm 60.6	1.423 \pm 0.040 ²
3	549.1 \pm 60.1	1.122 \pm 0.033 ²	443.8 \pm 45.7	1.103 \pm 0.028 ²
5	430.3 \pm 44.9	1.209 \pm 0.033 ²	406.3 \pm 32.4	1.185 \pm 0.054 ²
	(8 individuals)		(6 individuals)	
Days after implantation or transfer				
2	582.3 \pm 79.2	1.188 \pm 0.112	259.0 \pm 19.0	1.264 \pm 0.092 ²
4	502.7 \pm 106.7	0.744 \pm 0.055 ³	441.7 \pm 22.1	0.764 \pm 0.023 ²
	(5 individuals)		(6 individuals)	
				202.9 \pm 21.6
				242.8 \pm 23.1
				(6 individuals)

¹ For details, see text.² Significantly above unity at $P = 0.05$.³ Significantly below unity at $P = 0.05$.

TABLE XXIX. Body constituents in allatectomy- and normal-diapausing ♀ *Leptinotarsa decemlineata*¹

	Allatectomy-diapausing insects ²				Normal-diapausing insects ²			
	Lipid % ^a per dry weight	Trehalose % per dry weight	Glycogen % per dry weight	Lipid % per dry weight	Trehalose % per dry weight	Glycogen % per dry weight		
Days after the onset of diapause								
1	30.7	4.127 ± 0.562(S.E.)	40.765 ± 7.584(S.E.)		3.957 ± 0.580(S.E.)	6.147 ± 1.793(S.E.)		
3	35.5	2.357 ± 0.695	15.314 ± 14.953	44.9 ⁴	5.665 ± 1.859	5.772 ± 1.323		
5	30.9	3.228 ± 0.550	10.604 ± 3.609		2.918 ± 0.401	4.970 ± 0.565		
Days after im-plantation or transfer								
2	18.3	3.988 ± 1.334(S.E.)	10.190 ± 3.052(S.E.)	33.7	3.779 ± 0.260(S.E.)	5.123 ± 0.570(S.E.)		
Days after sham-implantation								
2	32.2	2.261 ± 0.124(S.E.)	25.026 ± 8.714(S.E.)					

¹ For details, see text.² 4 individuals for every age group.³ combined lipid extract for every age group.⁴ combined lipid extract for the 3 age groups.

diapausing and the control of transferred ones ($453.3 \pm 27.8 \mu\text{l O}_2/\text{g/hr}$ and $222.9 \pm 16.2 \mu\text{l O}_2/\text{g/hr}$ respectively).

The removal of the corpus allatum hormone factor, in either case, resulted in respiratory quotients significantly above unity indicating a possible conversion of carbohydrate into fat. However, the fat content of both diapausing groups (normal and allatectomized), quantitatively estimated in preliminary investigations, did not show a considerable increase in the course of time. Moreover, the

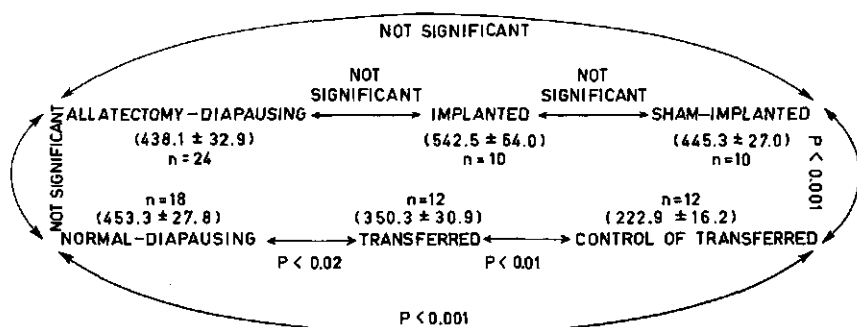


FIG. 11. Demonstrative diagram showing the differences between the average oxygen consumption (in $\mu\text{l/g/hr}$) of allatectomy-and normal-diapausing *Leptinotarsa decemlineata* females as well as some other interactions. For details see text.

carbohydrate content represented by both glycogen and trehalose seems to be conspicuously changed. The available data therefore suggest that the primary function of the corpus allatum in lipid metabolism is to promote the conversion of inert fat, probably by the desaturation of the fatty acids. In other words: the absence of the corpus allatum hormone may result in the saturation of the fatty acids formed from the carbohydrates which accounts for the R.Q. with a value persistently above unity, coinciding with the increased stable level of fat. The saturation of the fatty acids as well as the conversion of carbohydrates into fat give rise to a R.Q. of a value above unity (EDWARDS, 1953, p. 102).

The implantation of six active corpus allatum-cardiacum complexes did not bring about a significant increase in the oxygen consumption of the allatectomy diapausing insects, although it decreased the R.Q. to a value significantly below unity. By contrast, the transfer of normal-diapausing insects to long-day conditions brought about a significant increase in the oxygen consumption as well as a decrease in R.Q. to a value significantly below unity (see page 49).

3.8. FERTILITY IN MALE *LEPTINOTARSA DECEMLINEATA*

In this study it was observed that virgin females mated with normal-diapausing males deposited non-viable eggs, but in normal number and sequence.

By contrast, the male allatectomates and even the allatectomy-diapausing males proved to be as fertile as the intact long-day males. Virgin females, mated with either operated or intact males laid viable eggs in comparable numbers hatching after the same number of days of incubation.

4. GENERAL DISCUSSION

4.1. THE RESPIRATORY RATE AFTER ALLATECTOMY

Allatectomy in either sex of *Leptinotarsa* reduces the oxygen consumption throughout the period studied. Earlier work has also shown that allatectomy in *Leptinotarsa* females results in a decrease in the oxygen uptake (DE WILDE and STEGWEE, 1958).

By contrast, allatectomy in *Tenebrio* did not show any effect on the respiratory rate of both sexes.

The corpus allatum hormone in both insects studied (*Leptinotarsa* by DE WILDE and DE BOER, 1961; *Tenebrio* by MORDUE, 1965 a) is essentially involved in egg-maturation like in most adult female insects.

By contrast, no relationship between the corpus allatum and adult testes has been demonstrated so far (cf. SCHARRER and VON HARNACK, 1961). Allatectomy in males of various species shortly after emergence does not disturb the course of spermatogenesis (WIGGLESWORTH, 1936; THOMSEN, 1942; DAY, 1943; PFEIFFER, 1945 b). If mated with virgins, allatectomized males of the orthopterans *Bacillus* (FAVELLE, 1943) and *Leucophaea* (SCHARRER, 1946) can fertilize the eggs. Our observations on allatectomized male *Leptinotarsa* were consistent with these results. Allatectomized males were found to be fertile. However, as already mentioned allatectomy in *Leptinotarsa* males reduces the oxygen uptake, whereas it has no such effect in *Tenebrio* males. A question arises about the function of the corpus allatum in adult *Leptinotarsa* males. There is evidence of its influence on the flight muscles. Allatectomized *Leptinotarsa* males showed flight muscle degeneration (STEGWEE, personal communication, and own observations). This was not noticed in *Tenebrio* males (personal observations). Allatectomy in *Leptinotarsa* males brought about a reduction in the resting oxygen consumption of 67.3 %, whereas in females of as much as 77.0 %. This sexual difference was found not to be significant indicating that the greater part of the effect of the corpus allatum hormone on respiratory metabolisms is restricted to the flight muscle in females as well. STEGWEE et al. (1963) demonstrated in *Leptinotarsa* females a pronounced degeneration in the flight muscle sarcosomes after removing the post-cerebral complex of endocrine glands; the corpora cardiaca-allata complexes. Implantation of active complexes resulted in a very rapid regeneration of the muscle fibrils and new formation of sarcosomes. Moreover, STEGWEE (1964) argued that the thoracic tissues of active female beetles make up some 80 % of the total respiration.

Although the oöcyte production in *Tenebrio* females has been shown to be dependent on the corpus allatum activity (cf. MORDUE, 1965 a), removal of the gland did not result in a significant reduction in the oxygen consumption. Intact *Tenebrio* females, unlike intact *Leptinotarsa* females, did not show any cyclic changes in the oxygen consumption correlated with reproductive cycles. This might be due to the very low rate of oögenesis in this insect species, since the females deposited rather few scattered eggs. This rate is apparently too low to be

distinguished in the oxygen consumption. Moreover, intact non-ovipositing females showed oxygen consumption comparable with either intact ovipositing or allatectomized females. Allatectomy in these females did not bring about degeneration in the flight muscles (personal observations).

4.2. EFFECT OF MATING ON RESPIRATORY RATE AND OVIPOSITION IN *TENEBRIO*

Mating proved to have no significant influence on the oxygen consumption in both sexes and neither on oviposition in females.

Concerning oviposition, our results were found to be controversial with those of MORDUE (1965 a). This author pointed out that in *Tenebrio*, as in several other insect species, mating has a stimulatory effect on oögenesis and also prevents oöcyte resorption. It was suggested that this effect is mediated through the neurosecretory cells of the brain which regulate oöcyte growth by direct control of the protein synthesis (reflected in the haemolymph protein concentration and the protein uptake by the developing oöcytes). Their secretion also acts as a precursor for the corpus allatum hormone or stimulates production of the corpus allatum in some other way (MORDUE, 1965 b). In our observations, some of the mated females did not deposit eggs and most of the virgins oviposited at about the same time as mated ones. This could demonstrate that mating is not a 'must' in *Tenebrio* to oviposit. However, the possibility should not be excluded that our *Tenebrio* material might belong to a different strain.

4.3. THE RESPIRATORY RATE AFTER CASTRATION

Castration in both species studied, males or females, did not lead to a reduction in the oxygen uptake. Instead, it led to a significant late increase in the oxygen uptake of *Leptinotarsa* females. In *Tenebrio* females an increase seemed to occur throughout the period studied, but it proved to be insignificant. In fact, this increase in the oxygen consumption was not observed after allatectomy. Moreover, whereas castrated *Leptinotarsa* males increased their weight significantly, this increase was significantly less than that found after allatectomy. In *Leptinotarsa* females, on the other hand, castration induced an increase in the fresh body weight comparable to that induced by allatectomy. Furthermore, allatectomy in *Leptinotarsa* did not result in any significant sexual difference in the increase in the fresh body weight, whereas castration induced a significantly higher increase in females than in males. This could indicate that the metabolites accumulated after castration in females might be respiratory substrates in contrast to the inert metabolites accumulated after allatectomy.

The data on R.Q. seem to justify this interpretation. R.Q. was found to be significantly below unity in castrates similar to active long-day insects and significantly above unity in allatectomates as in inactive diapausing beetles. It follows that in *Leptinotarsa* females, the effect of the corpus allatum on res-

piration cannot merely be explained by an effect on the ovaries. On the contrary, the late increase in respiratory intensity observed in females castrates (fig. 6B) could rather point towards the reverse.

On the basis of the physiological evidence at hand it seems most probably that castration in *Leptinotarsa* females leads to a hyperactivity of the corpus allatum with regard to metabolism. A corollary would be, that the ovaries normally may have a restraining influence on the corpora allata.

Another possibility could be that the late increase observed is due to a homeostatic mechanism regulating the haemolymph composition and demanding more energy as the ovaries do not remove the precursor substances for oögenesis produced elsewhere. But it is difficult to see why this argument should not be valid in the case of allatectomates.

Although it has been observed in many cases that the size of the corpus allatum increases after castration (PFEIFFER, 1939; THOMSEN, 1942; BODENSTEIN, 1947; WIGGLESWORTH, 1948; THOMSEN, and HAMBURGER, 1955; VON HARNACK and SCHARRER, 1956; JOHANSSON, 1958; SCHARRER and VON HARNACK, 1961), it is by no means certain that this increase in size is correlated with an increase in activity (JOHANSSON, 1948; STAAL, 1961; MORDUE, 1965 a). We have, indeed, only indirect evidence to present on this point.

As mentioned before, the rate of oögenesis in *Tenebrio* might be too low to be reflected in the oxygen consumption. It seems, therefore, most probable that the ovarian influence may have escaped detection. In this regard, histological studies on the corpora allata of castrated *Tenebrio* females should be of great interest.

It is noteworthy that THOMSEN and HAMBURGER (1955) working with *Calliphora* females found that castration has no reducing effect on respiratory metabolism. They, however, did not demonstrate any later increase. These authors studied the effect on 7 days'old females. Had they prolonged their observation from the adult eclosion over a somewhat longer period it could have been possible that they might have found this increase.

Our results in *Leptinotarsa* females seem to be consistent with those of Sä-gesser (1960) in that the oxygen consumption of *Leucophaea* females increased after castration.

4.4. MAY DIAPAUSE IN *LEPTINOTARSA* be CONSIDERED A CASE OF 'PSEUDO-ALLATECTOMY'?

DE WILDE and DE BOER (1961) demonstrated that in adult *Leptinotarsa* the complete syndrome of diapause is produced upon surgical excision of the corpora allata. This is correlated with a standstill of ovarian functions, a low rate of oxygen consumption, and a change in behaviour from feeding to burying. These observations led the authors to describe diapause in this insect as a case of 'pseudo-allatectomy'. However, whereas allatectomy-diapause is simply reversed by implanting 2-4 active corpora allata, this leaves normal-diapause unbroken. For this reason, it was impossible for these workers to state decisively

that diapause in adult *Leptinotarsa* is merely a syndrome of corpus allatum hormone deficiency.

STEGWEE et al. (1963) and STEGWEE (1964) inferred another similarity, but on a sub-cellular level. They demonstrated a drastic desintegration in the flight muscle sarcosomes of both diapausing states in Colorado beetle and considered the low metabolic rate observed to be due to uncoupling oxidative phosphorylation.

The present study provides a further similarity from the physiological as well as the biochemical points of view. Removal of the corpus allatum hormone by surgical excision of the glands or by short-photoperiod treatment resulted in low values of oxygen consumption as well as R.Q.'s significantly above unity which suggests a possible conversion of carbohydrate into fat. Moreover, the preliminary chemical findings in females seem to justify this conversion.

Regarding *Leptinotarsa* males, there is only one distinct difference between allatectomy-diapausing and normal-diapausing insects. Whereas allatectomy-diapausing males proved to be fertile, normal-diapausing ones were not, although they responded to a mating behaviour with virgins.

More recently DE WILDE (1963, 1965) working with *Leptinotarsa* favours the idea that the neurosecretory material originating from the brain is essential in stimulating the corpus allatum to secrete its hormone (THOMSEN, 1952; SCHARER, 1958; HIGHNAM, 1962; MORDUE, 1965 b). He also stressed the importance of the active neurosecretory cells of the brain necessary for the maintenance of the activity of the glands. GRISON (1949) observed that diapausing *Leptinotarsa* start feeding and laying eggs after receiving brain implants from active individuals. DE WILDE (1953) demonstrated that young female Colorado beetles which are about to enter diapause in response to short-photoperiod may be induced to lay eggs by implanting six active corpora allata. But this effect decreases if short-photoperiod is continued after about two weeks.

Our failure to increase the oxygen uptake of allatectomy-diapausing female *Leptinotarsa* after implanting six active post-cerebral complexes and subsequent starvation, in contrast to the increase attained in normal-diapausing insects after transfer to long-day condition, may be explained as follows:

According to DE WILDE et al. (1959), starvation induces diapause and prevents activation of the corpora allata, while after break of normal-diapause (DE WILDE, 1949) it cannot be easily induced a second time.

In *Leptinotarsa* males it seems most likely that the corpora allata are not involved like in other insects, in the control of spermatogenesis. In this relation, there is no need to invoke a 'diapause hormone' to account for the evidence obtained (DE WILDE and DE BOER, 1961).

SLÁMA (1964 a) described the imaginal diapause in *Pyrhocoris* females as a syndrome of the endocrine deficiency of the cardiacum-allatum complex and not merely a corpus allatum hormone deficiency. He argued that allatectomized females retain a considerably higher respiration rate than either cardiac-allatectomized or diapausing insects. This conclusion, in our opinion, is rather questionable since he expressed the oxygen consumption per individual and not

per gramme of weight. Moreover, he did not show to what extent the individual variation in his insect groups would affect the data obtained. The respiratory rate of cardiacectomized *Pyrrhocoris* females with an inactive corpus allatum (considered as cardiac-allatectomized insects) does not seem to be significantly different from that of allatectomized insects. In this regard, reference should be made to the observations of JOLY (1945) on *Dytiscus* that cardiacectomy had the same effect on the ovaries as allatectomy. However, because of the degeneration of the corpora allata after cardiacectomy he thought this effect was induced by the degeneration.

Furthermore, SLÁMA observed that implanting active corpora cardiaca alone in cardiac-allatectomized females did not cause the oxygen consumption to reach the level of allatectomized females, whereas implanting an active cardiacum-allatum complex increased the oxygen uptake to the value of normal insects and renewed the reproductive cycles including normal oviposition. Nevertheless, the possibility should not be eliminated that diapause in *Leptinotarsa* is different from that in *Pyrrhocoris*. Diapause in *Leptinotarsa* is accompanied by a distinct degeneration of the flight muscles (STEGWEE et al., 1963), whereas the muscle tissues remain functional in diapausing *Pyrrhocoris* (SLÁMA, 1964 a). Moreover, diapause in adult *Leptinotarsa* is characterized by an entire lack of feeding activities as well as a complete cessation of mobility. *Pyrrhocoris* during diapause exhibits occasional feeding and mobility.

4.5. THE LOCATION AND NATURE OF THE 'DIAPAUSE MECHANISM' IN *LEPTINOTARSA DECEMLINEATA*

The most obvious differences found as a result of the surgical interferences in the two insect species studied (see table XXX) are manifested almost exclusively in the effects of allatectomy. Castration, on the other hand, does not seem to induce any important differences between species in as far as the oxygen consumption and the R.Q. are concerned. In the previous section, it has been argued that diapause in *Leptinotarsa* females may be seen as a case of 'pseudo-allatectomy'. Therefore, the differences encountered between allatectomized females of the two species studied may be considered a criterion in the analysis of the 'diapause mechanism' in *Leptinotarsa*.

Allatectomy in *Leptinotarsa* led to a reduction in the oxygen consumption, whereas it had no such effect in *Tenebrio*. Allatectomy resulted in a rise of the R.Q. to a value significantly above unity in *Leptinotarsa* but proved to have no effect on the R.Q. in *Tenebrio*. Allatectomy induced an increase in the fresh body weight in *Leptinotarsa* but it did not show this effect in *Tenebrio*.

It has been discussed earlier that these differences are partly caused by the degeneration of the flight muscles in *Leptinotarsa*. This might indirectly show the importance of the flight muscles in *Leptinotarsa* as an important location of the 'diapause mechanism'. The question arises what is the nature of this diapause mechanism. Is there, for instance, a biochemical mechanism in *Leptinotarsa* which is not present in *Tenebrio*?

So far, there is no decisive answer to this question. Detailed biochemical in-

TABLE XXX. Main interspecific differences caused by surgical interferences with *Leptinotarsa decemlineata* and *Tenebrio molitor*¹

		<i>Leptinotarsa</i>		<i>Tenebrio</i>	
		♂	♀	♂	♀
Allatectomy	O ₂ -consumption	Decreased	Decreased	Not affected	Not affected
	R.Q.	Raised (> 1.0)	Raised (> 1.0)	Not affected (< 1.0)	Not affected (< 1.0)
	Fresh body weight	Increased	Increased	Not affected	Not affected
	Flight muscles	Degenerated	Degenerated	Not degenerated	Not degenerated
Castration	O ₂ -consumption	Not affected	Not affected, but a late increase	Not affected	Not affected
	R.Q.	Not affected (< 1.0)	Not affected (< 1.0)	Not affected (< 1.0)	Not affected (< 0.10)
	Fresh body weight	Increased	Increased	Decreased ¹	Decreased

¹ For details, see text.

vestigations into the body metabolites after these surgical interferences are almost lacking as far as the author is aware. Though it is generally known that allatectomy results in fat body hypertrophy, mainly caused by accumulation of stored fat (see Introduction), the nature of this fatty material has not been critically investigated.

Similarly, there is substantial evidence in the literature showing that diapause is also accompanied by an increase in fat content (*Leptinotarsa* by DE WILDE, 1955; *Anopheles* by VINOGRADOVA, 1958; *Culex pipiens* by DANILEVSKII and GLINYANAYA, 1958; *Anthonomus* by BRAZZEL and NEWSOM, 1959; *Coccinella* by HODEK and ČERKASOV, 1960; and *Culex tarsalis* by HARWOOD and HALFHILL, 1964).

Our data suggest that a primary function of the corpus allatum hormone in fat metabolism might be the promotion of desaturation of the fatty acids synthesized from carbohydrates (see page 44). It is generally accepted that fat which is synthesized from carbohydrate tends to be saturated. This has also been found in certain higher animals (ELLIS and ISBEL, 1926; HEILBRUNN, 1947) and the insect *Heliothis* (DITMAN, 1938). Moreover, DITMAN and WEILAND (1938) pointed out that a lowered content of body water, increased quantity of fat, and increased saturation of the fatty acids are generally associated with the ability of insects to withstand hibernating conditions. LEATHES and RAPER (1925, pp. 118-120) stated that the saturated fatty acids are more stable than the unsaturated acids. The saturated acids contain a greater store of potential energy than the unsaturated ones. These writers also stated that it is almost certain that a process of desaturation takes place when reserve fat is called upon to yield its energy.

Concerning *Leptinotarsa*, there is considerable information to support that saturated acids are preponderant in the diapausing state, whereas unsaturated acids are dominant in the active state. DUCET and GRISON (1948) identified lecithin phosphatides in the adults of *Leptinotarsa*. The lecithin content in active insects of either sex is much higher than in diapausing ones. Moreover, it increases during adult life and reaches a maximum in the phase of reproduction. According to HEILBRUNN (1947) active muscles seem to contain more phospholipid than inactive ones, the phospholipid molecule serves as a transport vehicle for fatty acids. LAMBREMONT et al. (1964) working with diapausing boll weevil *Anthonomus* obtained as much as 90% of the extractable lipids in the form of neutral glycerides and free fatty acids. Furthermore, it is generally known that phospholipids are considered to be metabolically active forms of lipid, found in all cells, whereas the simple glycerides are characteristic of the relatively inert storage depots (cf. GILMOUR, 1961). TIMON-DAVID (1930) outlined that the fat content in *Leptinotarsa* increased from 3% of the live weight in larvae to 13.4% in adults. According to BECKER (1934), the fat content of *Tenebrio* decreased during metamorphosis; larvae contained 12-14% of the live weight, pupae 8.9% young beetles 8.1% and old beetles 4.6%.

It is of interest to mention that LANGENBUCH's investigations (1954, 1955) reveal that the fat content of the last larval instar of *Leptinotarsa* (L₄) is much higher than that of the third instar (L₃). There is substantial evidence that the

corpus allatum hormone titre during the larval stage attains the lowest level in the last larval instar (BODENSTEIN, 1957; WILLIAMS, 1958; SCHNEIDERMAN and GILBERT, 1959; VAN DER KLOOT, 1961). This low titre of the corpus allatum hormone may account for the fat accumulation in the last larval instar of *Leptinotarsa*.

Considering our data as well as the information from the literature, we propose the working hypothesis that in insects with the 'diapause mechanism', fat is the main source of energy, the absence of the corpus allatum hormone leading to a high fat storage. This results in a large store of potential energy and the insect subsequently enters into diapause. In the presence of the hormone the condition is reversed: fat being oxidized, the resulting energy is consumed and diapause is broken. Evidently, this hypothesis does not yet completely account for the data presented. Indeed, further studies are awaited to bring us to an understanding of the molecular events of metabolic control in diapause.

4.6. SITE OF ACTION OF THE CORPUS ALLATUM HORMONE

The preceding pages, clearly show from the data on *Leptinotarsa* that the corpus allatum hormone has a general metabolic effect since castration, in contrast to allatectomy, proved to have no reducing effect on the respiratory metabolism in this insect. Earlier work on *Leptinotarsa* females (DE WILDE and STEGWEE, 1958; STEGWEE 1960) also suggests a metabolic effect of the corpus allatum hormone. In the literature, there also is accumulating evidence that the corpus allatum hormone in a great variety of adult insects acts as a metabolic one (see section 1.2.). However, it should be borne in mind that the effect of a hormone on metabolism does not necessarily mean that the hormone is a metabolic one. Many myotropic hormones influence the degree of tissue respiration without acting directly on the metabolic centres.

From the evidence available at present, we may conclude that this metabolic effect only is normally manifested in the respiratory intensity of insects males or females, having metabolically active target organs. Therefore, it seems plausible that the corpus allatum hormone in *Leptinotarsa*, of either sex, has a more general effect on the intensity of respiration besides its gonadotropic influence in females. By contrast, such an effect is lacking in *Tenebrio* and apparently also in *Pyrrhocoris* mainly because of the inactivity of metabolic centres such as the flight muscles in these species.

The assumption of a possible gonadotropic effect in adult female insects favoured by some workers (see Introduction) has recently been supported by SLÁMA (1964 a) working with *Pyrrhocoris*. The results of this author are open, however, to some criticism. Firstly, he compared the respiration data of allatectomates with those of ovariectomates and the weights of each group with the weights of normal (intact) controls. He did not find remarkable difference between sham allatectomates and intact insects regarding growth, reproduction, and respiration but he did not perform sham-castrations. In this regard, it would have been more creditable if he had compared each operated group with

a relevant corresponding sham-operated control. Secondly, (discussed earlier on page 49) he compared most of his data on a specimen basis which could be influenced by individual variations. In our analysis of the effect of the increasing weight of allatectomized *Leptinotarsa* we were able to find that this increase in weight has no significant effect on the respiration data if it is taken in conjunction. Unfortunately, SLÁMA did not show the extent to which this would significantly influence his data on *Pyrrhocoris*.

In long-day *Leptinotarsa*, revealed by the data on the intact as well as the castrated females, the struggling respiration statistically was found to be entirely independent of the resting respiration, but their levels remain parallel. In *Leptinotarsa* allatectomates, males or females, the struggling respiration is reduced by a lower degree than the resting respiration. On the other hand, the struggling respiration was not demonstrated in both allatectomy-diapausing and normal-diapausing *Leptinotarsa*. All these findings support the suggestion that the corpus allatum influences the struggling respiration indirectly through the central nervous system which governs muscle activity. In this respect, it is most probable that the corpora allata activate the brain since their absence in allatectomy-diapause results in lethargy causing a lack of struggling respiration.

5. GENERAL SUMMARY AND CONCLUSIONS

This study essentially deals with two problems:

- a) Do the tissues of an adult insect with a 'diapause mechanism' react in a different way to low titre of the corpus allatum hormone than those in non-diapausing adult insects?
- b) Are the metabolic effects of the corpus allatum hormone merely caused by its effect on a primary target organ, e.g. the ovary, or is there also a more general effect on metabolism in other metabolic centres.

To investigate this two adult coleopterans were used; the Colorado potato beetle (*Leptinotarsa decemlineata* Say) having a 'diapause mechanism', and the yellow mealworm (*Tenebrio molitor* L.) in which this mechanism is absent.

The most important findings are summarized as follows:

1. All treated groups of both species studied exhibited fluctuations, in the respiratory rate, with the exception of diapausing *Leptinotarsa* of both sexes. These fluctuations were considered to be due to the struggling of the insects inside the metal gauze tubes. Evaluation methods were therefore developed to find the most basal respiration data from the original data obtained since it was almost impossible to secure the resting condition of the animals inside the gauze tubes.
2. The resting respiration rate in *Leptinotarsa* females is appreciably influenced by the activity of the gonads, whereas in *Tenebrio* this relation could not be demonstrated.
3. Mating in *Tenebrio* proved to have no influence on the respiratory rate of either sex or on oviposition in females.
4. Allatectomy in *Leptinotarsa* of both sexes led to a reduction in the oxygen uptake and to a rise in the R.Q. to a value significantly above unity, whereas such effects were not observed in *Tenebrio*.
5. Castration in newly emerged beetles did not show a reducing effect on the oxygen consumption of both sexes in the two species studied. However, it induced a late increase in *Leptinotarsa* females.
6. It was concluded that in *Leptinotarsa* females, the effect of the corpus allatum on respiration cannot merely be explained by its effect on the ovaries.
7. Some physiological as well as biochemical evidence is presented in favour of DE WILDE and DE BOER's assumption (1961) that diapause in Colorado potato beetle may be considered a case of 'pseudo-allatectomy'. However, it was found that males are infertile in diapause, while after allatectomy, fertility is retained.
8. Evidence was discussed in favour of the suggestion that the most probable location of the metabolic 'diapause mechanism' in *Leptinotarsa* besides the ovaries should be the flight muscles and the fat body.
9. A working hypothesis on the nature of the 'diapause mechanism' in *Leptinotarsa* is presented. This hypothesis is partly based on the assumption that the

primary function of the corpus allatum in fat metabolism might be the desaturation of fatty acids.

10. The corpus allatum hormone, besides its gonadotropic influence in females of both species studied, also has a more general effect on metabolism in *Leptinotarsa* of either sex.

11. Resting respiration in active insects is under direct control of the corpus allatum, whereas the struggling respiration might be under indirect control of the glands, presumably, through the central nervous system.

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SAMENVATTING EN CONCLUSIES

Deze studie houdt zich bezig met twee problemen:

- a) Reageren de weefsels van een volwassen insekt met een 'diapauze mechanisme' op een andere manier op een geringere titer van het corpus allatum-hormoon dan de weefsels van volwassen insekten zonder diapauze?
- b) Worden de metabolische effecten van het corpus allatum-hormoon voornamelijk veroorzaakt door zijn directe inwerking op het desbetreffende orgaan, b.v. het ovarium, of is er ook sprake van een meer algemene werking op het metabolisme in zijn geheel?

Om dit te onderzoeken zijn er twee soorten volwassen Coleoptera gebruikt: de Coloradokever (*Leptinotarsa decemlineata* Say) met een 'diapauze mechanisme' en de meelworm (*Tenebrio molitor* L.), die dat mechanisme niet heeft.

De belangrijkste vondsten kunnen als volgt samengevat worden:

1. Alle behandelde groepen van beide soorten vertoonden schommelingen in ademhalingsintensiteit met uitzondering van *Leptinotarsa*, ♀ en ♂, in diapauze. Wij menen, dat deze schommelingen te wijten zijn aan de pogingen van de insekten zich te bewegen in de metaalgazen kokertjes. Daarom zijn methoden ontwikkeld om het basaal metabolisme af te leiden uit de oorspronkelijk verkregen gegevens, daar het zo goed als onmogelijk was om de insekten in de kokertjes in staat van rust te houden.
2. Het basaal metabolisme van *Leptinotarsa* wijfjes wordt merkbaar beïnvloed door de activiteit van de gonaden, terwijl bij *Tenebrio* deze samenhang niet kon worden aangetoond.
3. Copulatie bleek bij *Tenebrio* van beide geslachten geen invloed te hebben op de ademhalingsintensiteit, noch op de ovipositie bij de wijfjes.
4. Allatectomie bij *Leptinotarsa* van beide geslachten leidde tot een vermindering van zuurstofopname en tot een verhoging van het respiratoir quotient tot een significant hogere waarde dan 1, terwijl zulke invloeden bij *Tenebrio* niet waargenomen werden.
5. Castratie van pas uitgekomen kevers liet bij beide seksen vermindering van het zuurstofverbruik zien bij de twee onderhavige soorten. Maar toch veroorzaakte het een later optredende toeneming bij *Leptinotarsa* wijfjes.
6. De conclusie werd getrokken dat het effect van de corpora allata op de ademhaling van de *Leptinotarsa* wijfjes niet kan worden verklaard door hun effect op de ovariën.
7. Enig fysiologisch en biochemisch bewijsmateriaal wordt naar voren gebracht ten gunste van de veronderstelling van DE WILDE en DE BOER (1961), dat de diapauze bij de Coloradokever beschouwd zou kunnen worden als een geval van 'pseudo-allatectomie'. Daarnaast werd gevonden, dat mannetjes onvruchtbaar zijn gedurende de diapauze, terwijl de vruchtbaarheid na wegnahme van de corpora allata behouden bleef.
8. Aanwijzingen worden besproken, ten gunste van de veronderstelling, dat de meest waarschijnlijke plaats, waar het metabolische 'diapauze mechanisme'

zich bevindt bij *Leptinotarsa*, de vliegspieren en het vetlichaam zijn.

9. Een werkhypothese over de aard van het 'diapauze mechanisme' bij *Leptinotarsa* wordt voorgesteld. Deze hypothese is gebaseerd op de veronderstelling, dat de voornaamste functie van het corpus allatum in de vetstofwisseling waarschijnlijk bestaat in het versnellen van het onverzadigd maken van vetzuren.

10. Het corpus allatum hormoon heeft behalve zijn gonadotrope invloed bij de wijfjes van beide soorten ook een meer algemene inwerking op het metabolisme van *Leptinotarsa* van beide seksen.

11. De basale ademhaling rust bij actieve insekten staat onder directe invloed van het corpus allatum, terwijl de ademhaling bij geforceerde beweging wellicht onder indirecte controle staat van deze klieren, vermoedelijk door middel van het centrale zenuwstelsel.

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