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NERVOUS CONTROL OF THE MECHANICAL PROPERTIES OF THE ABDOMINAL WALL AT FEEDING IN *RHODNIUS*

BY S. H. P. MADDRELL
*Department of Biology, Dalhousie University, Halifax, N.S., Canada, and Department of Zoology, Downing Street, Cambridge**

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INTRODUCTION

Recent work has shown that the mechanical properties of insect cuticle can change suddenly (Cottrell, 1962; Bennet-Clark, 1962). In the larva of *Rhodnius* it was found that the abdominal endocuticle rapidly becomes extensible when the insect starts to feed (Bennet-Clark, 1962); the abdomen can then expand greatly to accommodate the large meal of blood. Bennet-Clark suggested that this plasticization of the cuticle is to be attributed to the appearance in the haemolymph of some factor absorbed from the blood meal through the gut wall. Cottrell (1962) showed that the process of expansion of the newly emerged adult *Calliphora*, which involved a similar increase in extensibility of certain parts of the cuticle, was under nervous control. Later work on *Rhodnius* has shown that the properties of the abdominal cuticle in this insect can also be affected by the nervous system (Maddrell, 1962; Núñez, 1963*a*, *b*). This paper is an attempt to reconcile the various findings of work on *Rhodnius* and to describe more fully the way in which the mechanical properties of the abdominal cuticle are controlled in *Rhodnius*.

MATERIALS AND METHODS

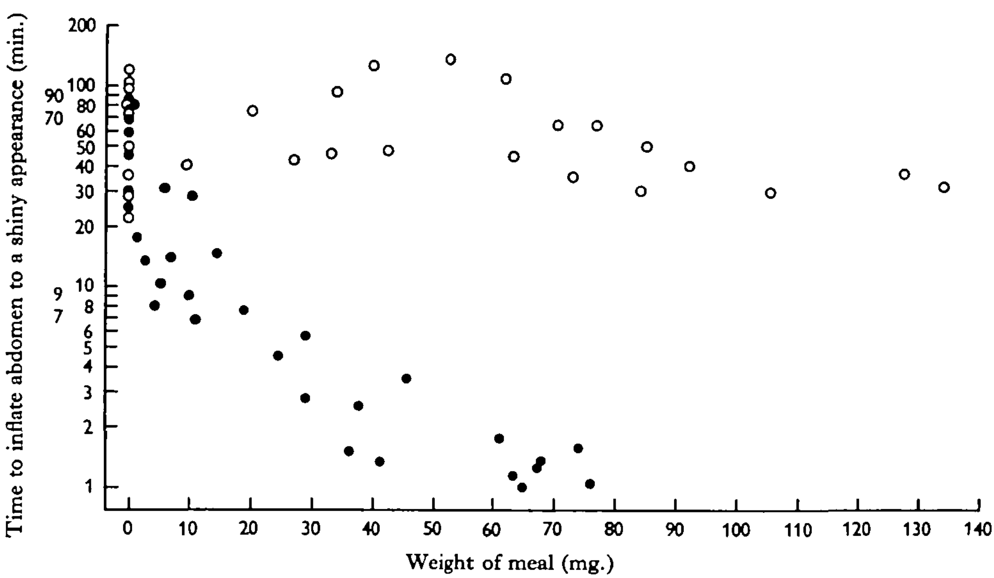
Fifth-stage larvae of *Rhodnius prolixus* from a laboratory culture were used in all experiments. The technique of nerve surgery on *Rhodnius* was as described earlier (Maddrell, 1963*b*). The abdominal nerves fan out from the mesothoracic ganglionic mass; it was relatively simple therefore to open the mesothorax ventrally and to denervate different parts of the abdomen without damaging the abdominal cuticle. The whole abdomen could be denervated by cutting either the connectives between the prothoracic ganglion and the mesothoracic ganglionic mass or all the abdominal nerves as they left the back of the ganglionic mass; the two methods gave the same results.

Insects were artificially inflated using the method developed by Bennet-Clark (1962). Air at a pressure of 10 cm. Hg was blown into insects through a 30 s.w.g. hypodermic needle pushed either into the anus or into a leg. The time taken for the abdomen to inflate until it had the shiny appearance that it has in a well-fed insect was taken as a measure of the extensibility of the abdominal wall (Bennet-Clark, 1962). All inflations were carried out at room temperature (25–27° C.). To avoid discrepancies arising from the variations in cuticular properties with age in the instar, insects taken from a uniform batch were used in all experiments which involved a comparison of cuticular properties.

* Present address.

RESULTS

If larvae of *Rhodnius* are fed after an operation in which a part of the abdomen has been denervated, the denervated areas fail to expand as much as the innervated areas (Pl. 1*a*; Maddrell, 1962; Núñez, 1963*a*, *b*). The longitudinal intersegmental muscles cannot be responsible for this effect because they lack fibres in an unfed insect (Wigglesworth, 1956), while the vertical tergosternal muscles cannot prevent stretching of the abdominal wall because they run at right angles to it. There is the further possibility that the effect is the result of some damage reaction; denervation might make abdominal cuticle less extensible.

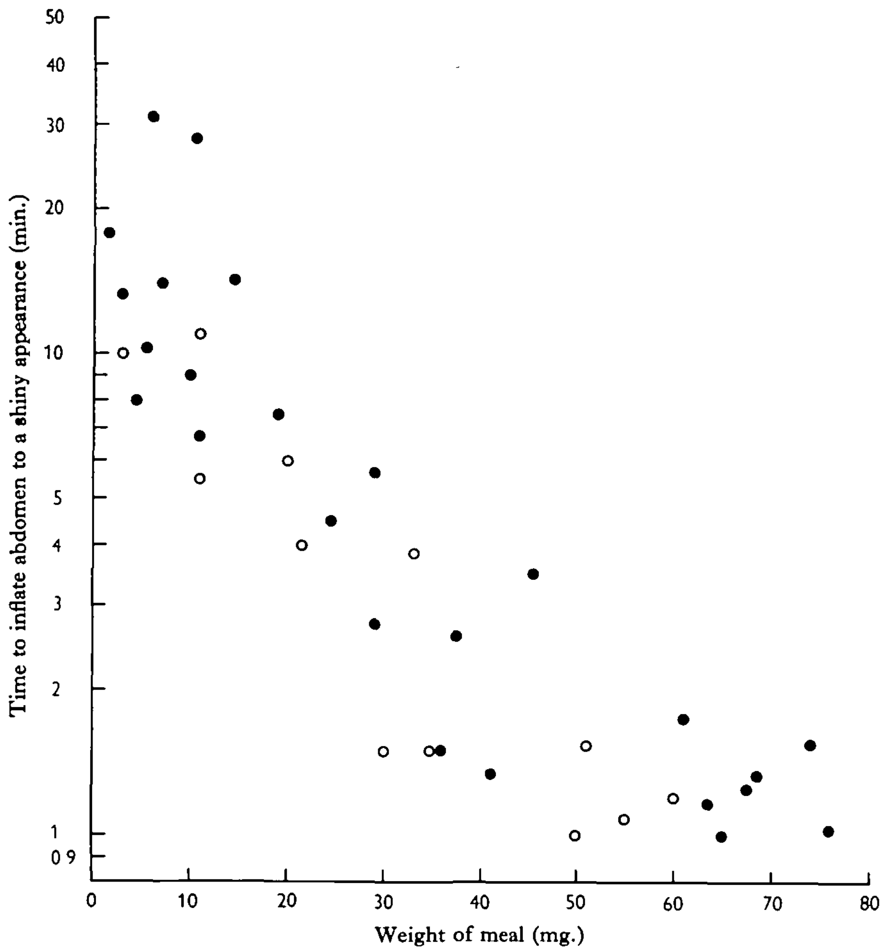


Text-fig. 1. The extensibility of the abdomina of fed and unfed insects whose abdomina had been denervated (○) compared with the extensibility of the abdomina of control insects (●).

To test this possibility, the extensibility of the abdominal wall in partly fed and unfed insects with denervated abdomina was compared with that of control insects. The results are shown in Text-fig. 1. They show that denervation does not affect the extensibility of the abdominal cuticle in unfed insects. Further, while a marked increase in extensibility of the abdominal wall follows feeding in intact insects, there is no such change in insects with denervated abdomina. To show that this failure is caused by the lack of nervous connexion and not by post-operational trauma, a control experiment was performed in which one only of the two connectives joining the prothoracic ganglion and the mesothoracic ganglionic mass was cut in each of ten insects. When these insects were allowed to take a small meal, the whole of the abdominal cuticle quickly became very extensible as judged by its rapid expansion when the insects were subsequently inflated with air at a pressure of 10 cm. Hg.

It may now be concluded that innervated areas of the abdomen are actively made more extensible during feeding and that denervation has no other effect than to prevent this active change. However, it is *not* possible to conclude from these results that the plasticization is solely dependent on the nervous supply. In view of Bennet-Clark's results it might well be that denervation makes the abdominal wall unable to respond to a plasticizing factor absorbed from the blood meal.

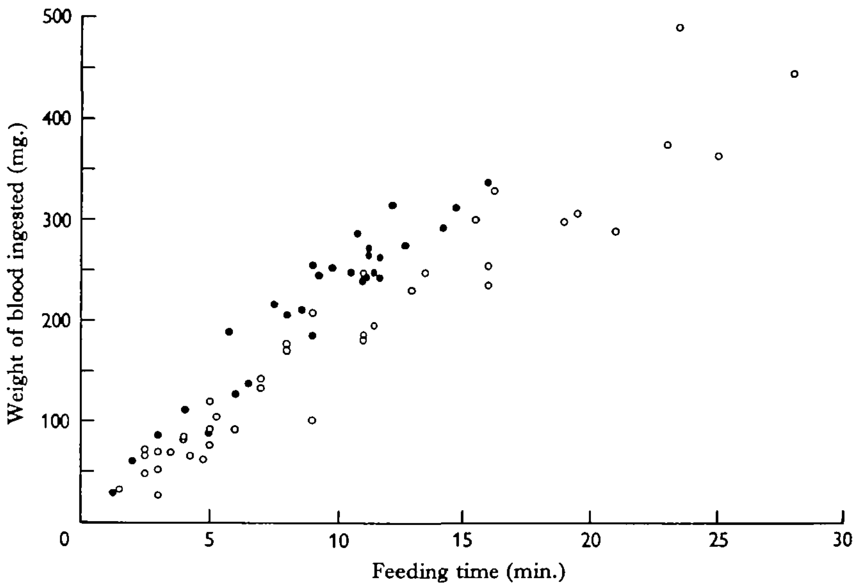
To test this possibility, five insects with denervated abdomina were given small meals of blood and air was blown into the haemocoel when, as before, the abdominal wall started to expand very slowly. However, when the hypodermic needle was



Text-fig. 2. The extensibility of the abdomina of insects given small meals of Ringer's solution (○) compared with the extensibility of the abdomina of insects given small meals of blood (●).

pushed further in so that it broke open the mid-gut, releasing the ingested blood into the haemocoel, the cuticle very quickly became extensible. So, although denervated abdominal cuticle is still able to respond to a factor from the blood meal, the gut wall of these insects prevents the factor from reaching the cuticle at an effective concentration, at least during feeding.

As a further test, weighed intact insects were given small meals of warmed Ringer's solution through a thin rubber membrane as described by Harington (1960). They were then reweighed to determine the size of the meal and inflated with air. The extensibility of the abdomen in these insects is plotted against size of meal in Text-fig. 2 together with similar results from insects given small meals of blood. Clearly, small meals of Ringer's solution are just as effective in causing plasticization of the abdominal wall as are meals of blood of the same size. The results presented thus far demonstrate that the plasticization of the abdominal cuticle in larvae of ***Rhodnius*** at feeding is effected by the nervous supply and is not the result of the presence in the haemolymph of any factor derived from the blood meal. This does not necessarily contradict the findings of Bennet-Clark (1962) but does provide a different conclusion. For a discussion of this point see p. 66.



Text-fig. 3. The rate of feeding of insects with denervated abdomina (O) compared with the rate of feeding of control insects (●).

In view of this conclusion, it came as a surprise to discover that insects with denervated abdomina were able to feed at a rate only a little slower than intact insects (Text-fig. 3), even when they were allowed to take the abnormally large meals characteristic of insects with denervated abdomina (Maddrell. 1963*a*). Apparently they can force blood into the mid-gut at a pressure high enough to stretch the denervated abdominal cuticle. Preliminary experiments have shown that this pressure is of the order of 10 cm. Hg, which compares with a pressure of around 2·5 cm. Hg developed during feeding in the abdomina of intact insects (Bennet-Clark, 1963). Denervated abdominal cuticle becomes white and opaque as it stretches, in contrast to the abdominal cuticle of intact insects which becomes transparent during feeding so that the dark-coloured meal shows through (Pl. 1 *b*).

The rest of this paper describes experiments to investigate first what it is that stimulates the nervous system to affect the cuticle and secondly how the nervous system affects the cuticle.

The stimulus to the nervous system

Since cutting the nerve-cord either in front or behind the mesothoracic ganglionic mass prevents the change in cuticular properties at feeding, it seems that nervous information from the anterior end of the insect is involved. In five insects with the nerve-cord cut in the neck feeding produced no change in the mechanical properties of the abdominal wall. It is concluded that nervous information from the head is essential to the plasticization of the abdominal cuticle.

Text-fig. 2 shows that the abdominal cuticle of intact insects becomes extensible during feeding to an extent which appears to depend on the size of the meal. This suggests that probing by itself is not an adequate stimulus and, indeed, in none of ten insects that probed for periods between 2 and 8 min. without gaining in weight had the abdominal wall become extensible.

Table 1. *Inflation of insects with ligated fore-guts after they had attempted to feed for various lengths of time*

Serial	Length of time during which sucking may have occurred (min.)	Time taken to inflate abdomen to shininess at 10 cm. Hg (min.)
1	½	20
2	1	15
3	2	2¼
4	2	4
5	4	2½
6	6	1½
7	8	1¾

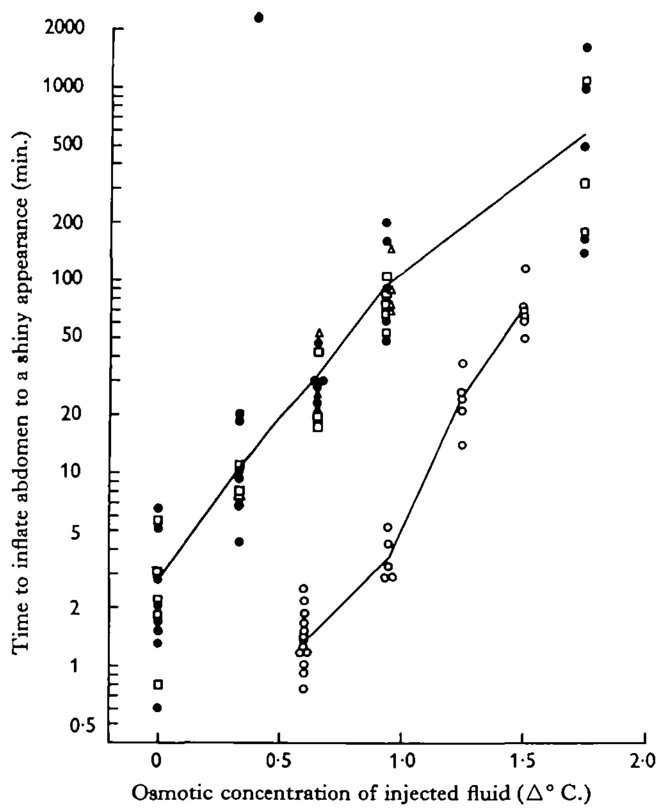
In a further experiment, five unfed insects were operated upon and the narrow fore-gut was ligated in the mesothorax. The insects were then allowed to attempt to feed for various lengths of time and were immediately inflated with air at a pressure of 10 cm. Hg. The times they took to expand to a shiny appearance are recorded in Table 1. Clearly, the abdominal cuticle had become extensible as little as 2 min. after sucking had started. Dissection showed that in all these insects there was blood solely in the fore-gut. This experiment demonstrates that it is not necessary for the plasticization of the abdominal wall that the mid-gut or abdomen be distended. Since a very small quantity of blood in the fore-gut is sufficient to cause extensive plasticization of the abdominal cuticle, it appears that the extent of plasticization depends on the length of time during which the insect is actually sucking and not on the amount of food that is ingested.

It is suggested, therefore, that the stimuli to the nervous system necessary to cause plasticization of the abdominal wall are the sensations which accompany feeding, and that these have a cumulative effect. Such sensations might be provided, for example, by sense organs similar to the stretch receptors in the pharynx of the locust (Clarke & Langley, 1963). A sensory system capable of detecting osmotic pressure changes in the cockroach crop has been demonstrated by Davey & Treherne (1963*a*, *b*) and it seems likely that other factors, such as hydrostatic pressure and the volume of the crop contents, are monitored and integrated by similar nervous mechanisms in this insect

(Davey & Treherne, 1964). A similar system sensitive to hydrostatic pressure in the fore-gut of *Rhodnius* could provide the basis for a sensory input leading to plasticization of the abdominal cuticle.

The stimulus to the abdominal wall

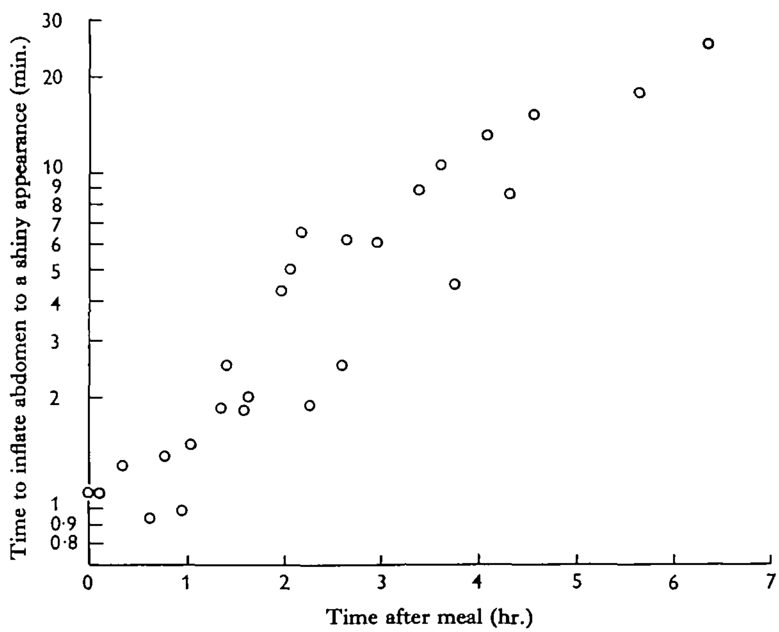
The fact that plasticization is brought about locally and peripherally by the abdominal nervous system demands the existence of an efferent nervous supply to or very close to the epidermis. Previously it has been suggested that all the nerve fibres actually on the abdominal wall terminate in primary sense cells (Wigglesworth, 1934, 1953). However, with the use of the electron microscope it has now been possible to



Text-fig. 4. The extensibility of the abdomina of unfed insects following the injection into the haemocoel of various solutions. Upper line: ●, intact insects injected with Ringer's solution; △, intact insects injected with initially half-strength Ringer's solution whose concentration was adjusted by adding sucrose; □, insects with denervated abdomina injected with Ringer's solution. Lower line: ○, intact insects injected with whipped ox blood.

find neurosecretory axons which run to the abdominal wall and penetrate beneath the basement membrane of the epidermis (Maddrell, 1965). It seems highly probable that it is these axons which cause the plasticization of the abdominal wall not only because they are neurosecretory but also because they are the only known efferent supply to the abdominal wall.

There is some evidence that the abdominal wall becomes plastic as a result of a change in its hydration; Bennet-Clark (1962) found that the cuticle of unfed *Rhodnius* became very extensible when distilled water was injected into the haemocoel and that dilute Ringer's solution was more effective in this respect than was more concentrated Ringer's solution. Text-fig. 4 displays the results of some similar experiments. Several determinations were made on insects with denervated abdomina to exclude effects attributable to the nervous supply, and to avoid the possible effects of changes in ionic concentration further determinations were made using samples of half-strength Ringer's solutions whose concentrations had been increased by adding various amounts of sucrose. The results show that the abdominal cuticle is made more extensible if the osmotic concentration of the haemolymph falls and vice versa. This



Text-fig. 5. The extensibility of the abdomina of insects at various times after they had taken a small meal.

supports Bennet-Clark's suggestion that the plasticization of the abdominal wall may well be caused by an increase in the hydration of the cuticle. Núñez (1963*b*) has suggested that during plasticization there is a change in the cohesion between the chitin-protein micelles of the cuticle; hydration could well produce such a result.

Text-fig. 4 also shows the striking effect of injecting whipped ox blood into the haemocoel. As Bennet-Clark (1962) discovered, this has a much greater plasticizing effect than has Ringer's solution of the same osmotic concentration. This plasticizing effect of blood is not affected by dilution of up to three times with isotonic Ringer's solution, but its effect is reduced when its osmotic concentration is increased by mixing it with more concentrated Ringer's solution (Text-fig. 4). This suggests that the action of the blood may merely be to cause an increase in the permeability of the epidermal cells so that water may more easily pass into the cuticle, though of course it is also possible that it may affect water movement in some other way.

Plasticization of the cuticle is not permanent, as the following experiment shows. Insects were allowed to feed until they had taken about 70 mg. of blood (sufficient to ensure full plasticization—Text-fig. 1). At various times after the meal they were inflated with air at a pressure of 10 cm. Hg. The extensibilities of their abdomina were recorded and are plotted in Text-fig. 5 against the length of time after the meal. Clearly after the first hour or so the abdominal cuticle becomes progressively less extensible.

DISCUSSION

The experiments described in this paper demonstrate that plasticization of the abdominal cuticle of *Rhodnius* at feeding does not depend on the presence in the haemolymph of a factor derived from the blood meal; this conflicts with Bennet-Clark's original suggestion (Bennet-Clark, 1962). His suggestion was based on three pieces of evidence: (*a*) that the abdomina of five insects given small meals of Ringer's solution did not become as extensible as did those of five insects given small meals of blood, (*b*) that blood injected into the haemocoel of unfed insects had a greater effect on the cuticle than had Ringer's solution, and (*c*) that dialysis of mammalian blood against an equal volume of Ringer's solution made the latter active, suggesting that it was quite possible for the active factor to cross the gut wall early after feeding begins.

With regard to (*a*), the present investigation has shown that the plasticizing effect of a small meal does not depend on its nature but is acutely affected by its size. It may well have been, therefore, that Bennet-Clark's batch of five bugs fed on Ringer's solution had taken slightly smaller meals than had his blood-fed bugs, especially since as he notes it is difficult to persuade *Rhodnius* to take Ringer's solution.

The facts in (*b*) and (*c*) are not in dispute; however, it is now clear that in fact *no* appreciable amount of the active factor from the blood appears in the haemolymph during feeding. It is a striking coincidence that the effect of the nervous system on the abdominal wall at feeding is so closely mimicked by the action that mammalian blood has when injected into the haemocoel.

As Text-fig. 3 shows, whether or not the abdominal cuticle is plasticized makes very little difference to the rate of feeding. One wonders then what is the significance of the process of plasticization. Perhaps it is an evolutionary relic and at an early stage in the insect's ancestry the pharyngeal pump was not so powerful as it is now so that plasticization of the abdominal wall had to occur if the animal was to take a large meal. So unless the process which leads to plasticization has some other effect on the insect, its only advantage now would seem to be that the pharyngeal pump does not have to exert its full force in inflating the abdomen.

The discovery that neurosecretory axons supply the abdominal wall is another example of the blurring of the distinction between neurosecretory axons, which were thought always to release their products into the haemolymph, and other nerve cells which release neurohumours not into the blood but directly to the tissues that they influence (for a discussion of this point see Johnson, 1963, and Johnson & Bowers, 1963).

SUMMARY

1. When larvae of *Rhodnius* feed, the abdominal wall rapidly becomes extensible. This change is brought about peripherally by the abdominal nervous supply; as a consequence, abdominal segments which have been denervated fail to stretch as much at feeding as do neighbouring innervated segments.

2. The stimulus for this change originates in the head. It appears that the sensations accompanying feeding are responsible and that they have a cumulative effect, so that the degree of plasticization of the abdominal wall depends on the length of time during which the insect has been sucking.

3. Neurosecretory axons are the only known efferent supply to the abdominal wall and so it is most probable that they cause the localized plasticization.

4. The evidence suggests that the plasticization of the abdominal cuticle is a result of an increase in its water content.

5. The increase in plasticity of the abdominal wall is temporary, the effect decreasing progressively after feeding.

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The later part of the work was done in the Department of Zoology at Cambridge and my thanks are due to Prof. C. F. A. Pantin for accommodation in the department. The author is now a Research Fellow of Gonville and Caius College, Cambridge, and of the Science Research Council.

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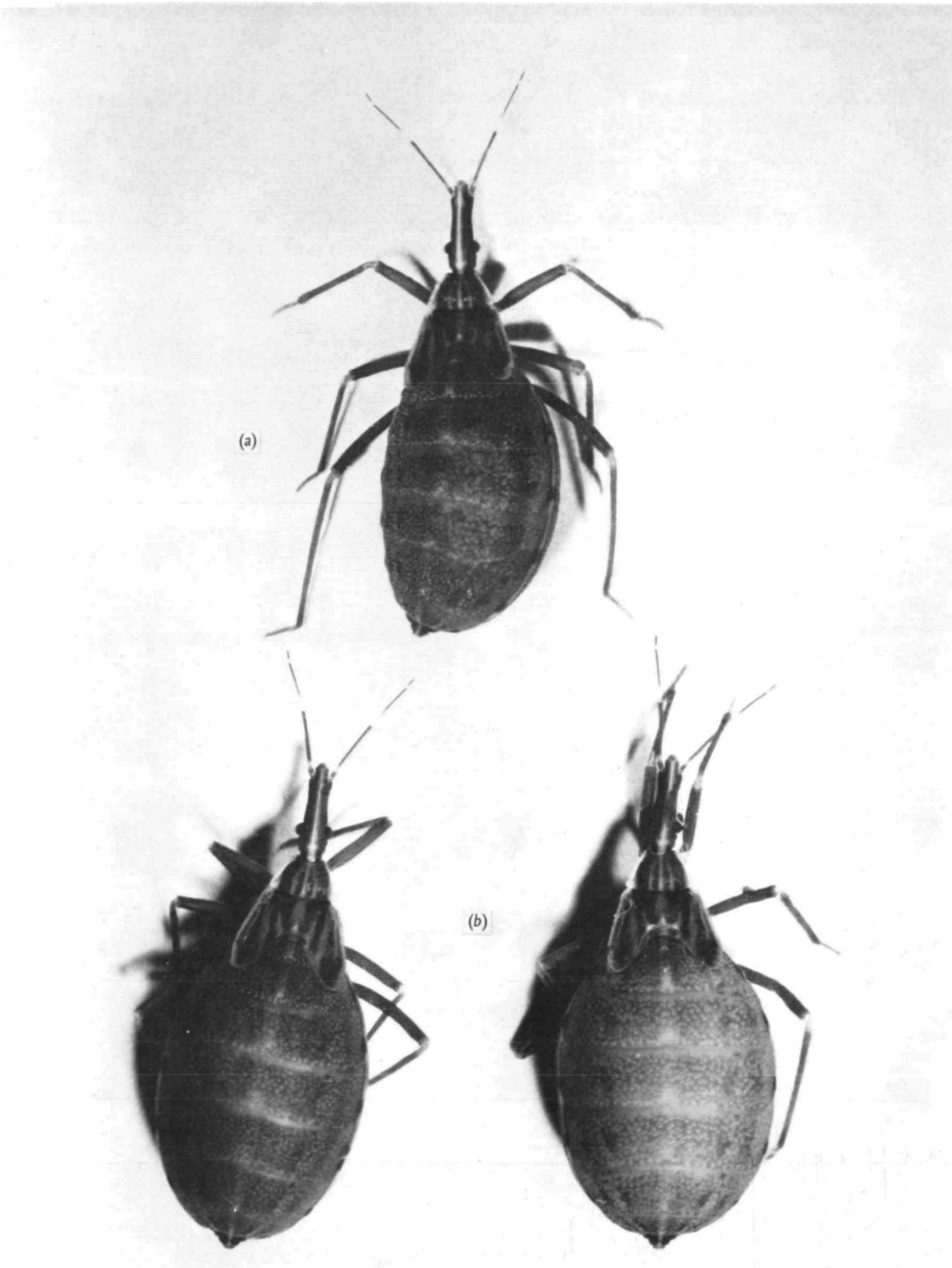
EXPLANATION OF PLATE

(*a*) The appearance after feeding of an insect in which the left-hand side of the abdomen was denervated before feeding.

(*b*) Two fed insects; on the left a normal fed insect and on the right an insect fed after its abdomen had been denervated. Note that the stretched denervated cuticle becomes opaque and white.


(a)
(b)