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Phil. Trans. R. Soc. Lond. B 1964 **248**, 279-314
doi: 10.1098/rstb.1964.0013

THE RECTAL COMPLEX OF THE MEALWORM *TENEBRIO MOLITOR*, L. (COLEOPTERA, TENEBRIONIDAE)

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(*Received* 29 *January* 1964)

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The rectal complex of the mealworm is an example of the 'cryptonephric' condition, wherein the distal ends of the Malpighian tubules are closely applied to the rectum and enclosed with it in a special chamber, the perinephric space, which is separated from the rest of the body cavity by the perinephric membrane. Wigglesworth has suggested that this arrangement serves to assist in the removal of water from the faeces by the rectal epithelium, but its physiological mechanism has remained virtually unexplored.

In the present work the ability of the rectal complex to remove water from the faeces has been quantified and it is shown that the faecal pellets are in equilibrium with an atmosphere of 90 % relative humidity (average) and sometimes of as low as 75 % relative humidity.

The concentration of the perinephric fluid is greater than that of the haemolymph (first observed by Saini); when the insect is deprived of water the freezing-point depression of the perinephric fluid may reach 8 °C at the posterior end of the rectal complex, and is mainly due to some non-electrolyte.

The freezing-point depression of the tubular fluid from the perirectal tubules is always very close to that of the perinephric fluid and can be almost completely accounted for as potassium chloride which may reach a concentration of over 2 M.

The perinephric membrane is relatively impermeable to water. Water injected into the rectal lumen is quickly absorbed into the perinephric space and eliminated via the tubules.

It is suggested that the physiological mechanism of the rectal complex is as follows. In water-deprivation the concentration of the haemolymph rises and this activates the rectal complex. Activation involves the secretion of potassium chloride from the haemolymph into the perirectal tubules (either directly or indirectly) whereby water is passively drawn into the tubules from the perinephric fluid. The concentration of the perinephric fluid is thereby increased and the work required to be done by the rectal epithelium in removing water from the faeces is decreased.

Vol. 248. B. 748. (Price 13*s*. 6*d*.: U.S. $2.00) 35 [Published 10 December 1964

1. Introduction

The Malpighian tubules of insects open into the alimentary canal at the junction between midgut and hindgut. In most insects the tubules for the greater part of their length lie freely in the haemocoel and do not come into any special relationships with other organs of the body. There are, however, several variants of the normal relationship, which have been classified and figured by Wigglesworth (1934). Notable among these is the arrangement described as 'cryptonephric' or 'cryptonephridial'* in which the distal, i.e. the blind, ends of the tubules are assembled and closely applied to the wall of the rectum and lie there in a space which is separated by a membrane from the rest of the body cavity. This association will be referred to as the 'rectal complex'. An admirable review of the occurrence of the cryptonephric condition in insects has recently been written by Saini (1962). The present work is concerned only with the cryptonephric condition as it exists in the larva and adult of the mealworm, *Tenebrio molitor* L.

The structure of the rectal complex has been worked out in considerable detail, as will be described in §2, but there has been little exploration of its physiology by experimental methods. Up to 1962 the only published experimental work was that of Patton & Craig (1939) who showed that ^{24}Na passed from the rectal lumen into the fluid bathing the rectal complex. In the absence of physiological facts speculation as to the function of the rectal complex has been unrestrained; an account of the various speculative theories may be found in Saini's thesis. Only one of these need be considered here, that first put forward by Wigglesworth (1934): '...the precise significance of the arrangement is not known; perhaps this serves to add the absorptive powers of the Malpighian tubules to those of the rectal epithelium'. Following up the idea Saini measured the freezing-point depression of the fluid collected from the space in which the perirectal tubules lie and found it to be greater than that of the haemolymph but less than that of the fluid in the rectum. He concluded: 'It appears that these reassociated Malpighian tubules, since they do not show any excretory products in their lumen, are simply secretory, secreting water and some salts from the perinephric chamber to their lumen. By doing so they increase the osmotic pressure of the fluid which becomes quite thick inside the perinephric chamber and consequently between the blood and the rectum there is the chamber which has an osmotic pressure higher than that of the blood but lower than that of the rectum. This higher osmotic pressure in the perinephric chamber enables the modified epithelium to do less work in the absorption of water and nutrient salts from the faecal waste and makes it possible for the epithelium to extract the maximum amount of water'. Saini's findings—but not all his interpretations—have been confirmed and extended in the course of the present work, and support is given to the suggestion put forward by Wigglesworth as to the function of the rectal complex.

2. The structure of the rectal complex

(*a*) *General*

In his thesis aforementioned, Saini has given an account of the discovery and early investigation of the cryptonephric condition in insects. It is found in some Coleoptera

* The terms 'cryptonephric' and 'cryptonephridial' have unfortunate morphological overtones. A better term 'cryptosolenic' was suggested by Lison (1937*a*) but does not seem to have come into general use.

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and in the larvae of some Lepidoptera. In *Tenebrio* the cryptonephric system has been most closely studied by Poll (1932, 1934), by Lison (1937*a*, *b*) and by Saini himself. A brief account will now be given of the main anatomical features about which there is general agreement.

The hindgut of *Tenebrio* is of relatively considerable length. From the pylorus (at the posterior end of the midgut) the intestine bends sharply to run forwards a short distance, then bends sharply again and runs back to merge gradually into the rectum. The intestine is narrow and very muscular and the circular muscle is normally contracted, relaxing only where a bolus from the midgut is in transit to the rectum. The rectum may be

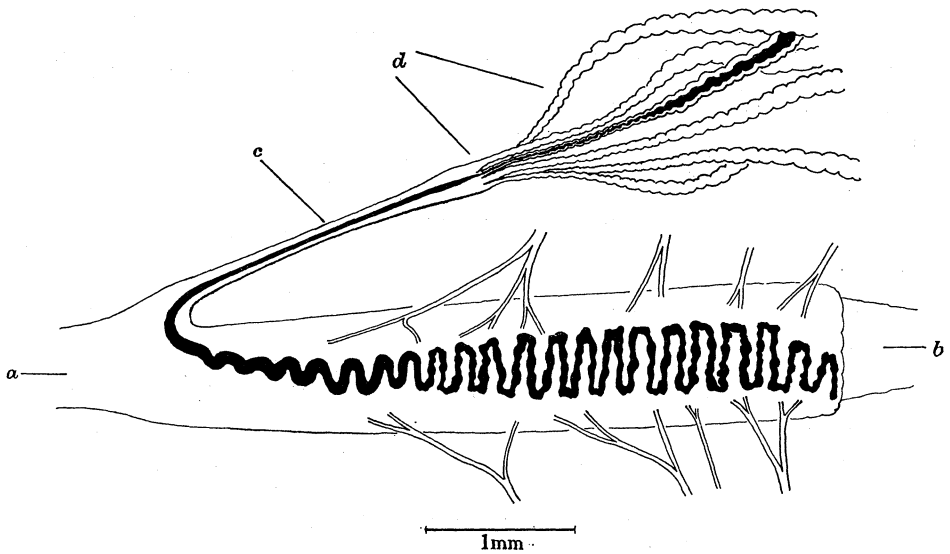

FIGURE 1. The rectal complex seen from the ventral side with the common trunk drawn to the left; one of the tubules injected with indian ink. *a*, intestine; *b*, anal canal; *c*, common trunk, *d*, region of peristalsis.

taken to be that part of the hindgut to which the Malpighian tubules are applied and which is enclosed, together with the tubules, by the perinephric membrane. There are six Malpighian tubules in *Tenebrio*, each of which has its distal end applied to the rectum under the perinephric membrane. These distal perirectal portions of the tubules are conspicuously different from the free portions which lie exposed in the haemocoel. The latter are of the usual diameter for insect Malpighian tubules and are fairly deeply pigmented by brown granules; the perirectal tubules are for the most part of smaller diameter and lack pigment. Their blind ends lie at the posterior end of the rectum and in this region they are closely folded upon themselves in chinese-cracker pattern and packed tightly together (figure 1). They have thick walls and their narrow lumina are dilated into what Lison has called 'boursouflures', to be described in more detail presently. About half way along the rectum the character of the perirectal tubules changes. Their folding and packing become looser, their walls become thinner and their lumina wider. Each tubule is now well separated from the others and each follows a zig-zag course to the anterior end of the rectum. Here all six are gathered together into a common trunk, still enclosed in an extension of the perinephric membrane, and run backwards ventral to the rectal

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complex nearly as far as its posterior end. The enclosing perinephric membrane now becomes vanishingly thin and the tubules separate from one another and presently assume the pigmented appearance of the free portions. Over the region where they emerge from the perinephric membrane they have contractile walls and are often seen to undergo peristalsis. The posterior boundary of the rectal complex is sharply distinct; thereafter a short and very muscular canal leads to the anus.

The boursouflures are arranged along each tubule as a series of closely spaced dilatations extending radially towards the perinephric membrane. Each boursouflure contains three cleft-like extensions of the lumen, elongated in the direction of the axis of the tubule, one medial and two lateral. Over the top of the boursouflure the perinephric membrane is thin and transparent and it comes into contact with the walls of the tubule in the region of the medial cleft. As they approach the perinephric membrane the walls of the tubule become thinner and in the zone of actual contact there is a little transparent window known as a leptophragma (figures 2, 3). This appears to be formed out of a single small cell whose nucleus is displaced to one side and hangs down into the lumen of the tubule. After treating the rectal complex with silver nitrate Lison (1937*a*) observed the formation in the region of the leptophragma of a white precipitate which later blackened upon exposure to light; he interpreted this as indicating the precipitation of silver chloride in the substance of the leptophragma.

In the present work no systematic attempt was made to re-investigate the structure of the rectal complex. But as a by-product of the physiological investigations certain structural details as yet undescribed have come to light, and these will now be presented.

(*b*) *The perinephric membrane*

What is meant by this term is not always clear. Some authors, e.g. Saini, use it to describe the outermost layer of the membrane which encloses the rectal complex and distinguish between it and 'pads of connective tissue' which extend inwards from it among the perirectal tubules. But if the membrane which covers the rectal complex is seized with fine forceps and stripped off, it comes away as one piece including the connective tissue pads. The view taken here is that the perinephric membrane is a complex structure and is made up of laminae, the lower laminae being folded inwards to produce the so-called connective tissue pads.

The rectal complex is well supplied with tracheae. Six small tracheae enter at the anterior end, following the bands of longitudinal muscle, and break up into tracheoles in the region anterior to the boursouflures. The perinephric membrane is supplied by numerous tracheal trunks which branch profusely over its outer surface. Wigglesworth (personal communication) has observed that these tracheae break up into dense bunches of tracheoles within the inner folds (connective tissue pads) of the perinephric membrane. No tracheae or tracheoles extend from these folds to the perirectal tubules, which appear to be without tracheal supply (also noted by Poll 1934). This arrangement underlines the concept of the unity of the perinephric membrane, which may be thought of as a folded, richly tracheolated, membrane investing the boursouflures of the perirectal tubules and apparently providing the only route by which oxygen can reach these structures and the muscles and rectal epithelium below.

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There are, however, regions where the outer layer separates from the lower layers and these are found over the boursouflures (figure 2*A*). Because the perinephric membrane undergoes considerable contraction after treatment with certain fixatives, drawings

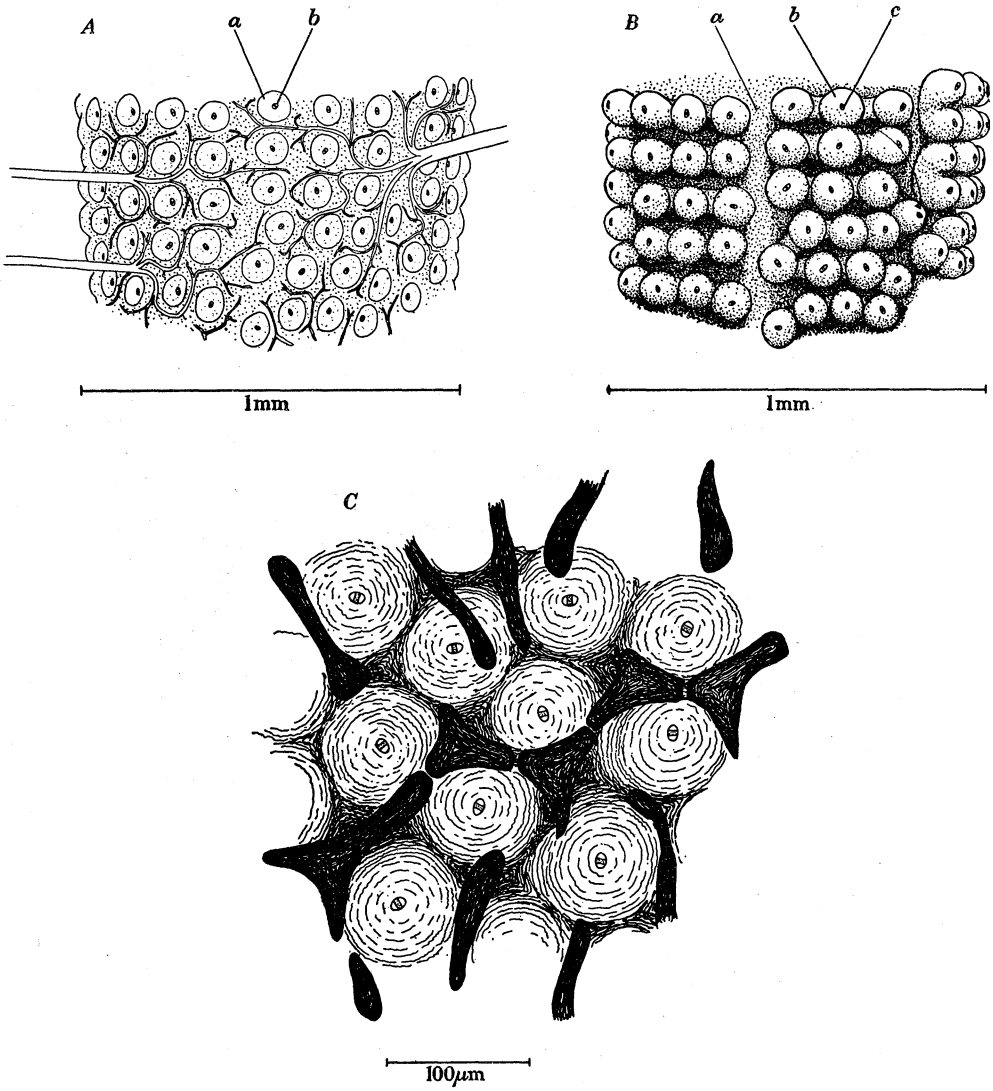

FIGURE 2*A*. Surface view of part of rectal complex, stained with silver to show leptophragmata; also showing the blisters covering the leptophragmata and the tracheae entering the perinephric membrane. *a*, blister; *b*, leptophragma.

B. Rectal complex, stained with silver to show leptophragmata; the perinephric membrane has been stripped off leaving the leptophragmata attached to the boursouflures of the perirectal tubules. *a*, rectal musculature; *b*, boursouflure; *c*, leptophragma.

C. Under the side of perinephric membrane, stripped from rectal complex and stained with osmic acid. The dark areas, which are close to the observer, are the folds which pass between the boursouflures. In this case the leptophragmata have come away with the perinephric membrane and are seen at the bottom of depressions into which the boursouflures fitted.

based on sections often fail to show that the outer layer is elevated to form a sort of blister over each boursouflure; but these blisters are very conspicuous in fresh material and can be seen in material fixed with osmic acid.

It is through these transparent blisters that the leptophragmata can be seen. When the perinephric membrane is stripped off, the leptophragmata sometimes go with the membrane, sometimes remain with the tubules, depending upon the previous treatment of the rectal complex. When the leptophragma is attached to the stripped perinephric membrane one can see that it is traversed by a band of some thicker material (figure 2*C*). When the leptophragma is left behind, the edges of the hole which it occupied in the perinephric membrane can be sharply focused. It therefore appears that the lower layers of the perinephric membrane end sharply upon the circumference of the leptophragma, an impression which is also gained from the examination of sections (figure 3).

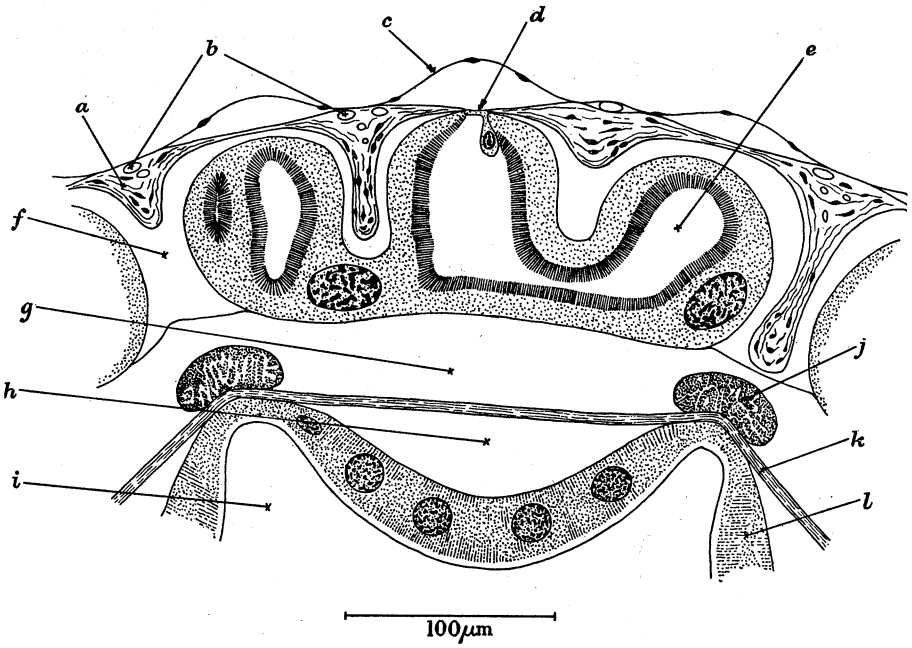

FIGURE 3. Transverse section of the wall of the rectal complex. *a*, perinephric membrane; *b*, tracheae; *c*, blister; *d*, leptophragma; *e*, lumen of perirectal tubule; *f*, peritubular space; *g*, perirectal space; *h*, subepithelial space; *i*, rectal lumen; *j*, longitudinal muscle; *k*, circular muscle; *l*, rectal epithelium. Partly after Lison (1937*a*). In most sections the tubular lumen underneath the leptophragma is very much narrower than shown here.

As will be described later, the perirectal tubules contain a solution of potassium chloride hich may be of high (up to 2M) concentration. The leptophragma being very thin it is not surprising that in Lison's silver staining method there is some interdiffusion of silver nitrate and potassium chloride within the thickness of the leptophragma, with precipitation of silver chloride and its subsequent reduction by light. According to Lison's procedure the rectal complex is first washed for about 30 s in distilled water and is then placed for about 30 s in dilute silver nitrate. In order to make this treatment physiologically less drastic the following variant was tried. A chloride-free Ringer was made up from the nitrates of sodium, potassium and calcium (for composition of normal Ringer see p. 305). After the rectal complex had been washed in this chloride-free Ringer it was transferred to chloride-free Ringer containing silver nitrate at a concentration of 0·0 1N The course of the staining of the leptophragmata was not affected by this change in procedure.

In further pursuance of this approach the rectal complex was left in chloride-free Ringer plus silver nitrate for longer periods, of up to 2 h. The deposit of silver at each leptophragma was now seen to be more extensive but no silver appeared anywhere else. The rectal complex was then washed in chloride-free Ringer and placed in photographic developer, whereupon it immediately went black all over; but this blackening was mainly restricted to the perinephric membrane, as could be seen when it was stripped off. After 15 min exposure to silver nitrate the blackening affected the outer layers of the membrane only, but after 30 min exposure the inner folds were also blackened. These observations may indicate a slow rate of penetration of silver ion through the perinephric membrane and lend support to the view, based upon physiological experiments to be described later, that it is in general a very impermeable structure. But the interpretation of silver staining is difficult and this problem will be taken up again in the Discussion.

The observation that silver is readily deposited at the leptophragma and less readily in the thickness of the perinephric membrane suggests that silver ion must pass quickly through the blisters of the perinephric membrane. If this is the case then it is surprising that no precipitate of silver chloride (and no deposit of metallic silver) has been seen at the surface of the blister or inside it. But if the preliminary washing in chloride-free Ringer is reduced from 30 s to 2 s the blisters blacken at once in photographic developer and after about half an hour a deposit of metallic silver gathers into the narrow space around the margin of the blister. The explanation appears to be that the blister membrane is extremely permeable to chloride and that a 30 s exposure to chloride-free Ringer is sufficient to allow nearly all the chloride to diffuse out.

(*c*) *The perinephric space*

It may be inferred from Saini's use of this term that he envisages the space between the perinephric membrane and the rectal epithelium as comprising a single compartment. Poll (1934) distinguishes a compartment between the rectal epithelium and the circular muscle. The separate existence of this compartment, as defined by the injection of indian ink, has been confirmed in the present work, and it will be called the subepithelial space. But this is the only compartment which Poll recognizes. There is, however, a very thin membrane which runs along the inner borders of the perirectal tubules; it is difficult to see in sections and Poll did not think that it was continuous. But injections of indian ink leave no doubt as to the continuity of this membrane which separates two compartments, to be called the peritubular space and the perirectal space. In the course of the present work attempts to inject the perirectal tubules with indian ink (as has been successfully accomplished in the case illustrated in figure 1) sometimes failed because the tip of the pipette had penetrated not into a tubule but into the space between tubules. The injection then spread diffusely between the tubules over the posterior region of the rectal complex and over the anterior region it formed a large irregular swelling. It never entered the perirectal space. Sections showed that the ink in the swelling lay between the perinephric membrane and the lower membrane whose existence is doubted by Poll. It is thus shown that there is indeed a continuous membrane separating a peritubular space from a perirectal space and that the thin-walled perirectal tubules of the anterior region are enclosed in the peritubular space which is effectively obliterated in the regions between the tubules

by the close apposition of the lower membrane to the perinephric membrane. Confirmation was provided by experiments to be described later (p. 295) in which the perirectal tubules were injected with mammalian blood. When the injection pressure exceeded 15 to 20 cmHg the thin-walled perirectal tubules burst, and when this happened the injected blood passed into, and distended, the peritubular space and never entered the perirectal space.

The perinephric space is thus seen to comprise three compartments: the peritubular space, the perirectal space and the subepithelial space. These compartments are defined as being separated by membranes impermeable to indian ink. This does not necessarily mean that there is any physiological significance in the subdivision of the perinephric space into compartments. With some fixatives, e.g. aqueous Bouin, an amorphous coagulum ('granulations' of Poll 1934) forms in the subepithelial and perirectal spaces and seems also to be present in the peritubular space though this is less easy to observe. The presence of this coagulum suggests that its precursor—presumably soluble protein—is distributed throughout the three compartments of the perinephric space and therefore that the membranes separating these compartments are permeable to molecules of large size.

Saini has concluded that the perinephric membrane is fused anteriorly to the surface of the intestine and that the perinephric space is isolated from the haemocoel. Poll (1934) observed that dyes added to the haemolymph penetrated into the anterior part of the perinephric space and concluded that over its anterior region the perinephric membrane was very permeable. My own experience is not in full accord with that of Saini or with that of Poll. I have never observed the passage of dyes from haemocoel to perinephric space. But I have found that if indian ink is injected into the perirectal space from a fine pipette thrust through the layer of tubules at the posterior end of the rectum the ink passes forward in the perirectal space but does not noticeably distend it. The ink presently escapes into the haemocoel from some ill-defined aperture a little way anterior to the point from which the common trunk originates. This observation has been many times repeated, with all possible precautions to avoid disturbance of the structures lying in this region, and there is no reason to believe that it results from damage to the perinephric membrane which is here very thin. If a ligature of thin nylon is tied around the rectal complex just anterior to the origin of the common trunk there is no escape of ink and the perirectal space becomes distended. This seems to indicate that the perinephric membrane is not fused to the surface of the intestine but merely invests it as a close-fitting sleeve. Such an arrangement would permit the escape of fluid accumulating in the perirectal space but would act as a valve to hinder movement of fluid in the opposite direction.

3. The ability of the rectal complex to remove water from the faeces

Tenebrio lives both as larva and adult in dry stored products such as meal and bran without access to liquid water. It produces faecal pellets which are to all appearances dry, like the material upon which it feeds. Wigglesworth (1932) noted the ability of the rectal glands of *Tenebrio* to reduce the faeces to 'a bone-dry powder' and, as already mentioned in §1, he suggested that the cryptonephric system contributed to this ability. The ability of the mealworm to remove water from its faeces was also commented upon,

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though without reference to the cryptonephric system, by Schulz (1930). Schulz determined the water content of the bran upon which mealworms were fed and of the faeces which they passed. He found that the faeces, which were largely of undigested bran, had a lower water content (7 to 9%) than that of the uneaten bran (10 to 12%), and he suggested that mealworms eat excessively in order to be able to make a net gain of water from this small disparity in water content. Schulz collected faeces from the culture vessels in which his mealworms were reared, and in making this suggestion he overlooked the fact that the faeces would have had time to come into equilibrium with the atmosphere in the culture vessel; before one can decide whether or not this is a practicable means of gaining water it is necessary to know the water content of the faeces as they leave the anus.

(*a*) *The water content of the faecal pellets*

The most detailed study of the ability of the rectal glands of insects to remove water from the faeces is that of Phillips (1964). His method was to fill the rectum with a fluid of known composition and to determine the changes in composition after lapse of time. He attempted to apply this method to the mealworm but was unable to withdraw any fluid from its rectum (personal communication). This is not altogether surprising. Water is removed so completely from the faeces that the spaces between the faecal pellets and the wall of the rectum become filled with air. Presumably water must be removed as water vapour, which suggests that the humidity maintained in the air spaces of the rectum would be a useful measure of the ability of the rectal glands to remove water from the faeces.

The possibility of inserting a hygrometer into the rectum was considered but was rejected on grounds of technical difficulty. A more promising line of approach seemed indicated by the fact that, as Schulz has shown, the faeces are hygroscopic. A relation must exist between the water content of the faeces and the water vapour pressure with which they are in equilibrium; if it were possible to determine the water content of a faecal pellet after its expulsion from the anus a maximum value for the water vapour pressure in the rectum could be obtained.

To make a reliable determination of the water content of a faecal pellet presents certain problems. The faecal pellet is about 0·3 mm in diameter and it is obvious that so small a body will very quickly come into equilibrium with the atmosphere to which it is exposed. As may be seen from figure 6 changes in weight of the order of 10% can take place within 30 s after a change from moist air to dry. It is therefore necessary to determine the water content within a very few seconds after the expulsion of the pellet. The simplest method of determining water content is based upon weighing. The average weight of a faecal pellet is 10μg and let us suppose that it is required to weigh it to ± 1%. This calls for a balance sensitive to 0·1μg. A general account of the techniques of microweighing is given by Kirk (1951). These techniques depend upon the use of quartz fibres. One method makes use of an orthodox balance with beam and scale pans suspended on a stretched quartz fibre; the disadvantage of this design for the present purpose lies in the relatively long period of the balance. Other methods depend upon the elastic properties of a quartz fibre in the form of a vertical helix or of a horizontal beam clamped at one end; this design is useful only if the weight of the pan is small compared with the weight of the object to be weighed. It was not found possible, even using aluminium leaf, to construct an adequate pan which

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weighed less than 10 μg. After much unfruitful experimentation with balances an extremely simple solution was found: if a quartz fibre is coated with a thin layer of grease the pellet will adhere to it, thus making it possible to dispense with the pan.

A horizontal quartz fibre clamped at one end was the basis of the balance used. The free end of the fibre was lightly smeared with petroleum jelly ('Vaseline'). The faecal pellet, falling from the animal's anus, was directed through a glass tube at the quartz fibre, and upon striking the fibre the pellet almost invariably adhered to it. The fibre was

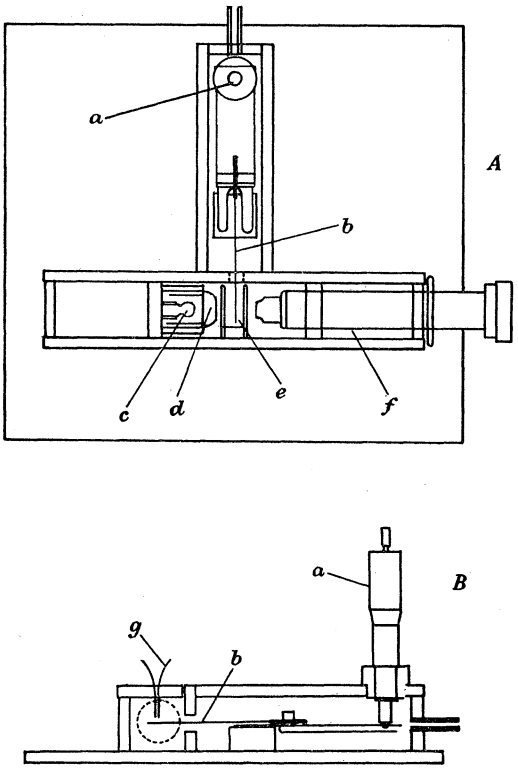

FIGURE 4. Apparatus for weighing faecal pellets. *A*, plan; *B*, vertical section. *a*, micrometer head; *b*, quartz fibre; *c*, lamp; *d*, condenser; *e*, fibre space; *f*, microscope tube; *g*, funnel. Further explanation in the text.

viewed through a horizontally placed microscope and when deflected by the weight of the pellet it could be brought back to the reference mark by tilting its fixed end. The tilting movement was driven by a screw micrometer and the deflexion of the fibre was recorded in terms of the movement of the micrometer (see figure 4). The fibre was 6 cm long and 25μm in diameter and the maximum deflexion of its free end did not exceed 3 mm; it was assumed that for such small deflexions the weight of the pellet was proportional to the movement of the micrometer. The deflexion produced by a 10μg pellet was about 1 mm and the micrometer could be read to the nearest 0·005 mm.

The humidity of the air surrounding the free end of the fibre (to be referred to as the 'fibre space') was roughly controlled by means of solutions of known vapour pressure soaked up in small sheets of filter paper which were arranged on either side of the fibre. These sheets of filter paper were mounted upon a brass framework which could be lowered into position through the roof of the apparatus. In the earlier experiments the filter paper

was moistened with distilled water. When it was found that the faecal pellets took up water from the fibre space thus conditioned, saturated KCl was used instead, the aim being to minimize the rate of change of weight of the pellet after its attachment to the fibre.

The eyepiece of the microscope was replaced with a fitting which incorporated a translucent screen having a phototransistor mounted in the centre. The image of the fibre was alined with a reference mark on the screen. In order to relieve the observer of the necessity of watching continuously for long periods for the arrival of a faecal pellet it was arranged that when a pellet dropped through the field of view its shadow fell upon the phototransistor and thereby operated a warning buzzer. I am indebted to Mr D. M. Unwin for the circuit of the transistor amplifier and relay required to operate the buzzer; this circuit did not incorporate any novel features of design and is therefore not reproduced here.

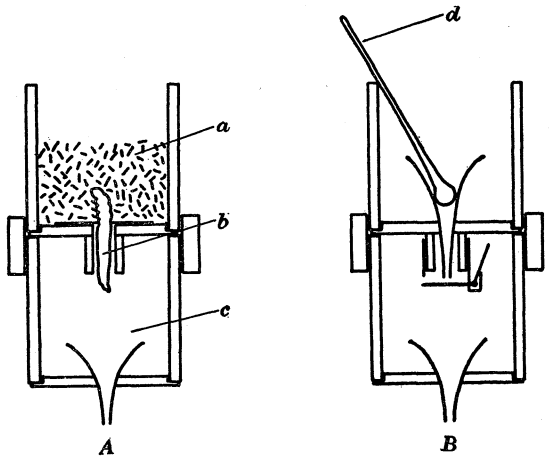

FIGURE 5. *A*, mealworm mounted in rubber diaphragm with access to bran; *B*, apparatus for equilibrating faecal pellets with atmospheres of known relative humidities before allowing them to drop into the weighing apparatus. *a*, bran; *b*, mealworm; *c*, animal space; *d*, stopper. Further explanation in the text.

The mealworm was mounted in a small Perspex vessel which is illustrated in figure 5*A*. The abdomen of the mealworm was passed through a hole in a thin rubber diaphragm which held the animal just behind the thorax. Above the diaphragm the vessel was filled with bran upon which the animal fed. This vessel carrying the mealworm fitted into a lower vessel having a glass funnel which served to collect the faecal pellets and to conduct them to the glass tube of the quartz-fibre balance. The lower vessel was lined with filter paper moistened with a solution of known vapour pressure which controlled the humidity within the vessel (to be referred to as the 'animal space'). About 1 in 3 of the mealworms so mounted continued to feed and to produce faeces for many days.

The apparatus shown in figure 5*B* was used for purposes of determining the relationship between the water content of faecal pellets and the water vapour pressure with which they were in equilibrium. In the upper Perspex vessel the rubber diaphragm was replaced with a glass funnel leading to a small platform of aluminium foil. A pellet introduced through the funnel settled on the platform, after which the funnel was closed with a stopper. The walls of the chamber were lined with filter paper moistened with a solution of known vapour pressure, and after sufficient time had been allowed for equilibration the

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platform was tipped, allowing the pellet to fall into the lower glass funnel and so to the balance. The tipping of the platform could be brought about without opening the apparatus by rotating the upper vessel upon the lower vessel so as to bring the 'handle' of the platform against a stop.

Procedure was as follows. Several mealworms were kept mounted in upper vessels (figure 5*A*). One which was observed to be defaecating regularly was selected and placed upon the lower vessel. 15 min were allowed for the humidity of the air in the animal space to reach its equilibrium value and the two vessels were then moved into

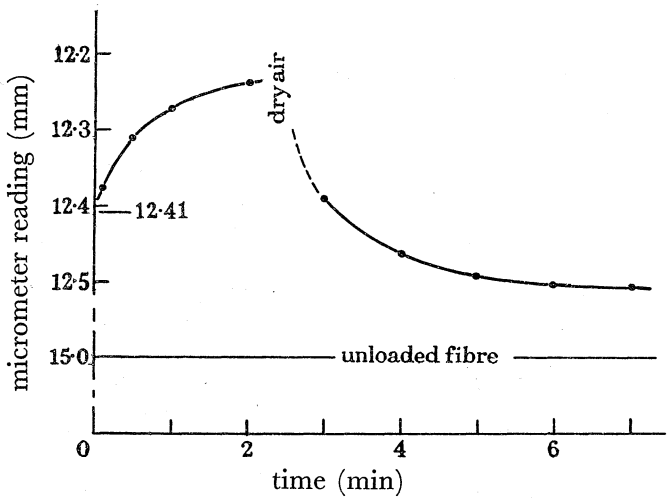

FIGURE 6. Record of changes in weight of a faecal pellet, obtained with the apparatus illustrated in figures 4 and 5*A*. Decrease in micrometer reading indicates increase in weight.

position over the balance. The fibre was brought to the reference mark and the reading of the micrometer was noted. When the buzzer announced that a faecal pellet had dropped, the observer (who sat within reach of the apparatus) started a stopwatch and as rapidly as possible adjusted the micrometer to bring the fibre back to the reference mark. As soon as this had been done the time was recorded and then the reading of the micrometer. The time to the first reading was usually of the order of 5 s and seldom exceeded 10 s.The second reading was taken at 30 s and the third and fourth at 1 min and 2 min respectively. These four readings were a sufficient basis for extrapolation back to zero time (see figure 6). After the fourth reading had been taken the metal frame carrying the moist filter paper which flanked the fibre was removed, and a slow stream of air from the laboratory compressed air supply was admitted to the balance housing. The relative humidity of this air supply was low (10 to 15% r.h.) and relatively constant. The micrometer reading after the pellet had been exposed to the air-stream for 5 min was used to give the 'dry weight'; the micrometer reading extrapolated to zero time was used to give the 'wet weight', and this was expressed as a percentage ($> 100\%$) of the dry weight. The apparatus was operated at room temperature which varied between 19 and 21 °C during the period of these experiments, but over the duration of any single determination the temperature was effectively constant.

For its validity the method depends upon there being no significant change in the water content of the pellet from the moment of its leaving the animal to the moment of its

contacting the quartz fibre. The fall of the pellet occupied only a fraction of a second and as far as this is concerned the required conditions were met. It was not impossible, however, that the pellet might have remained attached to the anal region (and thus exposed to the atmosphere in the animal space) for some time before it dropped. The act of defaecating was observed a number of times in a mounted mealworm viewed from below by means of a mirror on the stage of a stereomicroscope. Once it was fully extruded the pellet invariably dropped at once, but occasionally the expulsion of a pellet required several efforts and at each of which one end of the pellet was momentarily exposed. The possibility of some change in water content from the time the pellet entered the anal canal to the time when it contacted the quartz fibre could not be ruled out.

TABLE 1

humidity-controlling solution	relative humidity* at 20 °C (%)	average wet weight† of dropped pellets (% dry wt.)	average wet weight† of equilibrated pellets (% dry wt.)
distilled water	100	118·7 ± 5·54 (7)	> 150
Na tartrate	92	117·0 ± 2·86 (4)	119·9 ± 2·67 (6)
KCl	86·5	113·6 ± 3·30 (12)	112·4 ± 1·27 (8)
NaCl	76·5	114·5 ± 2·94 (4)	106·7 ± 1·25 (6)

All the figures in column 3 were obtained with one and the same mealworm. The figures in column 4 were obtained with faecal pellets taken at random.

* These figures are taken from Janisch (1938). It is to be noted that the relative humidities maintained by these solutions are virtually constant over the range 19 to 21 °C.

† Mean ± standard deviation (no. of observations).

The extent of such change in water content was revealed by comparing the wet weights under two different conditions of humidity in the animal space; wet conditions were provided by moistening the filter paper with distilled water, dry conditions by removing the filter paper and placing small pieces of anhydrous calcium chloride on the floor of the vessel. The filter paper of the fibre space was moistened with distilled water. The average of 6 wet weights over calcium chloride was 102·2%, the average of 7 wet weights over distilled water was 118·7%. It was therefore clear that the observed wet weights were greatly affected by the humidity of the air in the animal space.

The validity of the method is compromised by these observations, but not fatally so. Provided that the exposure of the pellet to the atmosphere in the animal space is not sufficient for equilibrium to be reached it is possible to make allowance for the effect of this atmosphere by taking observations over a range of humidities in the animal space. At each humidity the wet weights of the pellets dropped by the animal are compared with the wet weights of pellets which have been allowed to come into equilibrium with this humidity; if the wet weight of the dropped pellet is less than the equilibrium wet weight it may be concluded that the dropped pellet, when it left the rectum, was in equilibrium with some lower humidity than that prevailing in the animal space—and vice versa.

Some of the results obtained from experiments carried out along these lines are summarized in table 1 and in figure 7. The intersection of the curves in figure 7 suggests that the faeces are, on the average, in equilibrium with an atmosphere of about 90% r.h.

In table 2 are reproduced the original observations of wet weights of pellets dropped by three different mealworms in the presence of saturated KCl. These figures indicate that

variation is considerable, from one pellet to another and from one animal to another. From the physiological point of view interest attaches to the extremes as well as to the means. It will be noticed that there are two observations in table 2, one of 104·5 for mealworm (*a*) and the other of 106 for mealworm (*c*) (both observations made with saturated KCl in the animal space) which seem to indicate that the animal is capable on occasion of so drying its faeces that they are in equilibrium with an atmosphere of about 75% r.h.

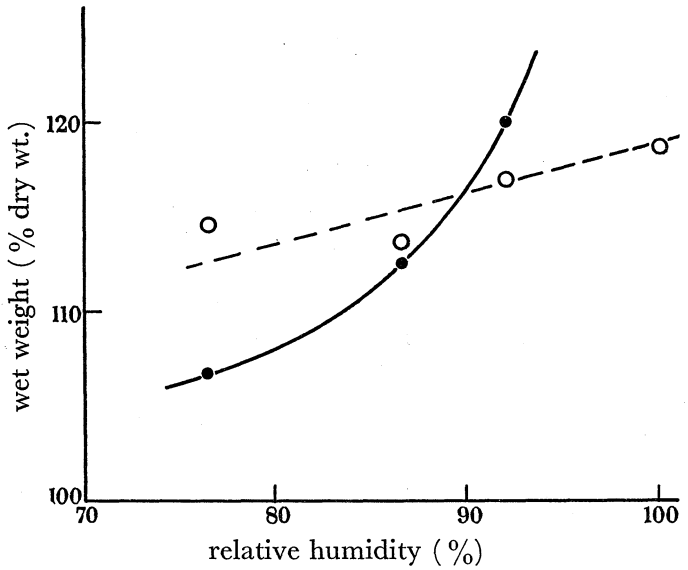

FIGURE 7. The relation between the wet weight (as % of dry weight) of faecal pellets and the relative humidities of the atmosphere to which they have been exposed in the animal space (figure 5). Further explanation in the text. ● equilibrated, ○ dropped.

It is possible that these observations are in error owing to some shortcomings of technique, but it is not easy to see what type of fault could produce a result of this kind. The data reproduced in figure 6 relate to the observation of 104·5 for mealworm (*a*). This particular observation was made in the middle of a series with the same mealworm, the observations immediately before and immediately after being both 115·5. Altogether nine micrometer readings were made on this pellet and they are obviously mutually compatible, which rules out an error in reading the micrometer. Prolonged exposure to the atmosphere in the animal space would not be expected to reduce the observed figure below 112. The humidity in the fibre space could not have been greater than 86·5% r.h. yet the pellet increased in weight while exposed to this atmosphere. It is therefore difficult to see why one should not accept this observation, and the other observation on mealworm (*c*), at their face values.

TABLE 2. WET WEIGHTS (AS % DRY WT.) OF PELLETS DROPPED BY THREE DIFFERENT MEALWORMS IN THE PRESENCE OF SATURATED KCl

Mealworm: (*a*) 115·5, 116·5, 112·5, 111·5, 113, 114, 117, 114·5, 113, 115·5, 104·5, 115·5
(*b*) 116·5, 118·5, 116·5, 116
(*c*) 115·5, 111·5, 106

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(b) The energy required to remove water from the faeces

The figures of 90% r.h. for the average and 75% r.h. for the extremes are in keeping with other observations. Mellanby (1932) found that mealworms came into steady state with an atmosphere of 88% r.h. and some insects maintain steady state at much lower values, e.g. 45% r.h. in the case of the prepupa of *Xenopsylla* (Edney 1947). The mealworm's ability to overcome forces tending to remove water from the body do not seem to be out of the ordinary; but since the faeces are voided at a relatively rapid rate it is of interest to know whether the removal of water from them requires a physiologically significant expenditure of energy.

The dry bran which the mealworm eats is passed back to the midgut where it is flooded with a relatively large volume of fluid which is isosmotic with the haemolymph. This water is removed during the passage of the faeces through the intestine and rectum; in the early stages dissolved materials are no doubt removed together with the water, but in the later stages, when the spaces in the rectum are filled with air, the water must be removed as vapour unaccompanied by non-volatile solutes.

For purposes of calculating the minimal energy required it is assumed that the following series of operations is performed isothermally and reversibly.

(i) a small mass, δm, of water is allowed to evaporate from the faeces at vapour pressure p.

(ii) this amount of water, as vapour, is compressed from p to p_0 where p_0 is the vapour pressure of the haemolymph.

(iii) the water vapour is allowed to condense into the haemolymph.

Since the latent heat of vaporization required for operation (i) is recovered during operation (iii) the net work is required for operation (ii).

Assuming that the water vapour behaves as a perfect gas the work required to compress 1 mole of water vapour from p to p_0 is

$$w = RT \ln \frac{p_0}{p}.$$

The work required to compress δm grammes of water vapour from p to p_0 is

$$\delta w = \frac{RT}{M} \ln \frac{p_0}{p} \delta m,$$

where M is the molecular weight of water—or at 20 °C—

$$\delta w = \frac{1{\cdot}98 \times 293}{18} \times 2{\cdot}303(\log_{10} p_0 - \log_{10} p)\, \delta m$$
$$= 132(\log_{10} p_0 - \log_{10} p)\delta m.$$

Let m be the number of grammes of water which have to be added to 1 g of dry faeces to bring them into equilibrium with an atmosphere of vapour pressure p at 20 °C. The relationship between p and m was established by equilibrating faecal pellets in bulk with various atmospheres of known vapour pressure and by determinations of freezing-point depression of suspensions of faecal pellets in known amounts of water. $\mathrm{Log}_{10} p$ was then plotted against m (figure 8). Using this graph the work required to bring 1 g (dry weight) of faeces from vapour pressure p_0 (corresponding to a water content of m_0) to vapour

pressure p_1 (corresponding to a water content of m_1) can be found as $w = 132A$, where A is the area bounded by the curve and by the lines $\log_{10}p_0$ and m_1. $\mathrm{Log}_{10}p_0$ was calculated on the assumption of 1·2 °C for the freezing-point depression of haemolymph; values of $\log_{10}p_1$ were chosen to correspond to integral values of relative humidity at 20 °C. The calculations gave figures as follows:

relative humidity with which faeces are finally in equilibrium (%)	energy required to dry 1 g of faeces to this value of relative humidity from being in equilibrium with haemolymph (cal.)
90	0·95
80	1·86
70	2·40
60	3·13

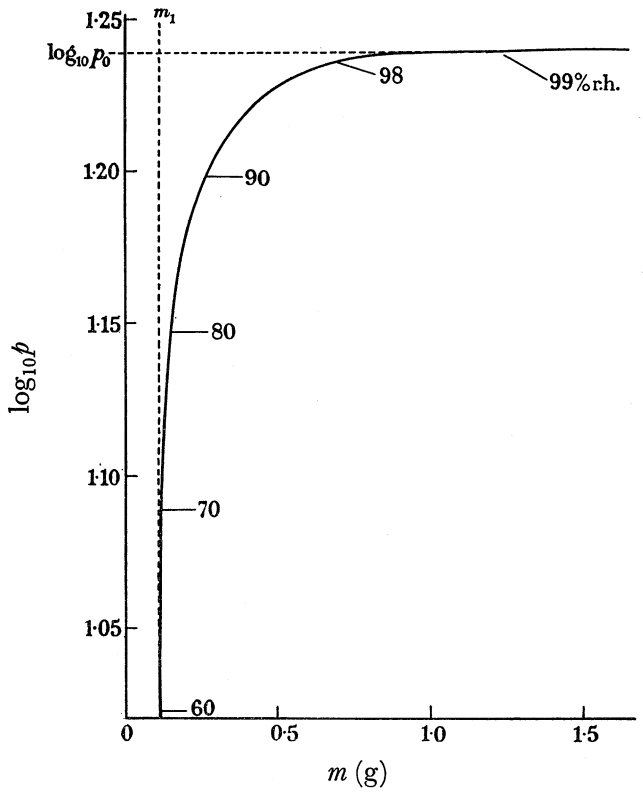

FIGURE 8. Graph relating m, the number of grammes of water added to 1 g of dry faeces, and p, the vapour pressure of water vapour (in mmHg) with which the faeces are in equilibrium at 20 °C. Further explanation in the text.

A mealworm has been observed to produce faeces at a rate of 0·112 mg dry weight/h. To bring 1 g (dry weight) of faeces from equilibrium with haemolymph to equilibrium with 60% r.h. requires 3·13 cal. To do the same for 0·112 mg requires $3{\cdot}5 \times 10^{-4}$ cal. The rate of oxygen consumption of the mealworm is 0·222 ml. $O_2\,g^{-1}\,h^{-1}$ at 13 to 14 °C (Heilbrunn 1952); assuming a Q_{10} of 2·5 this would give a value of 0·455 ml. $O_2\,g^{-1}\,h^{-1}$ at 20 °C. Taking 1 ml. O_2 consumed as equivalent to 4·9 cal. produced, the heat production is 2·23 cal. $g^{-1}\,h^{-1}$. The weight of an average mealworm being 0·1 g, the maximum energy

available to it is 0·223 cal./h. Expressed as a percentage of the maximum available energy, the energy required to dry the faeces to 60% r.h. is only 0·15%. Even if generous allowance is made for the inefficiency of the processes involved, there seems to be ample energy available.

I wish to record my thanks to Dr K. E. Machin for kindly checking the argument and calculations and for suggesting the method of graphical integration.

4. The physiological mechanism of the rectal complex

(*a*) *Preliminary observations*

Saini's measurements of freezing-point depression, referred to in §1, indicate that some relatively impermeable membrane must separate the perinephric fluid (of higher concentration) from the haemolymph (of lower concentration), and presumably this is the perinephric membrane. Low permeability of the perinephric membrane would also account for the marked difference between the free tubules and the perirectal tubules in their responses to gross concentration or dilution of the haemolymph. When the haemolymph is diluted with distilled water the free tubules become distended and any particles suspended in the tubular fluid are seen to be swept towards, and often into, the perirectal tubules. Conversely, when concentrated solutions of sucrose are added to the haemolymph the free tubules collapse while the perirectal tubules do not, and particles are seen to be swept in the opposite direction.

The effects of application of solutions of high concentration were further tested upon preparations in which ligatures had been tied around the intestine just anterior to the origin of the common trunk and around the common trunk itself. When such a preparation is flooded with 5 M sucrose (made up in Ringer, see p. 305) some decrease in the turgor of the perirectal tubules is detectable after about half an hour, and after 3 h the tubules are distinctly flattened. When the sucrose solution is injected into the perirectal space there is an immediate slight collapse of the perirectal tubules, followed by partial recovery.

These observations confirm the idea that the perinephric membrane is relatively impermeable to water. They suggest, by contrast, that the membrane separating the perirectal space from the peritubular space is very much more permeable. The failure of the perirectal tubules to collapse completely in response to the injection of 3 M sucrose into the perirectal space suggests that they contain fluid of high concentration; this is supported by measurements of freezing-point depression, to be described later.

While it seems natural to suppose that the tubular fluid flows from the perirectal tubules into the free tubules, this is not altogether easy to demonstrate. As mentioned in §1(*a*) there is a region, where the perirectal tubules emerge as free tubules from the common trunk, in which the tubules undergo peristalsis. Simple observation of the peristaltic region does not indicate that the movements are such as to propel the contents exclusively in one direction or the other. In order to observe the effects of these movements more closely recourse was had to the injection of visible materials, and for this purpose the most useful material was mammalian blood; under the high-power stereomicroscope the movements of individual corpuscles could be followed. Injection was usually made into one of the free tubules some distance from the region of peristalsis. When the injected blood

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penetrated to the region of peristalsis it happened more often than not that the injection broke through into neighbouring tubules and spread backwards along them. Some parts of the injection, however, would penetrate into the wide thin-walled parts of the perirectal tubules. It could then be seen that the principal effect of the peristaltic contractions was to produce a to-and-fro movement of the corpuscles superimposed upon which there might be a net movement in one direction. This could be either into, or out of, the perirectal tubules and could be in opposite directions in tubules lying side by side. Over long periods of observation, of the order of 1 h, it could generally be established that the injected blood was evacuated from the perirectal tubules into the free tubules.

It was also observed that the wide thin-walled parts of the perirectal tubules are normally in a state of turgor; when they are punctured, as with a micropipette, there is always a slight collapse of their walls even before any fluid is sucked out of them. On one occasion the injection pipette was successfully inserted into a tubule in the peristaltic region in such a way that 'reversible' conditions were established, i.e. the blood flowed into, or out of, the pipette according to the pressure applied. In this way it was established that the pressure in the perirectal tubules was of the order of 3 to 5 cmHg. This is definitely greater than the pressures measured in the tubules of the stick insect, where the corresponding figure is 1·5 cmHg (Ramsay 1954).

These observations do not suggest that the peristalsis operates so as to draw fluid out of the perirectal tubules, as supposed by Lison (1937*b*). On the contrary, they suggest that the effect of the peristalsis is to maintain turgor in the perirectal tubules and to allow a net escape of tubular fluid only when the pressure in the tubules exceeds a certain value.

(*b*) *The freezing-point depression* (Δ) *of the perirectal fluid in relation to availability of water*

As mentioned in §1, Saini collected fluids from the rectum and from the perirectal space and determined their freezing-point depressions, comparing them with the freezing-point depression of the haemolymph. His figures for *Tenebrio* are:

	Δ (°C)
haemolymph	0·916
perirectal fluid	1·65
rectal fluid	2·15

I have confirmed Saini's observations on the perirectal fluid and haemolymph, and indeed find that the difference in freezing-point depression can be even greater than Saini supposed.

In order to make collections of perirectal fluid the larva was opened under liquid paraffin. A sample of haemolymph was taken. The rectal complex was then quickly dissected free of its tracheal connexions and was pulled to one side. The common trunk was pinned away from the rectum, thus drawing the perirectal tubules and perinephric membrane away from the rectum on one side and making it possible to insert a pipette into the anterior part of the perirectal space, as shown in figure 9. Such collections were made on a number of animals kept under three different regimes: 'normal', being the laboratory stock culture; 'moist', fresh carrot being added to the bran; 'dry', the culture being enclosed in a desiccator over concentrated sulphuric acid. Freezing-point depression was measured according to the method of Ramsay & Brown (1955). The freezing-

point depression of the perirectal fluid was plotted against the freezing-point depression of the haemolymph, as in figure 10. It is clear from this figure that the relationship is an unusual one. As the freezing-point depression of the haemolymph increases up to 1·0 °C both fluids have the same freezing-point depression; as the freezing-point depression of

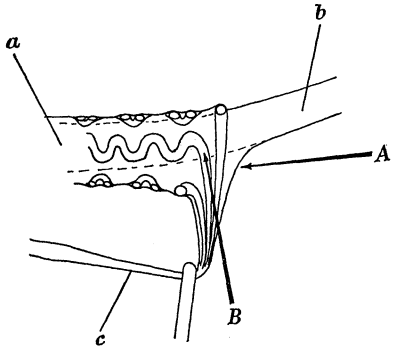

FIGURE 9. Anterior end of rectal complex with common trunk pulled to one side. Arrows indicate directions of penetration of pipettes for collection of: *A*, perirectal fluid; *B*, tubular fluid. *a*, rectum; *b*, intestine; *c*, common trunk.

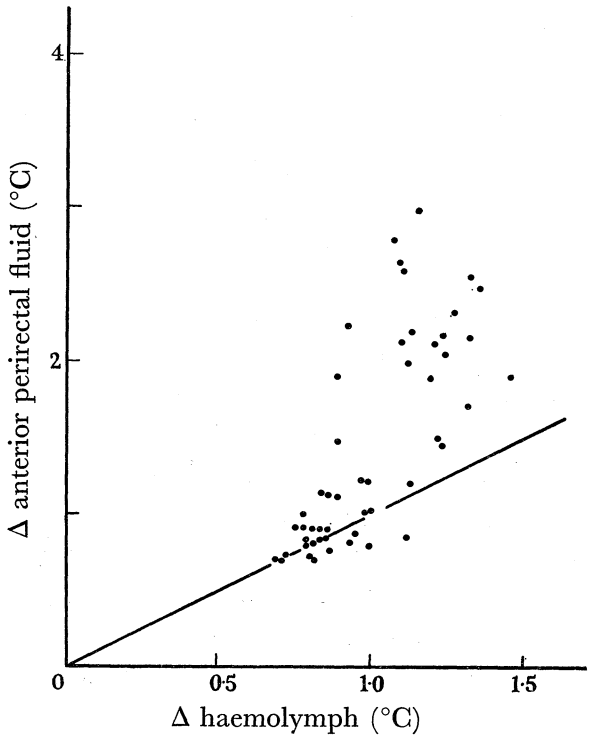

FIGURE 10. Freezing-point depression of the anterior perirectal fluid plotted against freezing-point depression of the haemolymph.

the haemolymph increases beyond 1·0 °C that of the perirectal fluid increases more rapidly. The freezing-point depression of the haemolymph never exceeded 1·5 °C, whereas that of the perirectal fluid was recorded as more than 4° C.

By inspection of the opened insect it can be seen that faecal matter passes down the intestine suspended in fluid, and that most of this fluid is removed in the anterior part of the rectum, where the faecal matter is first compacted into faecal pellets. It is reasonable

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to suppose that further water is progressively removed from the pellets as they pass along the rectum towards the anal canal. It is not surprising to find that the greater intensity of the drying process, which may reasonably be supposed to prevail in the posterior region of the rectal complex, is reflected in a still greater freezing-point depression of the perirectal fluid in the posterior region than in the anterior region.

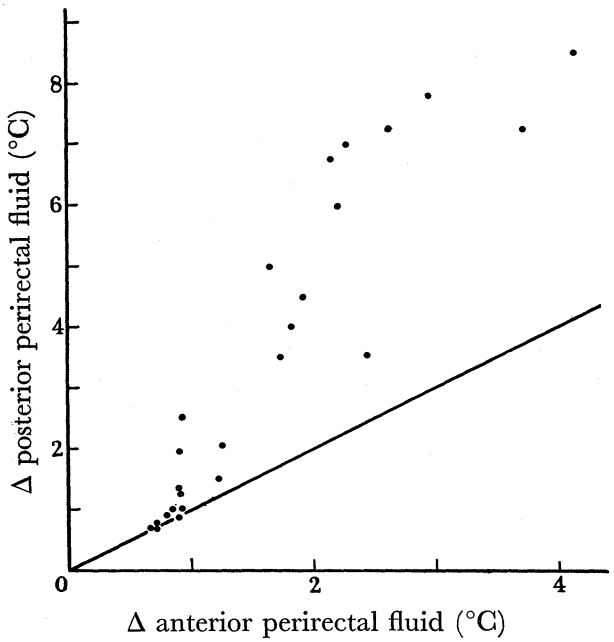

FIGURE 11. Freezing-point depression of the posterior perirectal fluid plotted against freezing-point depression of the anterior perirectal fluid.

Perirectal fluid is less easily collected from the posterior end of the perirectal space, which is narrower than the anterior end and contains very little fluid. In addition, it is not possible to see the tip of the pipette where it lies under the thick-walled perirectal tubules. The method of collection was as follows. The insect was opened under oil and a sample of anterior perirectal fluid was taken as already described. A silica capillary, such as is used to contain the sample for determination of freezing-point, was then inserted through the hole already punctured in the perinephric membrane and was passed along the perirectal space to the posterior end. Negative pressure was briefly applied and the

* When small ice crystals are observed under the microscope, as in the freezing-point method of Ramsay and Brown, one notices that large crystals grow at the expense of small ones and that the edges of the crystals are rounded—the natural consequences of surface tension at the water-ice interface. The change of state between solid and liquid is perfectly temperature-reversible. In these respects the tubular fluid collected from the perirectal tubules is entirely normal. By contrast, the crystals which appear in fluid from the anterior perinephric space tend to have a jagged outline and large crystals do not grow at the expense of small ones. Furthermore, the system is not temperature-reversible. As the temperature is raised the crystals decrease in size, but as the temperature is lowered they do not increase in size. After the temperature has been lowered by a few degrees the crystals suddenly begin to grow rapidly. On occasion undercooling of the order of 10 °C was observed (in the continued presence of small crystals) and then suddenly the whole sample appeared to solidify instantaneously. This effect is even more marked with fluid from the posterior perirectal space and from the midgut, and it is not uncommonly seen with haemolymph. Its cause and physiological significance (if any) are as yet unknown. In the present work it provided a very useful means of checking that samples of tubular fluid were not contaminated with perirectal fluid.

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tube was then quickly snatched out. In most cases the tip contained sufficient fluid for determination of freezing-point. The posterior perirectal fluid was often noticeably viscous; when it was frozen it became generally opaque and one could not make out individual ice crystals.* The thawing point could only be estimated by observing the return of the normal unfrozen appearance and for this reason the figures for the posterior perirectal fluid are not claimed to be more accurate than $\pm 10\%$. But this diminished accuracy does not obscure the fact that, as may be seen from figure 11, the freezing-point depression of the posterior perirectal fluid is generally greater than that of the anterior perirectal fluid and may be as great as 8·5 °C. The relationship between posterior and anterior perirectal fluid has similarities to the relationship between anterior perirectal fluid and haemolymph: as far as $\Delta = 1{\cdot}0$ °C they are the same; beyond that, the freezing-point depression of the posterior perirectal fluid increases more rapidly than that of the anterior perirectal fluid. The lower concentration of the anterior perirectal fluid is understandable, since it is in the anterior region that most of the water is reabsorbed from the rectal lumen.

These observations indicate the existence within the rectal complex of some mechanism which can produce a highly concentrated fluid in the posterior part of the perirectal space, and which can vary the concentration of this fluid in relation to the concentration of the haemolymph. The faeces being, on the average, in equilibrium with an atmosphere of 90% r.h., we may assume that a relative humidity of 90% obtains in the air spaces within the rectal lumen. A relative humidity of 90% corresponds to a notional freezing-point depression of 10·5 °C. In terms of freezing-point depression the posterior perirectal fluid stands about half way between the rectal lumen and the haemolymph, and clearly its high concentration should substantially decrease the work required to be done by the rectal epithelium in removing water from the faeces. Its 10-fold increase in response to a twofold increase in the concentration of the haemolymph would suggest that the mechanism is homeostatic, in the sense that desiccation of the insect, by increasing the concentration of the haemolymph, is followed by disproportionate increase in the concentration of the perirectal fluid, which may in turn promote a more effective withdrawal of water from the faeces.

(*c*) *The composition of perirectal fluid and of tubular fluid from the perirectal tubules*

The further exploration of the mechanism just described calls first for some knowledge of the composition of the fluids in the various spaces which have been recognized within the rectal complex. Of these the rectum itself does not (in the feeding insect) contain fluid apart from that which arrives at its anterior end from the midgut and is quickly absorbed. The peritubular space and the subepithelial space are extremely narrow and inaccessible and it has not been possible to obtain fluid from them. The posterior perirectal space, although accessible, provides volumes of the order of only 0·1 nl.* This leaves the haemolymph, the midgut fluid, the anterior perirectal fluid and the tubular fluid of which it is practicable to obtain samples of sufficient volume for more extended analysis.

The collection of anterior perirectal fluid has already been described; tubular fluid can be collected from the same preparation by penetrating the wide thin-walled parts of the

* 1 nl. (nanolitre) = 10^{-9} l.

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perirectal tubules as indicated in figure 9; collection of midgut fluid presents no problem. For each of the four fluids volumes of at least 10 nl. were obtainable and these were analyzed for sodium and potassium by the method of Ramsay, Brown & Falloon (1953) and for chloride by the method of Ramsay, Brown & Croghan (1955); in addition a determination of freezing-point depression was made on each fluid. The results of these analyses are assembled in table 3 and some of them are plotted in figures 12 and 13.

TABLE 3. Na, K, Cl IN MEQUIV/L. Δ IN DEGC

serial	conditions of culture	tubular fluid Na	K	Cl	Δ	perirectal fluid Na	K	Cl	Δ
1	dry	37	455	354	1·345	93	70	152	1·585
2	dry	42	430	229	1·11	99	45	154	1·175
3	normal	34	630	578	1·78	107	141	281	1·855
4	normal	39	697	916	2·245	108	239	438	2·165
5	dry	22	330	224	0·94	75	80	150	1·025
6	dry	56	1070	855	3·115	85	270	440	3·035
7	dry	45	815	771	2·705	109	171	292	2·545
8	dry	58	700	665	2·43	109	102	243	2·465
9	moist	26	250	158	0·735	47	76	105	0·77
10	moist	55	205	158	1·06	72	30	123	1·095
11	moist	27	180	156	0·72	58	69	106	0·745
12	moist	29	188	151	0·835	56	67	103	0·84

serial	conditions of culture	haemolymph Na	K	Cl	Δ	midgut fluid Na	K	Cl	Δ
1	dry	60	87	95	0·79	—	—	—	—
2	dry	84	49	143	1·145	77	198	111	1·145
3	normal	81	59	132	0·835	32	206	79	1·27
4	normal	78	94	113	1·105	33	230	110	1·255
5	dry	75	65	160	1·165	73	108	77	1·28
6	dry	84	78	134	1·36	23	132	73	1·48
7	dry	80	82	125	1·13	36	253	105	1·76
8	dry	76	92	122	1·225	66	200	82	1·305
9	moist	57	32	80	0·76	12	142	96	0·72
10	moist	61	43	112	1·195	19	62	55	1·20
11	moist	58	52	81	0·755	39	87	64	0·71
12	moist	61	43	85	0·82	67	52	96	0·78

Accuracy (as standard error): Na, K ± 10 %, Cl ± 2 %, Δ ± 1 %.
(Serial 4, tubular fluid, chloride. The figure of 916 is anomalous; the chloride determination was repeated with the same result; it is presumed to be due to contamination subsequent to the other determinations).

From these analyses the following conclusions can be reached:

(i) the freezing-point depression of the tubular fluid can be accounted for almost completely by potassium chloride (figure 12*A*);

(ii) the freezing-point depression of the tubular fluid is always very close to that of the anterior perirectal fluid (figure 13*D*); it is therefore usually very much greater than the freezing-point depression of the haemolymph (figure 10);

(iii) the freezing-point depression of the anterior perinephric fluid cannot be accounted for by the inorganic ions (figure 12*B*) and is presumably mainly due to non-electrolyte;

(iv) the concentration of potassium is always greater (figure 13*A*), and the concentration of sodium is always less (figure 13*B*), in the tubular fluid than in the anterior perirectal fluid.

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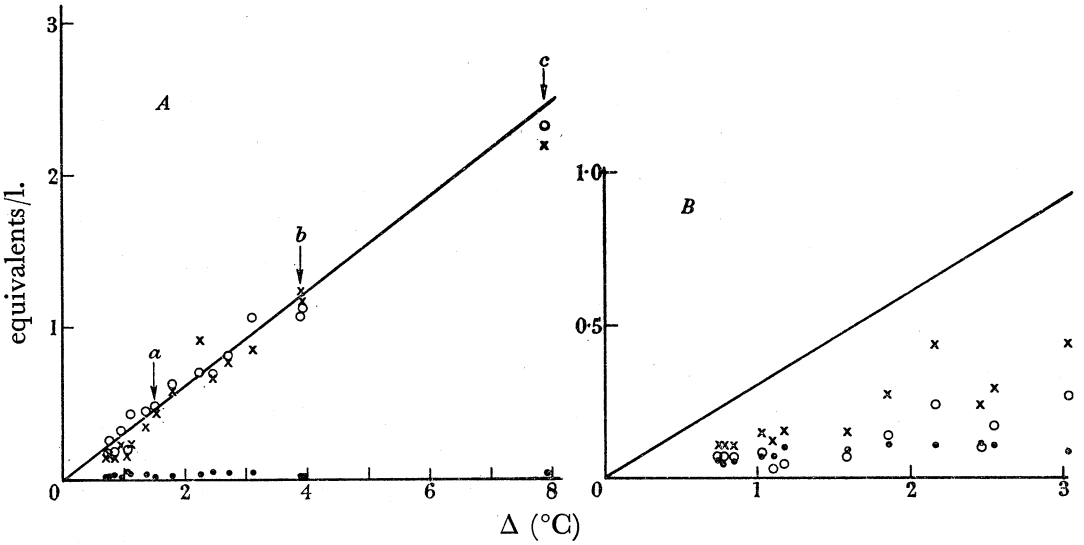

FIGURE 12. Concentrations of sodium (●), potassium (○) and chloride (×) plotted against freezing-point depression for: *A*, tubular fluid; *B*, anterior perirectal fluid. The solid line indicates the relationship between concentration and freezing-point depression for solutions of potassium chloride. The data are all taken from table 3 except for the four sets of points marked *a*, *b*, and *c*, in *A*, which were obtained in other experiments; set *c*, is taken from the data in figure 19*D*.

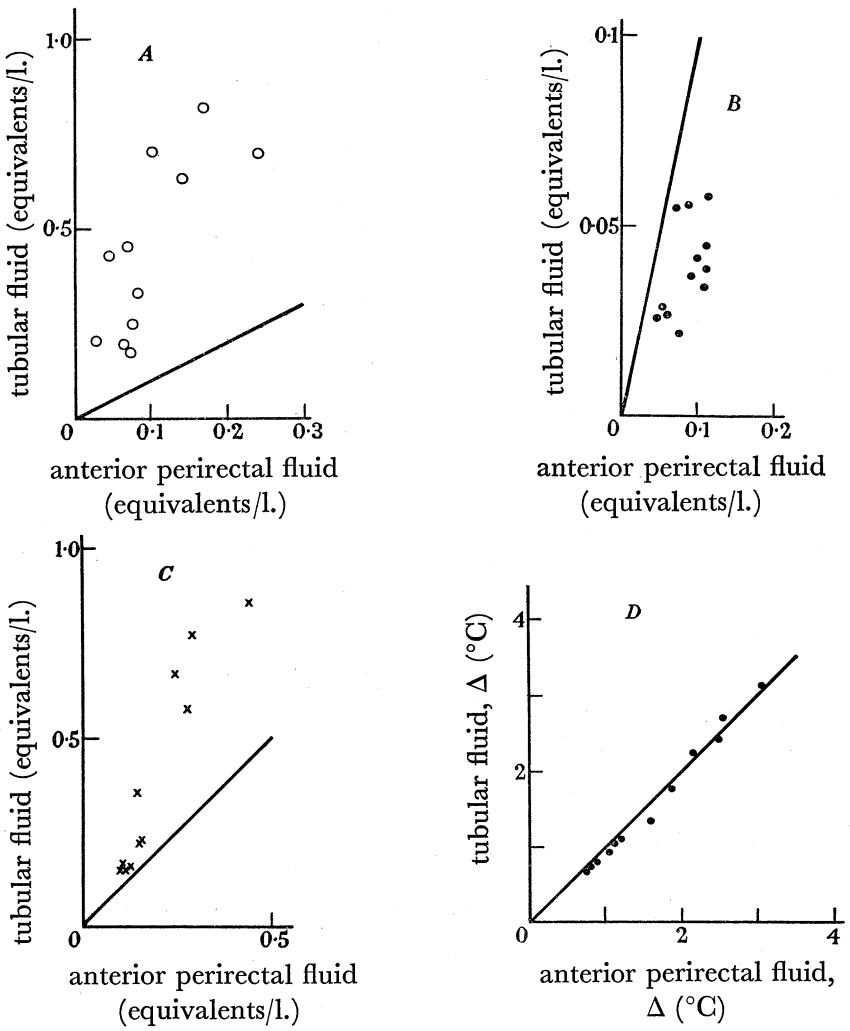

FIGURE 13. Values of: *A*, potassium concentration; *B*, sodium concentration; *C*, chloride concentration; *D*, freezing-point depression, of tubular fluid plotted against corresponding values for anterior perirectal fluid. Data from table 3.

In the Malpighian tubules of the stick insect, the only case for which comparable data are available (Ramsay 1954, 1955), the tubular fluid is slightly hypo-osmotic to the medium in which the tubules are bathed, and the concentration of potassium is always greater (and that of sodium is always less) in the tubular fluid than in the medium. If the perirectal tubules of the mealworm resemble the tubules of the stick insect in these respects it would follow that the perirectal tubules could be in normal physiological relationship with the perirectal fluid, but could not be in normal physiological relationship with the haemolymph, by reason of the disparity in freezing-point depression.

(*d*) *Methods for continuous collection of tubular fluid from the perirectal tubules*

The information provided by analyses of fluids collected by micropuncture can be further interpreted with the help of some knowledge of the rates of flow.

In order to study the rates of flow and the composition of the tubular fluid, and the effects thereon of changes in the medium bathing the rectal complex, it would clearly be advantageous to set up a preparation of the rectal complex in isolation from the rest of the insect, whereby it could be bathed in a relatively large volume of medium whose composition would not be appreciably altered by the activities of the tubules or by exchange with other tissues of the body. Such a preparation is relatively easy to make, but its performance is unsatisfactory in that the flow is maintained for only very short periods, of the order of an hour. This may well be because the rectal complex, being deprived of its tracheal connexions, suffers from lack of oxygen. In view of this it was necessary to use preparations in which the rectal complex remained in the body of the insect with its tracheal connexions intact; the disadvantage of working in an incompletely controlled medium had to be accepted. After much experimentation two such preparations were devised, one making use of the larva and the other making use of the adult.

Larval preparation

In order to hold the larva in a convenient position for operations upon it the device illustrated in figure 14*A* and *B* was constructed. The main features of this device were: (i) a loop of thin tungsten wire which passed over the body just behind the anus and was attached to a spring, thus serving to secure the posterior end of the larva, (ii) a fork on the end of a spring serving to hold down the thorax, and (iii) four hooks of platinum wire serving to hold open the body wall. The larva was thus held down on its back and was opened along the mid-ventral line. The nervous system and the midgut were removed and a thread was tied to the anterior end of the intestine whereby the latter was pulled anteriorly and straightened. The common trunk was then exposed where it ran backwards over the ventral surface of the rectal complex and the free tubules were cut a little way beyond the region of peristalsis.

A receptacle in which the tubule fluid could be collected was then built up out of petroleum jelly worked by means of a hot wire loop. A shallow cup was first constructed just anterior to the rectum. The cut ends of the tubules were then lifted up and turned forwards so as to lie in the cup. Then the lip was further built up, hot jelly being run over and around the tubules where they crossed the lip, and further jelly was then added all round to produce a cup about 1 mm across and 1 mm deep. Finally, the cup was

completely roofed over with a thin layer of jelly. The body cavity was then filled up with the medium to be tested and the gape of the body wall was roofed over by liberal application of jelly. This sealing of the body cavity was never completely reliable, since it could be disturbed by slight movement of the insect; but the sealing of the cup was always effective and evaporation of water from the tubular fluid accumulating in it was prevented. The

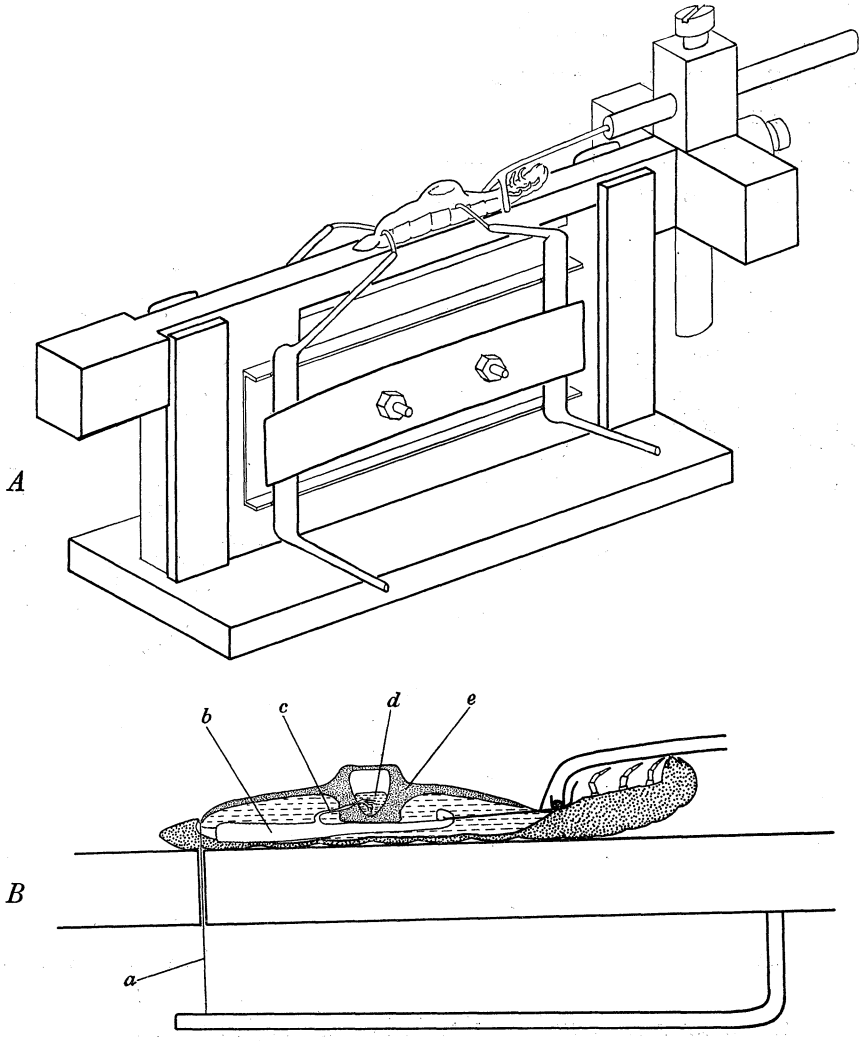

FIGURE 14. Preparation for the continuous collection of tubular fluid from the larva. *A*, general view of the device for holding the larva; *B*, section showing tubular fluid from the common trunk accumulating in the petroleum-jelly cup. *a*, tungsten loop; *b*, rectum; *c*, common trunk; *d*, tubular fluid; *e*, petroleum jelly.

lid of the cup was removed to allow the accumulated tubular fluid to be drawn off and was then sealed back into place. Extensive tests with dyes showed that the seal around the common trunk was effective.

Adult preparation

When the elytra and wings are cut away from the adult beetle the soft cuticle of the dorsal abdominal wall affords easy access to the contents of the body cavity. This cuticle was first cut along the mid-dorsal line and was reflected to the sides; it was then fastened

with wax to two inclined plates of thin brass foil previously coated with wax. This arrangement, which is not easily described in words, is illustrated in figure 15, *A* and *B*. The side walls, provided by the brass plates, were extended with wax applied by hot wire and in this way a bath was constructed upon the back of the insect. When this bath was filled with Ringer the viscera floated freely in it, and the rectal complex, lying dorsal to the genital organs and midgut, was readily accessible. A fine wire hook passing through the wax wall engaged a loop of the intestine and so held the rectal complex steady. The common trunk, lying ventral to the rectal complex, was less easily accessible but could be grappled with a fine tungsten hook and hauled out between the tracheae; after the free tubules had been cut through, the end of the common trunk could be sealed into a cup of petroleum jelly in the same way as has already been described for the larval preparation.

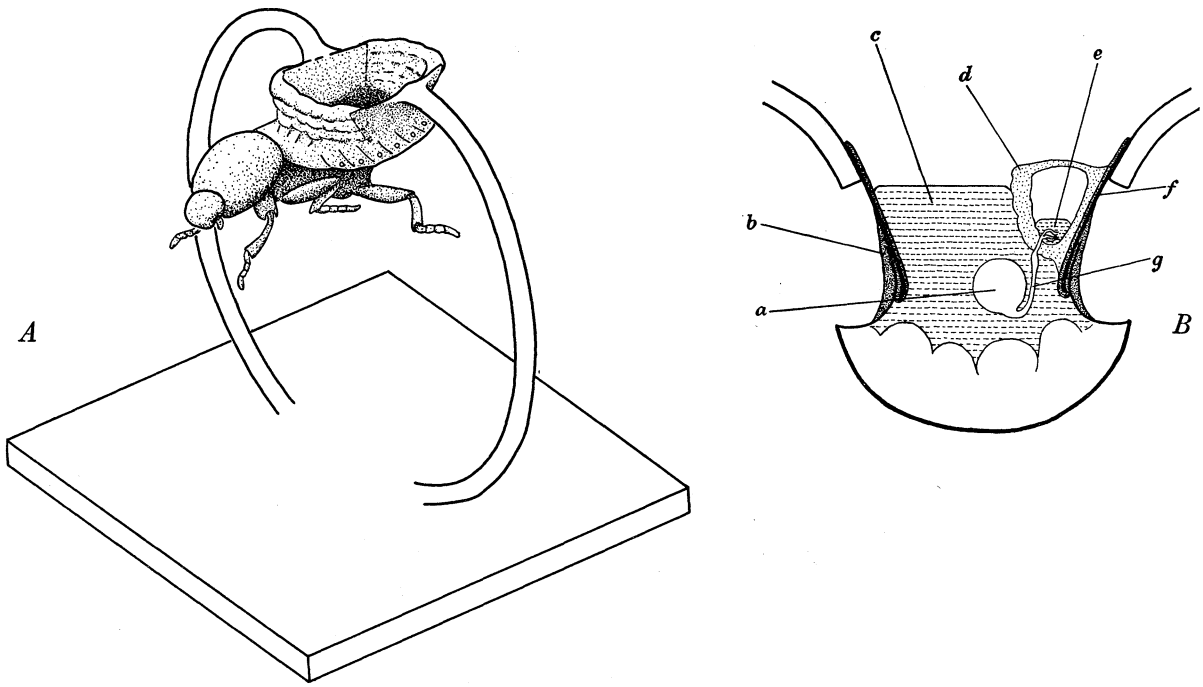

FIGURE 15. Preparation for the continuous collection of tubular fluid from the adult. *A*, general view of the adult beetle with wax trough built up upon its back; *B*, transverse section showing tubular fluid from the common trunk accumulating in the petroleum-jelly cup. *a*, rectum; *b*, wax; *c*, Ringer; *d*, petroleum jelly; *e*, tubular fluid; *f*, brass foil; *g*, common trunk.

Nearly all the work herein reported was carried out on the larval preparation. The adult preparation was particularly useful for observation of the movements of the intestine and rectum and of the passage of faeces through them. It was also used for some experiments with isotopes, briefly reported on p. 305.

Composition of Ringer solution

It is convenient at this juncture to give the composition of the Ringer solution used in this work. It is based upon the data assembled by Duchâteau, Florkin & Leclercq (1953) and was made up as follows: NaCl, 3·8 g; Na_2HPO_4, 1·4 g; KCl, 4·1 g; $CaCl_2$, 1·1 g; $MgCl_2.6H_2O$, 2·0 g; water 1000 ml. The precipitate of earthy phosphate was filtered off

and the filtrate was brought to pH 7·1. Since in the present work only sodium, potassium and chloride are considered, the relevant figures for basic Ringer are:

Na	85	mequiv/l.	(nominal)
K	55	mequiv/l.	(nominal)
Cl	160	mequiv/l.	(nominal)
Δ	0·47 °C		(measured)

Variants of this basic Ringer were also made up: (i) by using different amounts of water, e.g. Ringer × 4 contained the aforementioned weights of salts in 250 ml. of water; (ii) by replacing one ion with another, e.g. potassium-free Ringer in which potassium chloride was replaced by an equivalent amount of sodium chloride.

(*e*) *Freezing-point depression and rate of flow of tubular fluid in relation to ambient media*

It is clear that faecal matter enters the rectum suspended in water and leaves the anus in the form of dried pellets; considerable amounts of water must therefore be reabsorbed from the rectal lumen. The observations described under (*a*) above indicate that the perinephric membrane is relatively impermeable to water. It is therefore natural to take the view that the fluid which issues from the perirectal tubules comes from the rectal lumen and not from the haemolymph. But in the experiments presently to be described it is found that the perirectal tubules continue to produce fluid for some hours after the intestine has been cut and ligatured, which indicates that water may also reach the tubules from the haemolymph. Equally, the existence of the leptophragmata suggests that there may be exchange of materials between tubular fluid and haemolymph. We must therefore admit the possibility that the water and solutes which leave the rectal complex by the perirectal tubules may come either from the rectal lumen or from the haemolymph or from both.

As a means of revealing the paths by which these materials move the tracer method at once suggests itself. In principle it would be possible either to add tracer to the haemolymph or to inject it into the rectum and then follow its appearance in the tubular fluid. In practice this approach is likely to be successful only if the removal of the tracer via the tubular fluid is considerably more rapid than its exchange between the rectal lumen and the haemolymph. In a short series of experiments, for which the adult preparation was used, ^{22}Na and ^{42}K were separately injected into the rectum via the intestine and the rate of increase in radioactivity of the medium in the bath was followed. From these experiments the half-time of exchange of ^{22}Na was found to be of the order of 30 min and that for ^{42}K to be of the order of 10 min. Since the minimum time required for the accumulation of a usable quantity of tubular fluid is about 30 min it is clear that the rate of exchange is too rapid in relation to the rate of flow for the tracer method to be useful, and these experiments were therefore discontinued.

After the abandonment of the tracer method attention was directed towards the responses of the perirectal tubules to changes in the fluids with which they might be supposed to have physiological relationships, namely, the haemolymph and the perirectal fluid. By reason of the requirement that the rectal complex must be studied *in situ* with its tracheal connexions intact it was not possible to impose precisely defined changes in the composition

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of these media. Changes were made by injecting various solutions into the rectal lumen (via the intestine) and into the perirectal space, and by adding them to the haemolymph. The effects of these changes were assessed by measurements of the rate of flow and the freezing-point depression of the tubular fluid, making use of the larval preparation described under (*d*) above.

In all the experiments to be described in the present section the intestine was ligatured and cut close to the midgut. The tubular fluid was collected at appropriate intervals. varying from 30 min to 3 h or more, depending upon the rate at which it accumulated, The volume of each collection was estimated by measuring the diameter of the droplet suspended in liquid paraffin (the error of this method is of the order of $\pm 10\%$ of volume; Ramsay 1955). Thereafter a sample was taken for determination of freezing-point depression, and in a few cases the collection was also analyzed for sodium, potassium and chloride.

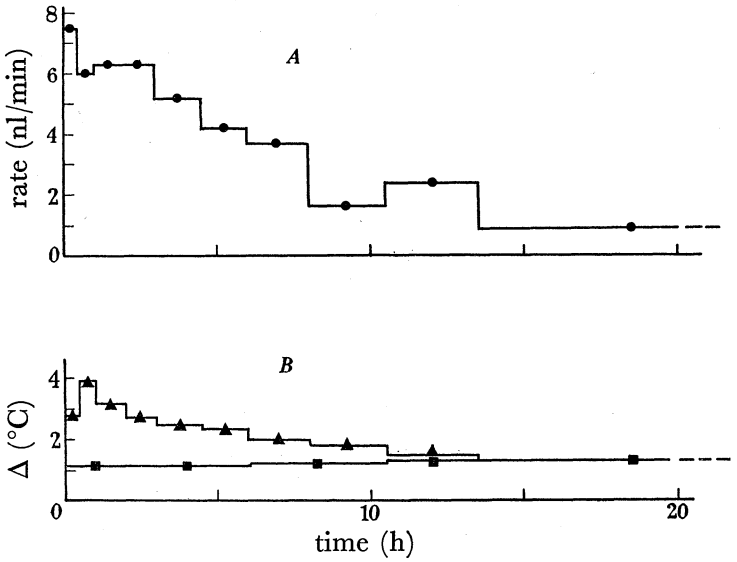

FIGURE 16. Continuous collection of tubular fluid from a larval preparation under standard conditions (see text). *A*, rate of flow (●) in nl/min. *B*, freezing-point depression, (▲) of tubular fluid, (■) of medium. The rate of flow gradually declines and the freezing-point depression of the tubular fluid gradually approaches that of the medium.

In order to evaluate the effects of changes of medium it is first necessary to establish the normal course of events under some chosen standard conditions. The standard fluid used to fill up the body cavity was Ringer × 3. The course of a standard experiment is illustrated in figure 16. The preparation continues to secrete tubular fluid for at least 15 h, but the rate of flow declines irregularly over this period, as also does the freezing-point depression, which eventually becomes the same as that of the medium. One must therefore be cautious in interpreting the effects of changes which result in a fall in rate of flow and a fall in freezing-point depression; significance may be more readily attributed to changes which result in increase of these quantities.

(i) *Injections into the rectal lumen*

To make these injections a cannula was inserted into the intestine and fluid was forced in under pressure so as to distend the rectum. A ligature was then tied around the intestine

just anterior to the rectum so as to retain the injection. The injected fluid was deeply coloured with indigo carmine so as to reveal any leakage. After about 10 min it could be seen that the distension of the rectum was distinctly reduced, indicating a fairly rapid absorption of the injected fluid.

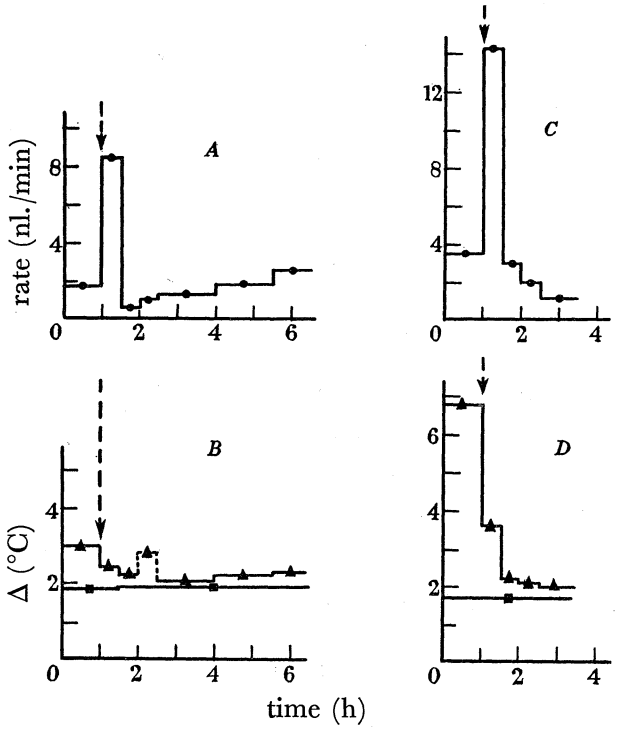

FIGURE 17. Effects of injections into the rectal lumen upon the rate of flow and freezing-point depression of the tubular fluid. *A* and *B*, injection of distilled water; *C* and *D*, injection of 3M sucrose. (●) rate of flow in nl./min; (▲) freezing-point depression of tubular fluid; (■) freezing-point depression of medium. Vertical arrows show time of injection.

Figure 17, *A* and *B*, shows the results obtained after the injection of distilled water. This is followed at once, i.e. within the next 30 min, by a rapid flow of tubular fluid with some fall in freezing-point depression (which in this experiment is hardly significant). Injection of 3M sucrose (figure 17, *C* and *D*) is also followed by a rapid flush of tubular fluid and in this case the fall in freezing-point depression is much more rapid than that seen in the standard preparation. It therefore appears that some of the water injected is rapidly removed via the perirectal tubules, and from the rapid fall in freezing-point depression seen in figure 17*D* it seems that sucrose is not removed by this route; it would not be surprising if the sucrose remained in the rectal lumen.

(ii) *Injections into the perirectal space*

For this purpose a long, gently tapered, pipette was thrust far into the perirectal space as described under (*b*) above, and the injection was continued until the coloured fluid emerged at the point of puncture. It was not possible to close this wound by ligature, but the amount so escaping was very small in relation to the volume of the surrounding medium. Once again, the injection was followed by a rapid flush of tubular fluid and a

fall in freezing-point depression (figure 18, *A* and *B*); and in this case the freezing-point depression fell below that of the surrounding medium, which is adjudged to be significant. Injection of 3 M sucrose was followed by complete cessation of flow of tubular fluid.

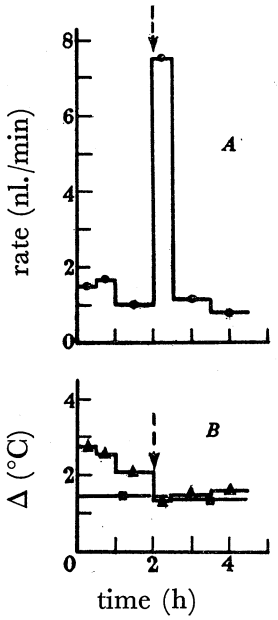

FIGURE 18. Effects of injection (↓) of distilled water into the perirectal space upon (*A*) the rate of flow and (*B*) the freezing-point depression of the tubular fluid. (●) rate of flow in nl./min; (▲) freezing-point depression of tubular fluid; (■) freezing-point depression of medium.

(iii) *Dilution of the surrounding medium*

Whereas distilled water causes a rapid flush of tubular fluid when it is injected into the perirectal space, the addition of distilled water to the medium surrounding the rectal complex is not followed by any obvious change in the rate of flow (figure 19*A*). The freezing-point depression begins to fall rather rapidly about an hour later (figure 19*B*), but this fall is barely significant in relation to the gradual decline seen in the standard preparation.

(iv) *Addition of sucrose to the surrounding medium*

Whereas 3 M sucrose injected into the perirectal space abolishes the flow of tubular fluid, it causes a barely significant decline in the rate of flow when added to the surrounding medium (figure 19*C*). On the other hand there is a marked rise in the freezing-point depression of the tubular fluid (figure 19*D*). It will be noted that the freezing-point depression of the tubular fluid is raised by about the same amount (4 degC) as the freezing-point depression of the surrounding medium. On figure 19*D* are included the results of analyses (see also figure 12*A*) which show that the freezing-point depression of the tubular fluid is almost entirely accounted for by potassium chloride, indicating that sucrose does not enter the tubules in significant amount.

(v) *Alteration of sodium/potassium ratio in the surrounding medium*

Alterations of sodium/potassium ratio were brought about by changing the normal Ringer × 3 for Ringer × 3 in which sodium was replaced by potassium and then for Ringer × 3 in which potassium was replaced by sodium. The results of these changes of

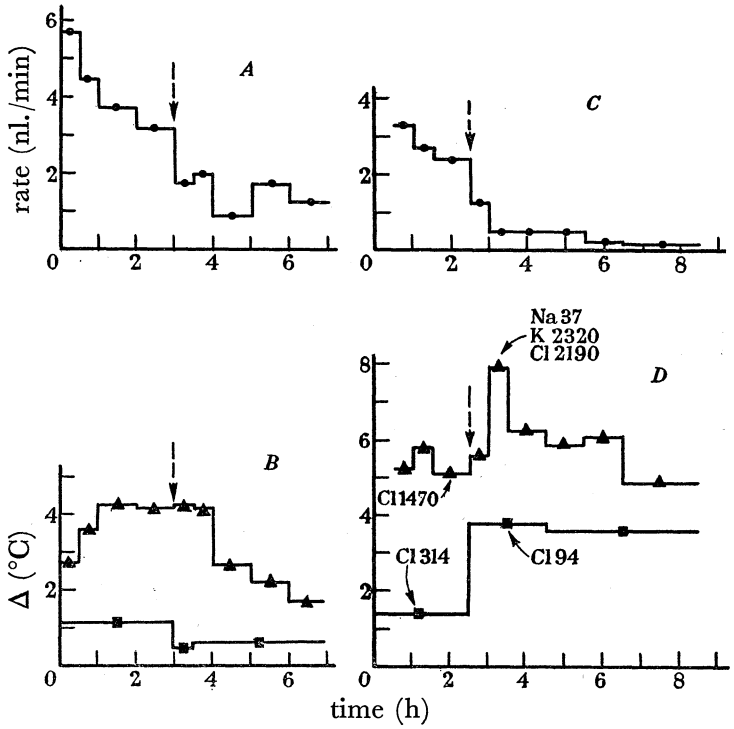

FIGURE 19. Effects of changes in the concentration of the surrounding medium (↓) upon the rate of flow and freezing-point depression of the tubular fluid. *A* and *B*, effect of adding distilled water; *C* and *D*, effect of adding 3 M sucrose. (●) rate of flow in nl./min; (▲) freezing-point depression of tubular fluid; (■) freezing-point depression of medium.

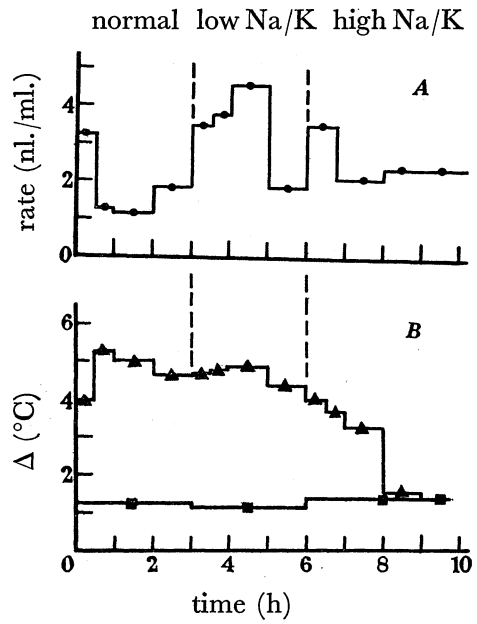

FIGURE 20. Effects of changes in the ionic ratios of the surrounding medium upon (*A*) the rate of flow and (*B*) the freezing-point depression of the tubular fluid. Low Na/K was produced by replacing part of the normal Ringer × 3 with Na-free Ringer × 3; and high Na/K by replacement with K-free Ringer × 3. (●) rate of flow in nl./min; (▲) freezing-point depression of tubular fluid; (■) freezing-point depression of medium.

medium are shown in figure 20. Decrease in sodium/potassium ratio is followed by increased rate of flow and increased freezing-point depression of the tubular fluid, notwithstanding that the freezing-point depression of the medium is slightly decreased. Increase of sodium/potassium ratio is followed by a fall in the freezing-point depression of the tubular fluid which soon becomes isosmotic with the medium. It cannot be claimed that there is a significant fall in the rate of flow.

The rapid flush of tubular fluid which follows the injection of distilled water or of 3 M sucrose into the rectal lumen indicates, as aforesaid, that water is rapidly absorbed from the lumen and is removed from the rectal complex via the perirectal tubules. Similarly, distilled water injected into the perirectal space is quickly removed by the tubules. The permanent cessation of flow following injection of 3 M sucrose into the perirectal space is interpreted as due to damage to the tubules.

The effect of changes in the surrounding medium are more difficult to interpret. The effect of decreased sodium/potassium ratio in increasing the rate of flow of the tubular fluid and the effect of sucrose in increasing its freezing-point depression suggest that some of the tubular fluid may be derived directly from the surrounding medium. Passage of water from medium to tubular fluid is further suggested by the fact that the flow continues for many hours notwithstanding that the intestine is ligatured and no water enters the rectal complex by this route; *a fortiori*, in view of their high concentrations in the tubular fluid, the same considerations apply in the case of potassium and chloride. This question could be settled if it could be demonstrated that the total amounts of water and potassium chloride eliminated from the perirectal tubules exceed the amounts which might conceivably have been present initially in the rectal complex; but this was not the case in any of the experiments carried out in the course of the present work.

The increased rate of flow in response to increased potassium concentration in the surrounding medium is consonant with the observation that potassium is actively secreted into the Malpighian tubules of insects in general (Ramsay 1953), but it implies that the perirectal tubules are effectively in contact with the surrounding medium. If they are, then the difference in freezing-point depression between the tubular fluid and the medium is unprecedented. It may be that the passage of water into the tubules from the medium encounters some unusual resistance; on the other hand there seems to be no unusual resistance to the passage of water into the tubules from the perirectal space.

5. Discussion

The experimental results presented in this paper demonstrate that the rectal complex has considerable power of removing water from the faeces, as has long been supposed. They also make it possible to suggest a role for the rectal complex in the water economy of *Tenebrio*, as follows.

When the insect is maintained on a moist regime the rectal complex is relatively inactive. The concentration of the haemolymph is of the order of $\Delta = 0{\cdot}75\ ^{\circ}\mathrm{C}$ and that of the perinephric fluid is not very much greater. Water is reabsorbed from the faeces, but these are distinctly soft and moist when passed. The water reabsorbed from the faeces, if it does not leave via the perirectal tubules, can escape from the perinephric space by passing between the intestine and the perinephric membrane anteriorly to the rectal complex.

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When the insect is maintained on a dry regime the concentration of the haemolymph may rise nearly to $\Delta = 1{\cdot}5$ °C. This is accompanied by the activation of the rectal complex, characterized by a great increase in the concentration (mainly due to non-electrolyte) of the perinephric fluid, especially at the posterior end of the rectal complex. Following the suggestion of Wigglesworth, this makes it easier for the rectal epithelium to remove water from the faeces, which are voided as hard dry pellets in equilibrium with 90% r.h. or less. The basic mechanism behind the rise in the concentration of the perinephric fluid is the active transport of potassium (either directly or indirectly) into the perirectal tubules from the haemolymph, without a proportionate amount of water. The concentration of the tubular fluid is thus increased and water diffuses from the perinephric fluid into the tubular fluid until the two fluids have the same concentration.

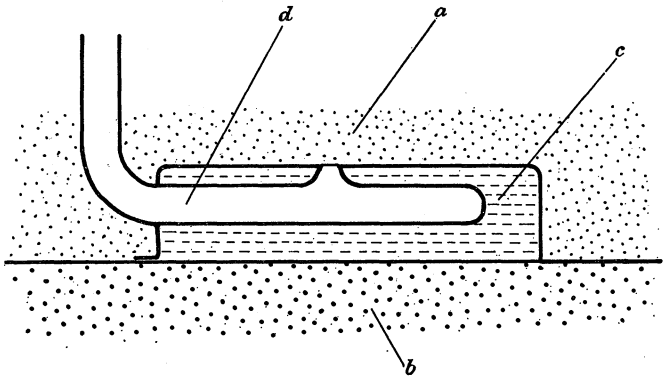

FIGURE 21. Diagrammatic representation of the rectal complex. *a*, haemocoel; *b*, rectal lumen; *c*, perinephric space; *d*, tubular lumen.

To reconcile this physiological mechanism with the microanatomy of the rectal complex is less easy. Stripped down to its essentials, and subsuming the peritubular, perirectal and subepithelial spaces under the perinephric space, the rectal complex is a 4-compartment system as indicated in figure 21. The four compartments are: (*a*) the haemocoel, (*b*) the lumen of the rectum, (*c*) the perinephric space, and (*d*) the lumen of the tubule. Exchanges certainly take place between (*a*) and (*d*) and between (*b*) and (*c*), and probably also between (*a*) and (*c*) and between (*c*) and (*d*). An experimental approach through a study of rates of exchange of tracers between compartments would encounter great difficulties of interpretation.

What enters the rectal complex is faecal matter suspended in a fluid which is isosmotic with the haemolymph. What leaves is dried faeces, hyperosmotic to the haemolymph, by the anal canal, and a solution of potassium chloride, hyperosmotic to the haemolymph, by the perirectal tubules. It therefore follows that under steady-state conditions either a hyperosmotic solution of potassium chloride must enter, or an isosmotic solution of potassium chloride must enter and water must leave, by some other route. Since it is likely (p. 310) that much of the water and potassium chloride which leaves by the tubules comes from the haemolymph it is simplest to suppose that, in effect, potassium chloride is actively transported from the haemolymph into the rectal complex accompanied by passive diffusion of water; there is no need to suppose that in addition there is active transport of water in the opposite direction. But on either view one cannot escape the conclusion that

active transport must take place somewhere on the membranes—perinephric membrane and/or leptophragmata—which separate the perinephric and tubular fluids from the haemolymph.

On grounds of their thinness and their reactions with silver nitrate it has been suggested by Lison and by others that in life the leptophragmata are the sites of exchange of materials between haemolymph and tubular fluid. But the interpretation of silver staining must be approached with great caution. The phenomenon known earlier as 'réduction vitale' of silver was shown by Koch (1934) to be associated with organs of salt uptake, and Krogh (1939) subscribed to the view that 'it appears most probable that the uptake of Ag represents a specific cation absorption'. Croghan (1958) working on the branchiae of *Artemia*, was less committal and attributed the phenomenon not to active uptake but to the passive permeability of the cuticle: 'The silver ions diffuse into the permeable cuticle, meet chloride ions derived from the animal via the branchial epithelium and form an AgCl precipitate within the cuticle. The whole phenomena is purely passive'. Following Croghan's interpretation the formation of a precipitate of silver chloride at the leptophragma indicates that this structure is permeable to silver ions or to chloride ions or to both. This does not mean that it was permeable to chloride ions—or to any other ion species—before the silver ions were brought into contact with it. Silver ions are known to damage secretory epithelia (e.g. branchiae of *Artemia*, anal gills of mosquito larvae) and the permeability seen after treatment with silver nitrate may be the result of injury. The striking feature of the silver reaction of the leptophragma—and no doubt the reason why it came to Lison's notice—is the rapid reduction of the silver chloride by light. This is probably a different phenomenon. When the rectal complex is treated with silver nitrate it may be observed that a precipitate, presumably of silver chloride, forms at the cut end of the common trunk; this precipitate, unlike that at the leptophragma, is not rapidly reduced by light. Some other process must be at work, but in view of the uncertainty which still surrounds the basic processes of photography it would be unprofitable to speculate on its nature.

The silver staining reaction of the leptophragmata shows only that in the presence of silver ion these structures are permeable to silver ion or to chloride ion or to both. It does not provide any evidence that potassium chloride is actively transported from haemolymph to tubular fluid by this route. Likewise the precipitation of silver chloride within the perinephric membrane does not indicate active transport of potassium chloride from haemolymph to perinephric fluid. Passive diffusion of chloride in the opposite direction could account for the results.

A further difficulty presents itself, namely that there are very few mitochondria in the perinephric membrane and the leptophragmata. Mitochondria are abundant in the rectal epithelium (Wigglesworth, personal communication) and in the brush borders of the perirectal tubules (as described by Saini) but nowhere else in the rectal complex. Thus we are left in the unsatisfactory position that on the one hand the physiological observations require that potassium chloride is actively transported into the rectal complex, while on the other no plausible suggestion can be made as to the site of this process.

If it should turn out that the basic mechanism is indeed the secretion of potassium chloride, unaccompanied by a proportionate amount of water, from the haemolymph into

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the rectal complex it would seem that the principle of the mechanism might have been put to work in a less complicated structure than actually confronts us. Secretion of potassium chloride directly into the perinephric fluid could be the means of raising its concentration, and this fluid, as it increased in volume by withdrawal of water from the faeces, could escape into the haemolymph through the narrow space under the perinephric membrane at the anterior end of the rectal complex; the involvement of the Malpighian tubules seems to be unnecessary. But an arrangement of this type envisages a perinephric fluid containing a high concentration of potassium chloride, and this is perhaps where the difficulty lies.

The ordinary cells of the body are intolerant of changes in the body fluid which involve alteration of ionic ratios or ionic strength. They are relatively tolerant of changes in the activity of water, as is seen, for example, in the well-known protective action of glycerol; the freezing-point of the body fluid can be lowered without injury to the cells provided that this is brought about by the addition of some non-electrolyte which, like glycerol, readily penetrates the cells. Secretory epithelia, while they may elaborate an 'unnatural' fluid on one side, are usually in contact with the 'natural' body fluid on the other. (By 'natural' in this context is meant only that the body fluid has a certain total ionic strength and maintains certain ionic ratios.)

The alternative arrangement contemplated above, whereby a high concentration of potassium chloride would be developed in the perinephric space, would expose the cells of the rectal epithelium to 'unnatural' media on both sides. The arrangement actually found avoids this by providing the perinephric space with Malpighian tubules which can remove the potassium chloride as a solution isosmotic with the perinephric fluid. By means of this device the concentration of the perinephric fluid is raised not by the net addition of potassium chloride but by the withdrawal of the water which accompanies the potassium chloride into the perirectal tubules. In the rectal complex the two main secretory epithelia—of the tubules and of the rectum itself—are placed as it were back to back, and the ionic ratios and total ionic strength of the perinephric fluid which bathes them lie within 'natural' limits, while on the one hand the rectal epithelium withdraws water from the faeces against a notional osmotic pressure of the order of 100 atmospheres and on the other hand the tubules secrete a fluid containing potassium chloride at a concentration of more than 2M.

I am very grateful to Professor V. B. Wigglesworth, F.R.S. for allowing me to see his preparations of the tracheoles of the rectal complex, and for much helpful and stimulating discussion.

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