und daß z. B. eine direkte Durchmesserbestimmung nur bei den vier größten möglich war, bei denen Werte zwischen 200 und 800 km gefunden wurden. Photometrische Methoden aber zeigen, daß wohl die große Mehrzahl dieser Zwergplaneten Durchmesser von weit weniger als 100 km besitzt. Für *Hermes* werden z. B. Werte von nur 500–800 m angegeben, so daß die Vermutung, es könnte sich bei diesem Kleinplaneten um ein Riesenmeteor gehandelt haben, nicht ganz abwegig ist.

Statistische Untersuchungen sind zurzeit noch im Fluß. Es betrifft dies vor allem die Verteilung der Bahnelemente im Raum und nicht zuletzt auch die immer wieder gestellte Frage nach der Herkunft dieser vermutlichen Weltensplitter. Unabgeklärt ist dann auch immer noch die Frage nach dem zehnten Großplaneten, den der junge KEPLER vor 350 Jahren zwischen Merkur und Venus «einschieben» mußte und der immer noch nicht entdeckt wurde, obgleich es an zahlreichen Versuchen nicht fehlte.

Neben alledem hat aber die Himmelsforschung allen Grund, denen dankbar zu sein, die vor einem halben Jahrhundert dafür gesorgt haben, daß dieses lohnende Feld der Sternkunde nicht vernachlässigt, sondern immer mit Hilfe tüchtiger und vor allem auch begeisterter Kräfte neu beackert und gefördert wurde.

Summary

After a historical survey on the discovery of the small planets and the international organization of the calculation of orbits the importance of the divergences from the ordinary orbits between Mars and Jupiter is discussed. On the one side those small planets which approach Mars, Jupiter, even Earth and Saturn, in strongly excentric orbits offer new methods for the mass determination of the large planets; e.g. the perigee of Eros made possible a new determination of the parallax of the sun (8″,79). On the other side the group of the Trojans e.g. gives new aspects for the problem of the three bodies. In the breaches of commensurability the statistical distribution of the orbits of the small planets hints to the problem of the stability of the planetary system.

Water Relations of Insects

By V. B. WIGGLESWORTH, Cambridge

It is very largely true to say that the physiology of insects is the story of their water relations. Small terrestrial animals, with a surface area great in proportion to their volume, they are hard put to it to maintain the normal water content of their tissues. An incessant struggle for water is reflected in every aspect of their organization.

As in other terrestrial animals, the demand for oxygen must be reconciled with the need for retaining water. Insects breathe through tracheæ, the external openings of which are closed by muscular sphincters, held firmly occluded most of the time and opened only enough to let in the needed oxygen[1,2]. If the spiracles are kept permanently open, as happens during exposure in an atmosphere containing 5 per cent of carbon dioxide, the insect soon dries up and dies[3,4].

Excepting such species as live in moist environments or which take abundant liquid in their food, insects conserve water with great care during digestion and excretion. They eliminate nitrogen mainly as uric acid, which is deposited in solid form. And insects from dry environments have the rectal portion of their alimentary tract furnished with a high columnar epithelium or with distinct "rectal glands" which reabsorb water from the excrement before it is discharged. Indeed, in insects like the clothes moth larva *Tineola*, or the mealworm *Tenebrio*, every trace of water is removed from the fæcal residue and this appears in the form of dry pellets[1].

A few insects, notably the eggs of certain Collembola and the eggs of some grasshoppers, can withstand the drying of the tissues. Eggs of the South African locust *Locusta pardalina* have been kept in this desiccated state for three and a half years; they hatch within two weeks if they are moistened. But in most insects the normal water content of the tissues is much the same as in other animals, and they cannot tolerate any great reduction below this level.

These physiological mechanisms for retaining water during respiration or reabsorbing it during excretion will be effective only if the insect has a body covering impervious to water loss. The insect cuticle is indeed specially adapted to this end. The cuticle is rigid, flexible or elastic according to the mechanical needs of the system; but it remains extraordinarily impermeable to water. It has been recognized for many years that it is the very thin outermost layer, the epicuticle, that is responsible for this impermeability, and that

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[1] E. H. HAZELHOFF, Regeling der Ademhaling bij Insecten en Spinnen, Thesis, Utrecht 1926.

[2] V.B.WIGGLESWORTH, Proc. Roy. Soc. B. *118*, 397—419 (1935).

[3] K. MELLANBY, Proc. Roy. Soc. B. *116*, 139—149 (1934).

[4] V.B.WIGGLESWORTH and J. D. GILLETT, Proc. Roy. ent. Soc. Lond. A. *11*, 104—107 (1936).

[1] V.B.WIGGLESWORTH, Quart. J. micr. Sci. *75*, 131—150 (1932).

lipoid materials in some form are associated with this impermeable layer[1].

In recent years a great deal has been learned about the nature of the insect cuticle. Its basic components are chitin and protein, intimately associated, perhaps chemically combined; although the protein (arthropodin) is readily extracted with water[2]. These form the endocuticle (Hauptlage or Innenlage). The outer parts, except in the soft or flexible regions, are hard and ambercoloured; they constitute the exocuticle (Pigmentschicht or Außenlage).

The hardness and coloration of the exocuticle result from a chemical change in the cuticular protein. It has long been known that the insect cuticle is extremely rich in phenolic substances (for example, dihydroxyphenylacetic acid) the function of which was obscure[3] except when they serve as chromogens in the production of melanin[4]. It appears from recent work that the physiologically important substances are not the polyphenols themselves but the quinones derived from them under the action of oxidases. Such quinones react with the proteins, linking together adjacent protein chains, occluding the reactive radicals, and converting a soft colourless soluble material into a horny brown insoluble and hydrophobe product. The protein of the exocuticle is in fact "tanned" protein; it has been termed "sclerotin"[5].

On the surface of the sclerotin is the thin epicuticle, usually not more than one micron in thickness. The epicuticle contains no chitin. Besides protein it is composed of lipoid material, the whole combined into a substance not unlike the cutin of the plant cuticle. This substance is excessively resistant and insoluble in all ordinary solvents including cold concentrated sulphuric or hydrochloric acids[6]. When subjected to destructive oxidation by warming in concentrated nitric acid saturated with potassium chlorate it is broken down and droplets of fatty material are liberated. This material was termed "cuticulin"[7]. It appears to consist of a polymerised lipo-protein which has been subsequently tanned with quinones[8].

It was earlier believed that it was this cuticulin layer which was responsible for the impermeability of the insect cuticle to water. The new cuticle formed before moulting is permeable almost to the time the old skin is shed. The impermeability acquired just before ecdysis was believed to be due to oxidative changes in the epicuticle at this stage[1]. But that explanation was mistaken. The true waterproof layer is a thin film of wax deposited on the outside of the histological epicuticle.

The outer layers of the cuticle are, as we have seen, very rich in dihydroxyphenols which will reduce ammoniacal silver oxide to give a deep brown stain. This is very evident when fresh sections of the cuticle cut with the freezing microtome are immersed in ammoniacal silver solution. But if the intact insect is immersed there is no staining. There is in fact an impervious layer between the solution and the "epicuticle." If, however, the surface of the insect is first rubbed very lightly with fine silica or alumina dust and is then immersed in ammoniacal silver oxide, all the prominent points and the crests of all the folds in the cuticle stain deeply (Fig. 1). The protective layer has been abraded so that the polyphenols are exposed[2].

The same thing happens if the insect is immersed in wax solvents, such as chloroform, except that now the exposure of the polyphenols is not confined to the crests of the folds.

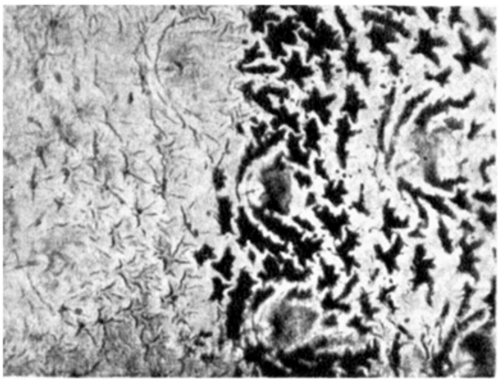

Fig. 1. Cuticle of *Rhodnius* treated with ammoniacal silver oxide, seen in surface view. Reduction is limited to the crests of the epicuticular folds of the area to the right which has been lightly rubbed with alumina dust.

In these insects, lightly rubbed with alumina dust or extracted with chloroform, there is no injury to the "epicuticle" that is visible with the microscope. And yet the impermeability of the cuticle to water is completely destroyed. A larva of the blood-sucking bug *Rhodnius* running on clean filter paper and exposed in dry air at 30° C lost only 2·1 per cent of its weight in twenty-four hours. If it were allowed to run on filter paper lightly dusted with alumina, so that as it ran along it rubbed the ventral surface of its abdomen, it lost 46·3 per cent of its weight and was in fact completely desiccated. (This effect could be largely prevented if the abdomen was supported by a tiny

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[1] W. KÜHNELT, Zool. Jahrb. Abt. Anat. *60*, 219—278 (1928); Verh. VII. Int. Kongr. Entom. Berlin, 1938, *2*, 797—807 (1939).

[2] A. R. TRIM, Biochem. J. *35*, 1088—1098 (1941).

[3] K. BHAGVAT and D. RICHTER, Biochem. J. *32*, 1397—1406 (1938).

[4] H. SCHMALFUSS, A. HEIDER and K. WINKELMANN, Biochem. Z. *257*, 188—193 (1933).

[5] M. G. M. PRYOR, Proc. Roy. Soc. B. *128*, 393—407 (1940).

[6] W. KÜHNELT, Zool. Jahrb. Abt. Anat. *60*, 219—278 (1928).

[7] V. B. WIGGLESWORTH, Quart. J. micr. Sci. *76*, 269—318 (1933).

[8] V. B. WIGGLESWORTH, Proc. Roy. Soc. B. (in the press) (1946).

[1] V. B. WIGGLESWORTH, Quart. J. micr. Sci. *76*, 269—318 (1933).

[2] V. B. WIGGLESWORTH, Nature, *153*, 493 (1944).

mound of wax so that it no longer rubbed the surface.)

These observations provide the explanation of the mode of action of fine inert dusts in protecting dry stored goods from insect attack. The silica or other fine dust gets into the moving joints and leads to the abrasion of the wax layer so that the insect dries up[1].

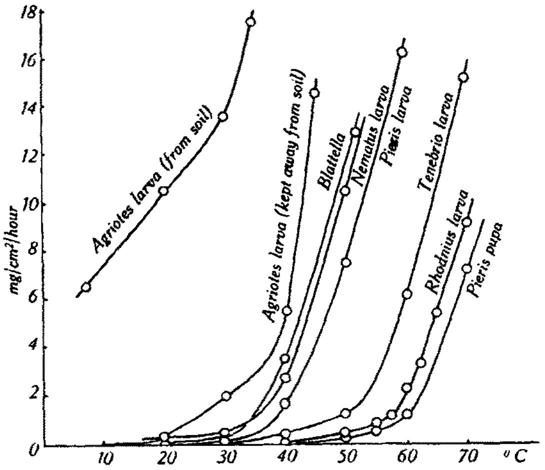

Fig. 2. The rate of evaporation of water from dead insects at different temperatures.

The first clear proof that the waterproofing of the insect was due to a layer of free lipoid on the surface of the cuticle was obtained in the cockroach[2]. When the cockroach is warmed to about 30° C there is a sudden increase in the rate of transpiration through the cuticle. This is the temperature at which there is a change of phase (melting or expansion) of the lipoid on the cuticle surface.

More recently it has been found that in almost all the insects studied there is a similar break in the curve relating rate of transpiration with temperature. There is a critical temperature above which the rate of water loss increases abruptly. This temperature may be around 30° C and so be demonstrable in the living insect. Or it may be around 60° C and become evident long after the insect has been killed by the high temperature (Fig. 2). In general the critical temperature is low in phytophagous insects from relatively moist environments, high in insects from dry environments. In the larva of the cabbage butterfly *Pieris* the critical temperature is about 37° C; in the pupa of the same insect, which has to withstand exposure in the open for many months, it is about 58° C[1].

The materials responsible for this waterproofing of the cuticle can be extracted from the cast skins of the insects with boiling chloroform. They prove to be waxes of the same general type as bees-wax, but they vary widely in physical properties. In the cockroach we have to do with a soft grease; in the leaf-eating larva of the sawfly *Nematus* or the cabbage butterfly *Pieris* they are soft pale yellow waxes without crystalline form; in the mealworm *Tenebrio* and in the bug *Rhodnius* they are hard, white and crystalline[1].

The extracted waxes can be laid down in the form of a thin film upon artificial membranes such as gelatine tanned with benzoquinone, or upon a piece of butterfly's wing from which all lipoids have been removed, and they render these materials highly impermeable to water. It appears that under the influence of the substrate membrane the wax molecules of the innermost layer are orientated at right angles to the surface and so closely packed in crystalline form that water molecules will not pass through. If these artificially waterproofed membranes are warmed they show the same phenomena as the intact insect. At a critical temperature there is a sudden increase in the rate of transpiration; and this temperature is the same in the isolated wax on the artificial membrane as it is in the normal insect from which the wax was obtained (Table 1).

Table 1

Approximate "critical temperature" for the increase in transpiration through the cuticle of intact insects (from Wigglesworth, 1945) and through films of the isolated waxes (from Beament, 1945).

	Intact insect	*Isolated wax*
Blattella	31° C	30° C
Calliphora prepupa	35° C	33° C
Calliphora pupa	47° C	49° C
Nematus larva	34° C	34° C
Pieris larva	37° C	39° C
Pieris pupa	58° C	57° C
Tenebrio larva	49° C	50° C
Rhodnius larva	57° C	57° C

There can be little doubt that it is these waxes which are responsible for the waterproofing of the cuticle. By relating the amount extracted with the surface area of the insect, it has been calculated that they form a layer about 0·25 μ in thickness. They have fairly definite melting points; but the critical temperature for the passage of water lies some 5 to 10° C below the melting point. At this lower temperature there is commonly a visible change in the wax which then becomes less opaque. It seems probable that at the critical temperature the intermolecular spacings in the crystalline waxes increase abruptly and the oscillating molecules begin to rotate freely[2].

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[1] V. B. Wigglesworth, J. exp. Biol. *21*, 97—114 (1945).

[2] J. A. Ramsay, J. exp. Biol. *12*, 373—383 (1935).

[1] J. W. L. Beament, J. exp. Biol. *21*, 115—131 (1945).

[2] A. Müller, Proc. Roy. Soc. A. *138*, 514 (1932).

In the bug *Rhodnius* the wax layer is not easily removed by brief immersion in lipoid solvents. It has been found that that is because there is yet another thin layer, a layer of cement, protecting the wax. The nature of this material is uncertain, and the extent to which it is present in other insects is not known, but in *Rhodnius* at least it is a very definite structure.

The "epicuticle" in *Rhodnius* has thus been shown to be a complex of at least four layers. At the time of moulting they are laid down as follows:—

(i) First the lipo-protein or cuticulin layer is secreted by the epidermal cells. This is the "epicuticle" visible in histological sections.

(ii) Then, after a substantial layer of the exocuticle has been formed below the cuticulin layer, a viscid secretion rich in polyphenols is poured out over the surface of the cuticulin. Extending from the epidermal cells through the endo- and exocuticle, are cytoplasmic filaments, the so-called pore-canals. These appear to penetrate the cuticulin layer also and the polyphenols are extruded from their tips in the form of tiny droplets which later fuse to form a continuous layer.

(iii) Shortly before the old skin is cast, a layer of wax is poured out, again by the epidermal cells, over the surface of the polyphenol layer. It is at this stage that the new cuticle is rendered waterproof; and immediately afterwards the insect moults.

(iv) Finally, within an hour after moulting, the numerous dermal glands discharge over the surface of the wax the protective layer of cement[1].

Thus, when the cuticle is newly formed, the presence of the pore canals ensures that it is alive right to the surface and the underlying cells can control the properties of the surface layers after the deeper parts of the cuticle have been formed. This state of affairs persists. If the cuticle of the living insect is cut the cells around show all the responses characteristic of wound healing[2]. It is interesting to note that they show the same responses when the surface of the cuticle has been so lightly rubbed with alumina dust that no visible injury can be detected. This slight abrasion abolishes the impermeability of the cuticle. But if the insect is kept in a moist atmosphere, it secretes through the substance of its cuticle a new layer of wax, less well organized than the original layer, so that the abraded area acquires a slight milky bloom, but effective enough to restore the impermeability in the course of two or three days almost to its original level[3].

The importance of abrasion of the cuticle in the life of the insect is very evident in those species that live in the soil[4]. The larvæ of insects from the soil usually dry up very rapidly when removed from their humid environment. The cuticle of wire-worm larvæ *(Agriotes)* is freely permeable, so that in watery solutions the larva swells or contracts like an osmometer[1]. This permeability is due to the natural abrasion of the cuticle surface which results from contact with soil particles (Fig. 3). If the wire-worm is allowed to moult out of contact with the soil its cuticle is impermeable and it shows a critical temperature for water loss just like other insects (Fig. 2)[2].

Fig. 3. Part of the cuticle of a wire-worm, *Agriotes*, treated with ammoniacal silver oxide, seen in surface view. Reduction by the exposed phenols reveals the extensive scratching of the cuticle.

These ideas have been extended to the study of the insect egg. The chorion of the egg of *Rhodnius* has proved extremely complex; but the principles of its structure have much in common with those arrived at in the study of the cuticle. Multiple layers are put down; lipo-proteins, polyphenols and tanned proteins form the different strata; and the final waterproofing is due to a thin film of wax. The film of wax, however, is laid down, not by the follicular cells over the surface of the egg, but by the oocyte itself on the inside of the shell[3,4].

The water relations of the insect consist in a balance between water loss and water gain. So far we have been considering the control of water loss—through the cuticle, through the respiratory system and by excretion. Two recent contributions to the study of gain of water by insects deserve notice.

When foodstuffs are oxidized in the course of metabolism, water is produced; and it has long been recog-

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[1] V. B. WIGGLESWORTH, Proc. Roy. Soc. B. (in the press) (1946).
[2] V. B. WIGGLESWORTH, J. exp. Biol. *14*, 364—381 (1937).
[3] V. B. WIGGLESWORTH, J. exp. Biol. *21*, 97—114 (1945).
[4] V. B. WIGGLESWORTH, Nature, *154*, 333 (1944*b*).

[1] A. C. EVANS, Ann. app. Biol. *31*, 235—250 (1944).
[2] V. B. WIGGLESWORTH, J. exp. Biol. *21*, 97—114 (1945).
[3] J. W. L. BEAMENT, Nature *157*, 370 (1946*a*).
[4] J. W. L. BEAMENT, In the press (1946*b*).

nized that this "metabolic water" is of great importance to insects feeding solely upon dry materials. Indeed, under dry conditions such insects as the beetles *Dermestes* and *Tribolium* or the caterpillar *Ephestia* eat more food to produce a given unit of body-weight than they do under moist conditions—because the food eaten is being utilized for water production and not for growth. Consequently the length of the larval period increases and the weight of the pupa decreases. Assuming that every trace of preformed water in the food is retained by the insect, it has been calculated that when the larva of the meal-moth *Ephestia* is reared at a relative humidity of 5 per cent, not more than 7·6 per cent of the water in the full-grown caterpillar can have been ingested with the food; at least 92·4 per cent is metabolic water[1].

At the other extreme of the humidity scale there are divergences of another sort. Certain insects, such as the meal-worm *Tenebrio*[2] and the grasshopper *Chortophaga*[3], if placed in an atmosphere of 85–90 per cent relative humidity after being desiccated, will actually increase in weight through the uptake of water. Since even in the most desiccated insect the tissues contain sufficient water to be in equilibrium with an atmosphere of at least 99 per cent relative humidity, the uptake of water from an atmosphere of 85–90 per cent will require the output of work in the form of active secretion.

The site of the secretory cells has not been demonstrated in any insect. But among the ticks (*Ixodes* and other genera) which show the same phenomenon, it has been proved that the epidermal cells are the active agents[4]. Water is taken up by the cuticle, presumably by some hygroscopic constituent, and then secreted by the cells into the body-fluid. The desiccated young tick can absorb water from an atmosphere as dry as 88 per cent relative humidity; it takes up what it requires to restore the normal composition of the tissue-fluids, and then takes no more. As the tick grows old this power diminishes until finally it can maintain its water content only in an atmosphere saturated with water vapour.

The cuticle of ticks is rendered waterproof in the same way as that of insects; in some species the lipoid layer restricts their loss of water by transpiration to a very low level. But added to this passive retention of water is the active secretion by the epidermal cells. At a certain "equilibrium humidity" (around 88 per cent R. H.) the desiccated tick neither gains nor loses moisture to the atmosphere; but even below this humidity the secretory activity of the cells assists in water retention. If a portion of the surface of the tick is lightly abraded with alumina dust, it can no longer absorb water from the air even at 100 per cent relative humidity. The wound shock to a part of the epidermis has put the whole epidermis temporarily out of action. It recovers its capacity for taking up water if it is kept for a few days in saturated air[1].

Zusammenfassung

Landinsekten konservieren Wasser, indem sie die Stigmen soweit wie möglich geschlossen halten und, außer wenn sie reichlich Wasser in ihrer Nahrung aufnehmen, Wasser von den Exkrementen absorbieren. Dieser Mechanismus setzt die Wasserundurchlässigkeit der Kutikula voraus. Neue Arbeiten über die Struktur und Eigenschaften der Kutikula werden besprochen.

Die «Epikutikula» ist der wasserundurchlässige Bestandteil. Sie hat eine komplizierte Struktur und ist bei der Wanze *Rhodnius* aus mindestens 4 Schichten aufgebaut:

1. Die histologisch sichtbare «Epikutikula», welche, so wird angenommen, aus polymerisierten und mit Quinonen «gegerbten» Lipoproteinen besteht.
2. Eine Lage reich an Polyphenolen.
3. Eine ungefähr 0,25 μ dicke Wachslage.
4. Eine Zementlage, die das Wachs beschützt.

Die erste, zweite und dritte Lage wird von den Epidermiszellen ausgeschieden, die vierte fließt aus den Hautdrüsen aus.

Die Wachsschicht ist die Lage, welche die Transpiration begrenzt, anscheinend als ein Resultat der Orientierung und dichten Packung der innersten Wachsmoleküle. Für jede Insektenart gibt es eine charakteristische Temperatur, bei der das Wachs Wasser leicht permeieren läßt. Diese Temperatur ist bei Insekten aus einer trockenen Umgebung höher; solche Insekten haben ein härteres Wachs mit einem höheren Schmelzpunkt. Wenn lebende Insekten mit gewissen feinen und inerten Pulvern bestäubt werden, wird die Wachsschicht abgerieben, und das Insekt stirbt durch Austrocknung. Insektenlarven aus dem Boden sind wasserdurchlässig als ein Resultat der natürlichen Abreibung durch die Erdpartikel.

Insekten wachsen langsamer in einer sehr trockenen Umgebung, weil ein Teil der Nahrung im Stoffwechsel als Wasser ausgenützt wird anstatt zum Wachsen. Wenn gewisse Insekten aus einer feuchten Umgebung etwas ausgetrocknet werden, können sie aktive Feuchtigkeit aus der Atmosphäre absorbieren. Bei Zecken wird dies verursacht durch eine nach innen gerichtete Sezernierung der allgemeinen Epidermiszellen von Wasser, das von außen durch die Kutikula eintritt. Bei diesen Tieren ist die Erhaltung von Wasser unter trockenen Bedingungen teilweise ein aktiver Prozeß.

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[1] G. FRAENKEL and M. BLEWETT, Bull. ent. Res. *35*, 127—139 (1944).

[2] K. MELLANBY, Proc. Roy. Soc. B. *111*, 376—390 (1932).

[3] D. LUDWIG, Physiol. Zool. *10*, 342—351 (1937).

[4] A. D. LEES, Parasitology, *37*, 1—20 (1946*a*).

[1] LEES, A. D., In the press (1946*b*).