TRANSPIRATION THROUGH THE CUTICLE OF INSECTS

By V. B. WIGGLESWORTH, Agricultural Research Council Unit of Insect Physiology, London School of Hygiene and Tropical Medicine

(*Received* 14 *November* 1944)

(With Plates 1 and 2 and six Text-figures)

The cuticle of most insects is an effective barrier to the evaporation of water. Since the work of Kühnelt (1928, 1939) it has been known that this impermeability is a property of the outermost layer. It is usually ascribed to the epicuticle—defined as the layer, of the order of 1 μ in thickness, that is not penetrated by the pore canals, contains no chitin, and resists solution in cold concentrated hydrochloric or sulphuric acids. From chemical tests Kühnelt concluded that the epicuticle contained fatty acids and cholesterol-like bodies. He therefore refers to it as a 'lipoid cuticula'.

The presence of lipoid material or of waxes in the outer parts of the insect cuticle has been indicated further by Wigglesworth (1933), Bergmann (1938), Pryor (1940), Hurst (1940) and others. But it has never been clear whether such materials were to be regarded as impregnating the lipoid-free substance of the epicuticle, as being chemically condensed or polymerized to compose the epicuticle or as forming a discrete layer upon its outer surface.

It was shown by Ramsay (1935) that the cockroach owes its impermeability to water to a thin and apparently mobile layer of lipoid on its surface. At a critical temperature of about 30° C. this lipoid seems to undergo a change of phase, and it then allows water to pass freely through it. This interesting observation has never been confirmed on other insects. It forms the starting-point of the present study.

PART I. THE EFFECT OF TEMPERATURE ON TRANSPIRATION

Material and methods

Representative insects of diverse groups from different habitats have been exposed for short periods in dry air and their loss of weight by evaporation measured at different temperatures. In most experiments the insects have been first killed with hydrogen cyanide and the spiracles occluded with cellulose paint. They have been placed in a small basket of metal gauze, usually three or four together, and suspended in a conical flask over phosphorus pentoxide. The flask is immersed in a water bath and the temperature recorded on a thermometer with the bulb close beside the suspended basket. The small opening to the flask, combined with the large surface of the desiccating agent and the relatively small air space, ensure the maintenance of an almost dry atmosphere around the insect.

When the same insects are used the same rates of evaporation are obtained whether they are exposed in this apparatus for 15, 30 or 60 min. Equilibrium is probably established very rapidly. In most experiments the short exposures have been employed in order that the entire series of readings may be taken without depleting the water content of the insect unduly. Provided the spiracles are sealed, there is no difference in the rate of evaporation from dead or living insects.

The rate of loss has been expressed in mg. per sq. cm. of surface per hour. The estimation of the effective 'surface' of the insect is a difficult matter. Where the rate of passage of water is very slow every little convolution and irregularity in the surface ought probably to be taken into account. Where the permeability of the cuticle is greater, the concentration of water vapour in the finer irregularities will make the gross surface a truer measure of the effective evaporating area. For the present purpose the fine foldings of the surface have been disregarded. The cuticle of an insect of known weight has been cut up and its surface area measured on squared paper. From this figure the value of k in the formula $S = k \times W^{\frac{2}{3}}$ has been found and so the surface area in sq. mm. (S) of any other member of the same species calculated from the weight in mg. (W). The following are examples of the values of k that have been employed: *Agriotes* larva, 11·0; *Tenebrio* larva, 8·4; *Calliphora* larva, 6·8; *Nematus* larva, 8·5; *Pieris* larva, 9·8; pupa, 8·4; *Rhodnius* nymph (recently fed), 8·1. This procedure gives only approximate values; but the specific differences in evaporation are so great that an error of 50% in the value of k will not affect the conclusions drawn.

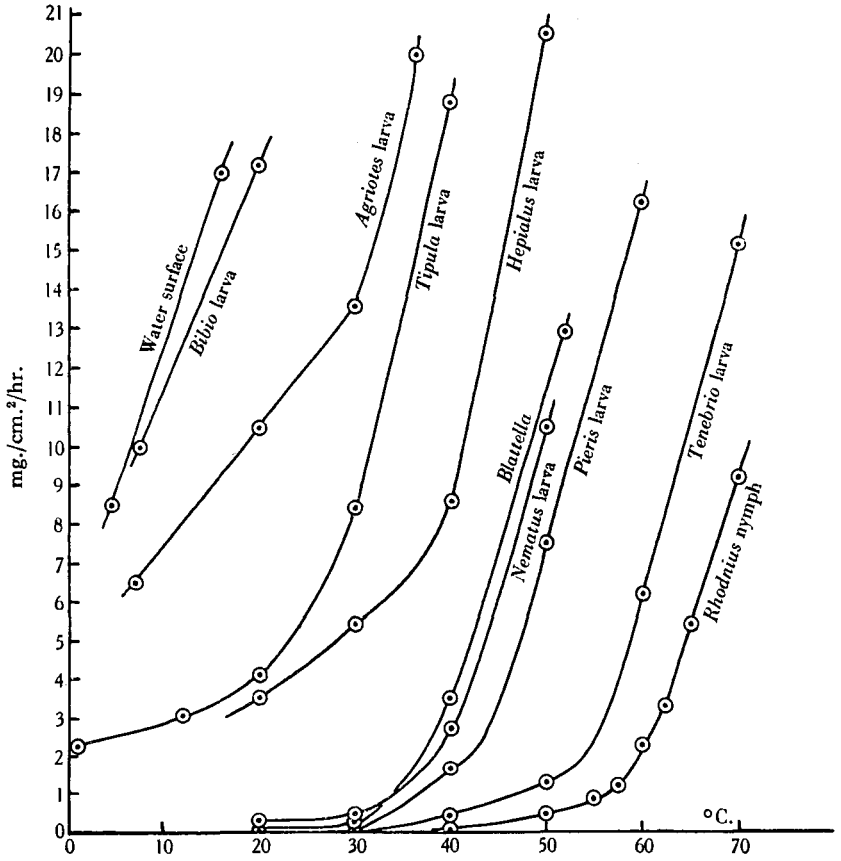
The rate of evaporation from a free-water surface was obtained by exposing a shallow capsule brimful of water in the same apparatus.

Results

Text-fig. 1 shows the results obtained on a small series of insects representative of different habitats. Each point represents a mean value from four or

eight individuals. The curve for water loss in *Blattella germanica* agrees fairly well with that obtained by Ramsay (1935) on *Periplaneta*; it shows a 'critical temperature', with an abrupt increase in evaporation a little above 30° C. The curve of the leaf-eating larvae of the sawfly *Nematus ribesii* and the caterpillar *Pieris brassicae* do not differ greatly from that of *Blattella*. But the insects from dry environments, which can survive long periods without feeding (larvae of *Tenebrio molitor* and nymphs of *Rhodnius prolixus*) show a critical temperature 20–30° C. *oleracea* and *Hepialus lupulinus*, the rate of evaporation at low temperatures is less and there is evidence of a rather ill-defined critical temperature.

Differences in the rate of transpiration through the cuticle and in the critical temperature may be well shown in the different stages of a given insect. This is illustrated in Text-fig. 2. In *Tenebrio* there is little difference in the transpiration curves of larva, pupa or adult. But there is a great increase in the waterproofing of *Bibio* on pupation; and in the pupa of *Pieris*, which has to withstand exposure in the open

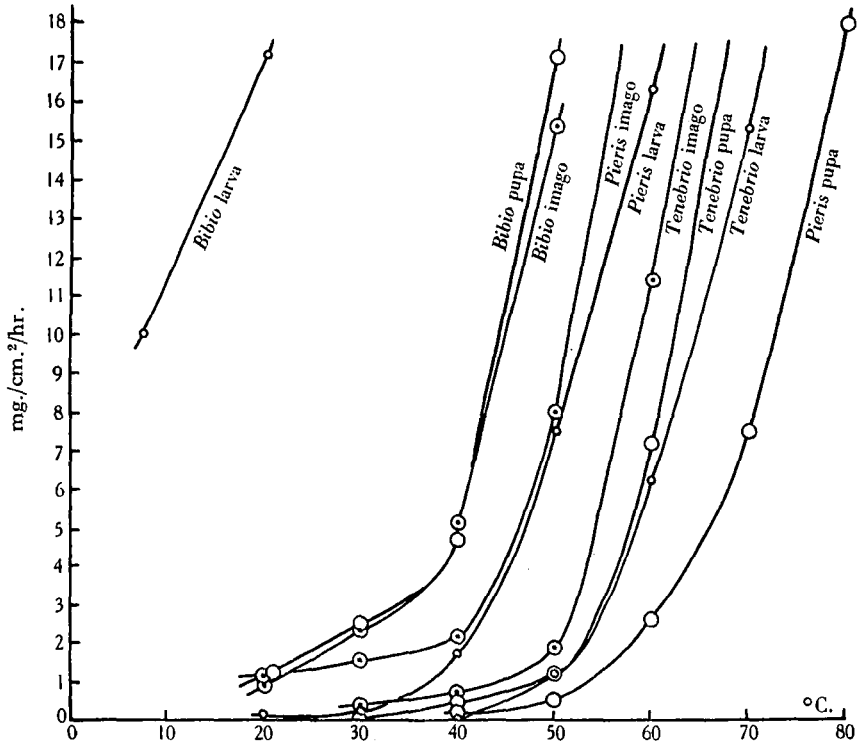


Text-fig. 1. The rate of evaporation of water from dead insects at different temperatures.

higher. Below the critical temperature the transpiration through the cuticle is very small in all these insects, though it is noteworthy that at say 20° C. the rate is generally higher in the insects with the lower critical temperature.

On the other hand, the group of insects from the soil, which normally exist in an atmosphere saturated with moisture, all show a high rate of transpiration even at the lowest temperatures. In the larvae of *Bibio marci* the rate approximates to that of a free-water surface, with no visible break in the curve. In the larvae of wireworms *Agriotes* spp., of *Tipula* for many months, the critical temperature exceeds even that of *Tenebrio* and *Rhodnius*. In these experiments the spiracles of the imagines of *Bibio*, *Tenebrio* and *Pieris* were not blocked. The true curve of evaporation for the adults should therefore probably lie slightly to the right.

These and other results are summarized in Table 1, in which the standard of comparison used is the temperature at which the loss of weight by evaporation amounts to 5 mg. per sq. cm. of surface per hour. This figure has been read off the evaporation curves; it gives a value perhaps 8 or 10° C. above the



Text-fig. 2. The rate of evaporation of water from dead insects in larval, pupal and adult stages at different temperatures.

Table 1. *Temperature in* ° C. *at which evaporation into dry air equals* 5 *mg. per sq. cm. per hr.*

Insect	Larva	Pupa	Imago
Bibio marci (Dipt. Bibionidae)	< 5	39·5*	40·5*
Pterostichus madidus (Col. Carabidae)	< 10	40·5*	40·0*
Agriotes sp. (Col. Elateridae)	< 10	44·5*	46·0*
Aphodius fimitarius (Col. Scarabaeidae)	15·5	—	—
Agrotis segetum (Lep. Noctuidae)	19·0	63·5	—
Tipula oleracea (Dipt. Tipulidae)	23·0	62·0	—
Phyllopertha horticola (Col. Scarabaeidae)	25·5	34·0	45·0*
Hepialus lupulinus (Lep. Hepialidae)	28·0	42·0	—
Blattella germanica (Orth. Blattidae)	—	—	42·5
Calliphora erythrocephala (Dipt. Muscidae)	41·0	57·0	43·5*
,, ,, (prepupa)	41·0	—	—
,, ,, (puparium)	41·0 (65·0)†	—	—
Nematus ribesii (Hym. Tenthredinidae)	43·5	—	—
Pieris brassicae (Lep. Pieridae)	46·5	66·5	46·0*
P. rapae (Lep. Pieridae)	48·0	65·0	—
Ephestia kuehniella (Lep. Pyralidae)	57·5	61·5	47·0*
Achroia grisella (Lep. Pyralidae)	59·5	57·0	—
Tenebrio molitor (Col. Tenebrionidae)	58·5	63·0	54·0*
Rhodnius prolixus (Hem. Triatomidae)	64·5	—	—

* Spiracles not blocked. † Three days old.

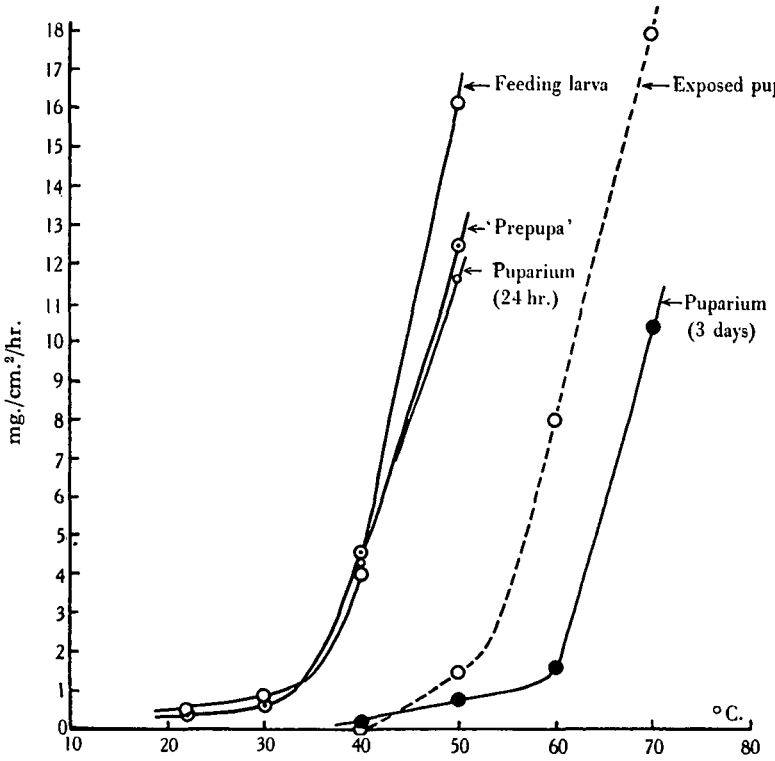
critical temperature, which is itself difficult to define without taking measurements at a large number of temperatures.

Evaporation from Rhodnius prolixus

The foregoing comparative results have been based on measurements of evaporation at a few temperatures only, usually at intervals of 10° C. More detailed studies were made on nymphs of *Rhodnius prolixus*. 4th stage and 5th stage nymphs 1 or 2 days after feeding have been used; they have identical curves of evaporation. As shown in Text-fig. 1, measurements were made at intervals of 2·5° C. around the critical temperature, each point being based on the averages of from four to twelve insects. It can be seen that there is an abrupt change in the rate of transpiration at about 57·5° C.; but even at 70° C. the evaporation is not more than one-sixth of that from a free-water surface at that temperature.

The rate of transpiration at a given temperature seems to be a definite figure which remains constant on repetition. Thus in a group of three 5th-stage nymphs with spiracles blocked the loss of weight in mg. per hour at 60° C. in four successive exposures of 15 min. was: 2·4, 2·8, 2·0, 2·8. The same insects exposed at 70° C. gave losses per hour in three successive exposures of 18·6, 17·6 and 17·6 mg.

On the other hand, this constancy is not altogether maintained if the insect is exposed alternately to high and low temperatures. After exposure to temperatures above the critical level there is some slight permanent increase in transpiration on return to a lower temperature; and this increase is greater the higher the temperature has been. Thus in successive exposures for 15 min. the loss of weight in mg. per hour in one group of 5th-stage nymphs was as follows: 50° C. 0·9; 65° C. 12·4; 50° C. 2·2; 80° C. 56·8; 50° C. 3·8. In another group the rates of loss were as follows: 50° C. 1·4; 80° C. 58·0; 50° C. 3·8; 50° C. (after keeping 24 hr.) 3·2; 50° C. (after keeping a further 3 days) 3·8. The exposure to high temperatures has clearly done some slight permanent injury to the water-proofing layer.



Text-fig. 3. The rate of evaporation of water from *Calliphora* in larval and pupal stages at different temperatures.

Evaporation from larvae and pupae of Calliphora erythrocephala

The results obtained with larvae and pupae of *Calliphora* are set out in Text-fig. 3. The insects have been killed with cyanide and the spiracles blocked. Feeding larvae removed from the meat, larvae (so-called 'prepupae') which have left the meat and evacuated the gut, and fully darkened puparia 24 hr. old, all show curves for evaporation that are substantially alike.

the other hand, there is a very striking displacement of the curve, with a great reduction in evaporation at high temperatures, in puparia 3 days old in which pupation has occurred. This increased water-proofing is due, not to any change in the puparium, but to the delicate cuticle of the true pupa; for when the puparial shell is peeled off and the young pupa exposed, there is only a relatively slight increase in permeability at high temperatures —such as would result from the removal of a comparatively permeable outer sheath.

The pupal cuticle in *Calliphora* is excessively fragile. These observations therefore afford further evidence of the importance of the thinnest layers in the prevention of evaporation.

Evaporation from larvae of Agriotes

Each point on the curve for evaporation from *Agriotes* larvae as given in Text-fig. 1 was derived from a separate group of larvae. For it was found that in these larvae the rate of evaporation at a given temperature diminished at each successive exposure. Thus a larva weighing 37·6 mg. was exposed for six successive periods of 15 min. at 30° C. The rates of loss expressed in mg. per sq.cm. per hour were as follows: 17·2, 11·6, 9·6, 7·6, 4·4, 2·8. The low final value may have been influenced by the depletion of water in the body; but the rapid initial fall must be due to a progressive reduction in the permeability of the cuticle*. A similar effect was seen in some of the *Aphodius* larvae; but the phenomenon has not been more closely studied.

The significance of the great and varied permeability of the insects living in the soil will be discussed further when the action of abrasive dusts has been considered. Further results on evaporation from the wireworm will then be given (p. 106 and Text-fig. 6).

PART II. THE EFFECT OF ABRASIVE DUSTS ON TRANSPIRATION†

It has been known for many years that insects in dry environments may be killed by exposure to certain chemically inert dusts, and it has been generally agreed that the cause of death is desiccation. The subject has been fully reviewed recently by Alexander, Kitchener & Briscoe (1944). These authors (and cf. Parkin, 1944) have made an extensive comparative study of the action of such dusts and have proved conclusively that they act by increasing the rate of transpiration through the cuticle. They suggest that this may be due to the 'crystalline forces' at the surface of the particles of dust exerting a specific attraction on the fatty film of the epicuticle and thus interrupting the continuity of the water-proofing layer.

In the present experiments the chief dust used has been alumina, which Alexander *et al.* (1944) have shown to be one of the most active, but some comparative observations have been made with powdered quartz and slate dusts.

Effect of dusts on evaporation in Rhodnius

Alexander *et al.* (1944) found that the dusts had no action on dead insects. This has been readily confirmed in *Rhodnius*. 5th stage nymphs of *Rhodnius* 1 day after feeding, with an average weight of 132 mg., were killed with cyanide and the spiracles sealed with cellulose paint. They were then kept at 30° C. over phosphorus pentoxide for 24 hr., half being thickly dusted with alumina and half serving as controls. The controls lost an average of 2·0 mg. (1·5 % of the weight), the insects covered with alumina lost 1·7 mg. (1·3 %).

Similar insects, with the anus blocked with paraffin, allowed to run on filter paper lightly dusted with alumina under the same conditions were completely shrunken and dead in 24 hr., having lost 46·5 % of their weight. Insects running on clean filter paper lost only 2·2 %.

But the living state of the insect does not appear to be the determining factor. Living 5th stage nymphs with normal spiracles were suspended in mid-air by sealing the thorax to the head of a bent pin with paraffin and sealing the point of the pin to the wall of a small capsule (Text-fig. 4A). Control insects suspended in dry air at 30° C. lost 1·8 % of their weight in 24 hr. Insects heavily dusted with alumina all over the body, including the spiracles, lost 1·9 %.

The insects suspended in this manner remain motionless during the experiment. It seemed likely that it is the movement of the insect which is needed for the action of the dust. It was observed that as the engorged *Rhodnius* nymph crawls on a surface the posterior region of the ventral abdomen touches the ground and is continually rubbed (Text-fig. 4B). If a small amount of paraffin wax is placed on this bearing surface so that it is held away from the ground (Text-fig. 4C) and the insect is then allowed to run on the dusted filter paper, it suffers comparatively little desiccation and instead of dying within 24 hr. in dry air it will survive for days. And this is so in spite of the fact that the whole of the body soon becomes covered with a film of dust.

Experiments along these lines, using alumina, finely powdered quartz (with particles of 0·5–1·0 μ)* and fine slate dust† are summarized in Table 2. As seen in the same table, a considerable increase in evaporation occurs if the insect runs on fine emery cloth.

* Dr D. L. Gunn has pointed out to me that a similar decrease in permeability to water following desiccation is seen in non-living membranes (cf. King, 1944).

† Certain of these results have been published in a preliminary note (Wigglesworth, 1944*a*). Confirmatory observations were reported by Kalmus (1944).

* Kindly supplied by Dr G. Nagelschmidt.

† Kindly supplied by Dr I. Thomas.

These experiments suggest that the dusts act as simple abrasives and that they increase the permeability of the cuticle only if this is rubbed upon the dusted surface. As seen in Table 2, there is some increase in evaporation even when the bearing surface is protected by wax. As will be shown later (p. 103), this is due to the chance abrasion of other parts and to the dust getting between the moving surfaces of the limb joints and causing their abrasion. A similar difference can be seen when fed and unfed nymphs are allowed to run on dusted filter paper. In the unfed insect the abdomen is held above the surface and only rubs against it occasionally (Text-fig. 4D). In one such experiment, during 24 hr. in dry air at 30° C., the loss of weight was as follows:

Fed insects on clean filter paper	6·5 mg.
Fed insects on dusted filter paper (alumina)	47·7 mg.
Unfed insects on clean filter paper	0·4 mg.
Unfed insects on dusted filter paper (alumina)	7·0 mg.

As can be seen in Table 2, the quartz dust is less effective than the alumina, and the slate dust less effective still. A difference can be readily appreciated by the tip of the finger drawn across the dusted paper. That treated with alumina feels like exceedingly fine sandpaper; the quartz dust feels less abrasive; the slate dust smoother still.

The necessity for abrasion by the dust can be demonstrated on the dead insect. Dead 4th stage nymphs, recently fed, and with the spiracles blocked, in dry air at 30° C. for 5 hr. lost 0·8% of their initial weight. Similar insects pressed firmly down into a thick layer of alumina lost 0·9%. Insects pressed lightly against dusted filter paper and at the same time drawn along, lost 30·0%. In this last experiment the dust was removed from the cuticle within less than a minute of application by brushing with a camel's-hair brush under a stream of water.

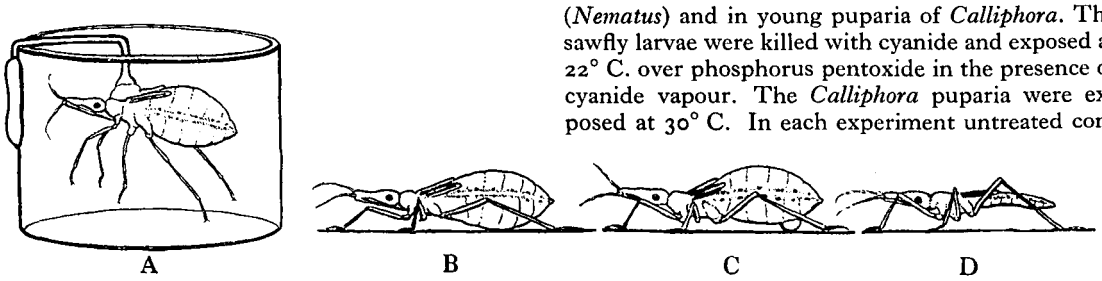
Table 2. *Average percentage loss of weight in 24 hr. in dry air at 30° C. from recently fed 5th stage nymphs of* Rhodnius

Running on clean filter paper	2·1
Running on emery cloth no. 0	7·8
Running on filter paper dusted with slate dust	8·4
Running on filter paper dusted with slate dust with mound of wax on the bearing surface	4·3
Running on filter paper dusted with fine quartz dust	17·8
Running on filter paper dusted with fine quartz dust with mound of wax on the bearing surface	5·2
Running on filter paper dusted with alumina	46·5
Running on filter paper dusted with alumina with mound of wax on the bearing surface	7·6

Effect of dusts on evaporation in some other insects

It is clear that the impermeable film on the surface of *Rhodnius* is interrupted only by the mechanical action of the dusts. Dust in stationary contact with the cuticle will not adsorb lipoid material and so break the film. But the cuticular wax of *Rhodnius* is a hard material with a high melting-point (Beament, 1945) and a high critical temperature. It is possible that adsorption alone may serve to remove the waterproofing layer in insects with waxes of lower melting-point.

This has been tested in larvae of the sawfly (*Nematus*) and in young puparia of *Calliphora*. The sawfly larvae were killed with cyanide and exposed at 22° C. over phosphorus pentoxide in the presence of cyanide vapour. The *Calliphora* puparia were exposed at 30° C. In each experiment untreated controls were compared with insects rubbed on filter paper dusted with alumina and with insects sprinkled with the dust. The results (Table 3) show that in



Text-fig. 4. A, method of suspending 5th stage nymph of *Rhodnius*. B, 5th stage nymph of *Rhodnius*, fed; showing how the abdomen rubs against the ground. C, the same; abdomen held away from the ground by a mound of paraffin. D, 5th stage nymph, unfed; abdomen out of contact with the ground.

Table 3

Insect and treatment	Loss of weight % in 24 hr.
Nematus larva	
A. Control	8·2
B. Rubbed with alumina	66·5
C. Sprinkled with alumina	8·3
Calliphora puparium 24 hr. old	
A. Control	14·6
B. Rubbed with alumina	51·0
C. Sprinkled with alumina	15·0
Calliphora puparium 3–4 days old	
A. Control	4·5
B. Rubbed with alumina	4·0
C. Puparial shell largely removed	6·9

[redacted] insects, as in *Rhodnius*, rubbing with the dust increased greatly the rate of evaporation but that sprinkling with the dust had no action. In the puparium of *Calliphora* 3–4 days old, in which pupation has taken place, rubbing with the dust naturally has no effect.

In the cockroach, as was shown by Ramsay (1935), the waterproofing lipoid is far more mobile; for example, it will spread over the surface of water droplets sprayed on to the cuticle and protect them from evaporation. Table 4 shows the results of an experiment with the cockroach (female *Blatta*), killed with cyanide and exposed to dry air in the presence of cyanide. Control insects (A) were compared with insects (B) in which the dorsal and ventral surfaces of the abdomen were rubbed on filter paper dusted with alumina and the dust then wiped off, and with others (C) sprinkled all over with alumina. It can be seen that in the cockroach simple dusting without rubbing causes rapid desiccation. This is apparent within the first 4 hr. after dusting. During this period the rate of water loss was greater in the insects which had been rubbed with the dust; but during the next 20 hr., whereas the rate of loss from the rubbed insects had diminished, the loss from the insects sprinkled with the dust had increased. During the ensuing 24 hr. the rubbed insects showed a further diminution; the sprinkled group would probably have lost water at a still greater rate but for the fact that they had become completely dried up.

Table 4. *Evaporation from dead cockroach* (Blatta) *at* 20° C. *in dry air expressed as mg. per hr. during successive periods of exposure. Average initial weight:* 880 *mg.*

Treatment	First 4 hr.	Next 20 hr.	Next 24 hr.	Total loss %
A. Control insects	1·9	1·5	1·4	8·4
B. Tergites and sternites of abdomen rubbed with alumina	9·7	5·2	3·4	32·0
C. Sprinkled with alumina	6·6	9·9	(7·2)*	46·8

* Almost fully desiccated.

Visible effects of dust on the cuticle

The cuticle of *Rhodnius*, where it has been rubbed with alumina by allowing the 5th stage nymph to crawl on dusted filter paper or by pressing the insect against the filter paper and drawing it along, has been examined microscopically in surface view and in sections. No injury to the epicuticle or to the bristles can be detected. The layer which prevents evaporation probably lies, therefore, on the outer surface of the epicuticle.

Now it has been shown by Schmallfuss and others (1933) that the outer layers of the insect cuticle commonly contain dihydroxyphenyl-alanine, dihydroxyphenyl-acetic acid or other polyphenols which provide the chromogens used in the production of melanin; and Pryor (1940) has found evidence to show that the quinones produced by oxidation of the polyphenols, by 'tanning' the cuticular proteins, are responsible for the hardening of the cuticle after moulting. The polyphenols are readily demonstrated by exposure to ammoniacal silver hydroxide, which they reduce to give a deep brown stain (Lison, 1936). Pl. 1, fig. 3, shows a section of a fresh *Rhodnius* cuticle, cut with the freezing microtome and immersed for 1 hr. in ammoniacal silver hydroxide. The epicuticle is everywhere stained dark brown.

If the living *Rhodnius* nymph, however, is immersed in freshly prepared 5% ammoniacal silver hydroxide, washed in distilled water, and the cuticle then dissected off, fixed in Carnoy's fixative and mounted flat, there is no sign of staining. The polyphenols are clearly separated from the solution by a protective layer (Pl. 1, fig. 2). But if the insect has been rubbed with alumina, or allowed to crawl on filter paper dusted with alumina or on fine emery cloth, there is very obvious brown staining over the rubbed areas. This staining is confined to the prominent points of the cuticle: the domes of the raised plaques from which the bristles arise and the crests of the stellate folds into which the epicuticle is thrown (Pl. 1, figs. 1, 4). The protective layer has clearly been rubbed off these points.

When the insect has been crawling on dusted filter paper there are brown staining patches also where the tibia and tarsus have been drawn across the surface, and between the articulations of the limb joints where these have rubbed together. The insects that have crawled on emery cloth often suffer a more brutal abrasion and obvious scratches can be seen (Pl. 1, fig. 5).

In section the brown staining of the prominent crests is seen to be confined to the epicuticle (Pl. 1, fig. 4). Nymphs rubbed with the dust have been immersed in the silver solution for periods ranging from 5 min. to 2 hr. The prolonged exposure causes only a slight increase in the extent of the brown staining areas. These spots therefore probably represent the true limits of the areas from which the protective layer has been abraded.

Similar results have been obtained with other insects. *Calliphora* puparia show no staining on immersion in the silver solution; but after rubbing gently on the dust, brown staining spots appear on all the prominent ridges (Pl. 1, fig. 6). Larvae of *Tenebrio* kept for a few hours on dusted filter paper show brown staining areas where the coxae rub against the thorax (Pl. 1, fig. 7), between the limb joints, and at all those points on the ventral surface of the abdomen where this touches the ground as the larva creeps along. Larvae of *Ephestia* on dusted filter paper are very rapidly affected and become desiccated within a few hours. Treatment with silver shows that not only has the protective layer been abraded from the

prolegs and wherever the cuticle has touched the ground, but the dust has also got into the surface folds everywhere so that staining occurs wherever one point on the surface rubs against another. In general, the rate of water loss from an insect treated with the dust runs parallel with the extent of the abraded areas as shown by treatment with silver.

Recovery of impermeability after treatment with dust

5th stage nymphs of *Rhodnius* 24 hr. after feeding were treated with alumina by laying them on their backs on dusted filter paper and drawing them over the surface for a standard distance while pressing them gently down with the finger. In this way a rounded area of fairly constant extent on the dorsal surface is abraded. The dust was removed at once with a camel's-hair brush under a stream of water. The rate of evaporation in dry air at 30° C. was measured immediately after abrasion and after the insect had been kept at 25° C. in saturated air for several days. The results are shown in Table 5.

Table 5. *Loss of weight of 5th stage nymphs of* Rhodnius, *with mean initial weight of* 120 *mg., during* 24 *hr. in dry air at* 30° C.

A. Immediately after rubbing with alumina	31·1 mg.
B. After keeping 1 day in moist air at 25° C.	5·2
C. After keeping 2 days in moist air at 25° C.	3·4
D. After keeping 3 days in moist air at 25° C.	2·4
E. Normal controls	1·5

Similar recovery has been demonstrated in larvae of *Tenebrio* and in pupae of *Pieris brassicae* after rubbing with the dust (Table 6).

Table 6. *Loss of weight during* 24 *hr. in dry air at* 30° C.

1. Pupae of *Pieris brassicae* (mean initial weight 370 mg.):	
A. Immediately after rubbing with alumina	42·1 mg.
B. After keeping 2 days in moist air at 20° C.	8·6
C. After keeping 6 days in moist air at 20° C.	4·3
D. After keeping 2 weeks in moist air at 20° C.	3·2
E. Normal control	0·7
2. Larvae of *Tenebrio molitor* (mean initial weight 90 mg.):	
A. Immediately after rubbing with alumina	46·5 mg.
B. After keeping 4 days in moist air	5·5
C. Normal control	1·0

It is clear that to a large extent the impermeability of the cuticle can be restored, although recovery never seems to be complete. This recovery is dependent on the activity of the epidermal cells and takes place only if they are living. Thus 4th stage nymphs of *Rhodnius* were dusted by rubbing as described above and the dust removed immediately. Some of these were exposed to dry air at once; the remainder were kept in saturated air for 24 hr., half of them living and half of them after exposure to hydrogen cyanide. At the end of 24 hr. those exposed to cyanide appeared dead, but the gut was still showing peristaltic movements. Table 7 shows the evaporation from these three groups of insects.

Table 7. *Loss of weight of 4th stage nymph of* Rhodnius, *with mean initial weight of* 95 *mg., during* 24 *hr. in dry air at* 30° C.

A. Alive: immediately after rubbing with alumina	36·1 mg.
B. Killed with HCN: immediately after rubbing with alumina	37·3
C. Alive: after keeping in moist air for 1 day	9·2
D. Killed with HCN: after keeping in moist air for 1 day	36·6

The importance of the living cells is shown also by experiments in which the cuticle has been abraded by dusting in the later stages of moulting, at a time when the old cuticle has become detached from the cells and the new cuticle is being laid down but has not yet become impermeable. The 5th stage nymph moults at about one month after feeding. Table 8 shows the absence of recovery in insects dusted at three weeks.

Table 8. *Percentage loss of weight of 5th stage nymphs rubbed with alumina and the dust removed at once, kept for 1 day at* 25° C. *in moist air and then exposed for* 24 *hr. in dry air at* 30° C.

A. 3 days after feeding	3·8
B. 3 weeks after feeding	31·9

We have seen that in *Rhodnius* and most other insects adsorption by the dust will not by itself break down the protective film. But once this film has been interrupted by abrasion the presence of the dust may seriously interfere with the recovery process. This may be shown by comparing the efficiency of recovery in insects in which the dust has been removed immediately after dusting, with others in which the dust has been left on (Table 9). When the dust is left on the cuticle, recovery is very incomplete even after many days.

Table 9. *Percentage loss of weight of 5th stage nymphs rubbed with alumina, exposed for* 24 *hr. in dry air at* 30° C.

A. Immediately after rubbing; dust left on	48·2
B. Immediately after rubbing; dust removed	47·5
C. After keeping in moist air for 2 days; dust left on	16·8
D. After keeping in moist air for 2 days; dust removed	3·1

In the cockroach, on the other hand, as shown in Table 4, adsorption by the dust quickly leads to increased evaporation. The high degree of mobility in the waterproofing grease of the cockroach not only leads to its removal by adsorption, but, as Table 4 also shows, it allows a considerable amount of recovery in the dead insect—by the spreading of the oil over the abraded areas.

Visible changes during recovery

If the dust has been removed from the *Rhodnius* nymph as completely as possible immediately after application, and the insect then kept in moist air for a few days, the rubbed cuticle develops a milky bloom. The effect is more striking if the dust is left on; what is at first an almost imperceptible film is then converted within 2 or 3 days into a conspicuous chalky white patch, more or less firmly adherent to the surface of the cuticle. In both cases this waxy secretion is more evident if the insect after recovery is kept in a dry atmosphere, just as certain plants (which likewise are able to regenerate their waxy coating) produce less wax if they are grown in a damp atmosphere (Haberlandt, 1914).

As the new wax is secreted the cuticle becomes less readily wetted by water. At first droplets of water will adhere to the surface; after a few days the droplets fall from the cuticle without wetting it. Measurements of the angle of contact between water and the cuticle (kindly made for me by Mr Beament) show that this increases from 102° to nearly 120° during the first 48 hr. as the wax is being secreted. It is of interest to note that the angle increases also, though more slowly, on the normal cuticle as moulting proceeds; by 21 days after feeding it has increased gradually from 102° to 117°. An extra quantity of wax thus appears to be secreted on to the surface of the old cuticle before the epidermal cells separate from it and so lose the power of making good any abrasion that may occur.

The presence of the wax may be demonstrated also with fat stains. If the cuticle of the insect which has recovered from dusting is heated with Black Sudan B at 50° C., the waxy deposits, chiefly on the sides of the epicuticular folds, are deeply stained. This staining is very much more conspicuous if the dust has not been removed (Pl. 1, fig. 10).

As the wax is secreted, and the impermeability of the cuticle is restored, the patches that stain with silver become smaller; and after recovery has proceeded for a few days no reduction takes place on immersion in the silver solution. Pl. 1, figs. 8 and 9 show two stages in this process. From the way in which the individual star-shaped areas on the crests of the folds are broken up it is evident that the secretion which covers them is coming through the substance of the cuticle everywhere. It clearly does not spread out from the openings of the ducts of the dermal glands; it must be the product of the general epidermal cells.

During the process of recovery after abrasion there are striking changes also in the underlying epidermal cells. In spite of the fact that there is no visible injury to the epicuticle the underlying cells behave as though they had been wounded (Wigglesworth, 1937). They migrate towards those regions that have been most severely rubbed, leaving areas with sparse cells between. This response is not the result of injury to the cells by desiccation, for it occurs equally in insects that have been kept in air saturated with moisture. Indeed, the cells behave as though their living substance extended not only by way of the pore canals up to the epicuticle, but through the epicuticle up to the protective film on its surface.

During the next day or so small droplets which stain with fat stains and reduce osmic acid appear in the epidermal cells most affected. This is probably a further reaction to injury; the droplets do not seem to be related with the secretion of wax that is occurring at this time. Another change is the failure of the epidermal cells under the rubbed area to develop the large amounts of red pigment or the white granules (believed to be uric acid) which they normally contain.* At no stage is there any sign of renewed activity in the dermal glands.

Natural abrasion of the cuticle in soil insects

It is evident from Text-fig. 1 that the larvae of insects from the soil lose water very readily at quite low temperatures. The question naturally arises whether this is the result of abrasion by particles of earth. Immersion of the larvae in ammoniacal silver hydroxide reveals in all of them extensive areas over which the layer of polyphenols has been exposed, and in most of them these areas are made up of obvious scratches.

In *Hepialus*, after immersion in the silver solution, there are dark brown areas over all the projecting folds of the body, particularly towards the anterior end (Text-fig. 5A). On microscopic examination these areas are seen to be made up of scratches crisscrossing in all directions. Scratches are very evident also on the outer surface of the true legs (Pl. 1, fig. 11). In *Agrotis* the results are the same. It is noteworthy that in both these caterpillars the cuticle is much more resistant to scratching over the tubercles and over the head where there is a hard exocuticle. In *Aphodius* and *Phyllopertha* similar results are obtained: reduction of the silver appears in the form of scratches on the prominent lateral folds, on the anal extremity and the legs, but particularly over the surface of the rounded back (Text-fig. 5B; Pl. 1, fig. 13). The scratching of the back is less evident in *Phyllopertha*, in which the protective spicules are more strongly developed.

In the wireworm *Agriotes* the body is covered

* There is also an interesting effect on growth. The moulting of the insect is in no way delayed by extensive abrasion of the epicuticle, provided desiccation is prevented. But the process which leads to the determination of new plaques is upset. The number of plaques on one-half of an abdominal tergite when the 4th instar nymph moults to a 5th instar increases from about 200 to 250 (Wigglesworth, 1940). In a nymph which had been rubbed on one side of the abdomen only there were 206 plaques on the rubbed side, 258 on the normal. Injury to the cells caused by rubbing the layers *outside* the epicuticle has prevented the determination of new plaques.

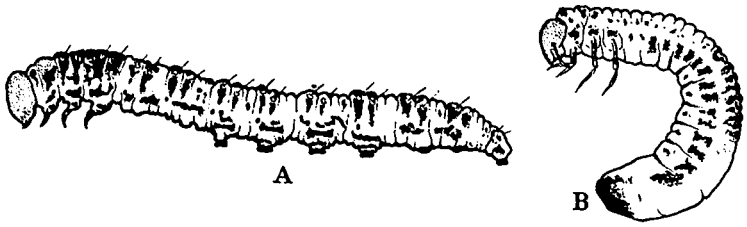
almost everywhere with hardened cuticle which resists extensive abrasion. But there are ventro-lateral lines of soft cuticle and some areas of soft cuticle at the base of the limbs which always show extensive browning in the silver (Pl. 1, fig. 12). And when examined with a higher magnification the hardened cuticle everywhere shows innumerable fine scratches. The degree of this visible scratching runs parallel with the rate of evaporation. Thus in one series of wireworms exposed at 40° C. the most permeable lost 39·2% of its weight in 15 min.; another lost only 17·2%. Pl. 2, figs. 1 and 2, show corresponding areas of cuticle from these two insects. The more permeable is much more severely scratched.

In the larva of *Pterostichus* the hard cuticle is restricted to plates or sclerites set like islands in the soft body surface (Pl. 2, fig. 3). Here the abrasion of the soft areas is so extensive that in many places they show a uniform brown staining with the silver, and individual scratches cannot be seen. But where there are depressed folds, as between the body segments, abrasion is incomplete and in some places linear scratches appear. These scratches are sometimes continuous with the scratches on the sclerites. On the sclerites, as on the hard parts of the other insects, abrasion is limited to fine scratches, running mostly in the long axis of the body (Pl. 2, fig. 4).

The larva of *Bibio* is everywhere covered with little plaques bearing rows of blunt spines. The plaques show some abrasion, and in the soft cuticle between them there are innumerable small areas which stain with the silver (Pl. 2, fig. 6). These areas do not take the form of scratches, but in view of the observations on the soft cuticle of other insects they are probably the result of abrasion. Likewise in the larva of *Tipula*, there are small scattered points over the cuticle which stain with the silver; in many places these lie in rows and are almost certainly due to abrasion (Pl. 2, fig. 5).

In all these insects the rate of evaporation of water at low temperatures runs parallel with the extent of the silver staining areas (Text-fig. 1). In *Bibio* and *Pterostichus* evaporation is so great that there is no sign of a critical temperature. In *Agriotes*, *Tipula*, *Hepialus*, etc., in spite of the high rate of water loss at the lowest temperatures, the curve turns steeply upwards as soon as the critical temperature is reached.

It is concluded therefore that the permeability of the cuticle in soil insects is the result of abrasion. If this is so, the insect which has moulted and been kept out of contact with the soil ought not to show any notable permeability to water until the critical temperature is reached. This has been tested on the wireworm. Larvae of *Agriotes* were placed singly in specimen tubes with a pledget of moist cotton-wool and a small piece of carrot. The date of moulting was noted and the larva was then kept for 2 weeks. Text-fig. 6 shows the temperature-evaporation curve for such a larva. It falls into line with that of insects from other environments: there is little evaporation until the critical temperature is reached. In an experiment done less than 24 hr. after moulting there was considerable evaporation at low temperatures and exposure to silver showed irregular diffuse areas of brown staining.



Text-fig. 5. Larva of *Hepialus* (A) and *Aphodius* (B) from the soil, after immersion in ammoniacal silver, showing the distribution of the chief areas where the wax layer has been abraded.

Abrasion of the cuticle and the entry of insecticides

Not only is the loss of water by evaporation increased after abrasion by rubbing with the dusts, but also the uptake of liquid water. For example, 5th stage nymphs of *Rhodnius* killed with cyanide 24 hr. after feeding and then immersed in distilled water for 24 hr. showed an average increase in weight (on an initial weight of about 120 mg.) of 0·4 mg. in the normal insect, and 4·1 mg. after rubbing gently on filter paper dusted with alumina. This observation falls into line with the osmotic uptake of water by normal (and therefore abraded) wireworm larvae living in the soil (Evans, 1943).

It was to be expected that this increased permeability of the cuticle would be reflected in an increased susceptibility to insecticides. This has been tested with nicotine and with rotenone. 5th stage nymphs of *Rhodnius* with uniformly thick cuticles were fed, and glass capsules of the type previously described (Wigglesworth, 1942) sealed to the dorsum of the abdomen with cellulose paint. A solution of 2% nicotine, in distilled water, was introduced into the capsule and the top sealed with a cover-glass. The normal insects were slightly affected in 6 hr.; they

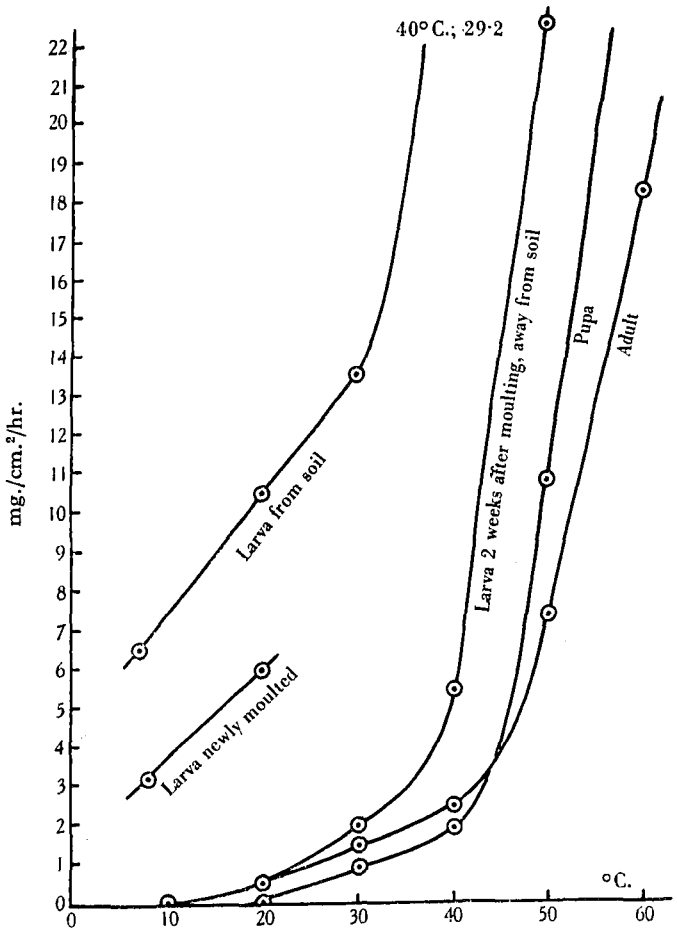
w[redacted]badly affected but had not collapsed in 24 hr. In[redacted]ts in which the back had first been lightly rubbed with alumina dust collapsed and were unable to walk within 20 min.

The experiment was repeated using dry powdered rotenone (Stafford Allen and Sons 90 % rotenone) as the insecticide. The normal insects were unaffected at the end of 3 weeks; those rubbed with the dust showed weakness in 8 hr. and were dead in less than 24 hr.

PART III. THE EFFECT OF SOLVENTS AND DETERGENTS ON TRANSPIRATION THROUGH THE CUTICLE

Since oils and waxes are responsible for maintaining the impermeability of the insect cuticle to water, lipoid solvents and detergents should break down the protective layer.

Experiments along these lines have been made chiefly on *Rhodnius*. Chloroform was selected as a good general solvent for oils and waxes. Table 10



Text-fig. 6. Evaporation from the wireworm larva, pupa and adult at different temperatures.

These observations suggest that the activity of insecticidal powders may be enhanced if an abrasive dust is incorporated with the filler. Special studies will be needed to prove whether this happens in practice, but it is well known that the efficiency of a given insecticide is greatly influenced by the nature of the filler (Turner, 1943). Hockenyos (1939) noted that hydrated lime increased the rate of entry of sodium fluoride through the cuticle of the cockroach, and attributed this to an effect on the oil film.

Table 10. *Loss of water during* 1 *hr. in dry air at* 30° C. *from* 5*th stage nymphs of* Rhodnius 1 *day after feeding. Average initial weight* 130 *mg.*

A. Dead, normal controls	0·2 mg.
B. After extraction with chloroform for 15 min. at 20° C.	2·7
C. After extraction with chloroform for 15 min. at 50° C.	34·6

shows that there is a considerable increase in evaporation after extraction with chloroform at room temperature, a very great increase after extraction at 50° C. Insects from these three groups were subsequently immersed in ammoniacal silver hydroxide and the cuticle of the abdomen examined. The controls as usual showed no staining. Those extracted for 15 min. at room temperature showed staining over the muscle insertions (Pl. 2, fig. 7) but very little elsewhere. Those extracted for 15 min. at 50° C. showed well-marked staining over the muscle insertions and widespread staining elsewhere, particularly in the hollows of the epicuticular folds (Pl. 2, fig. 8).

The wax extracted with hot chloroform from the cast cuticle of *Rhodnius* is readily soluble in chloroform in the cold (Beament, 1945). The foregoing experiments show that extraction from the surface of the cuticle is very much more difficult, and that the ease of extraction varies in different regions. The structural basis of these observations will form the subject of a separate paper (Wigglesworth, 1944*c*); but the general conclusion may here be stated: that the wax layer overlying the epicuticle is further protected by a layer of cement which varies in hardness and resistance to removal in different insects and in different parts of the same insect.

The hardness of the wax in *Rhodnius* is such that the permeability of the insect is not very greatly affected if it is killed with chloroform vapour. But in the soft-skinned larva of the sawfly *Nematus*, and in the cockroach *Blatta*, exposure to the vapour of chloroform at room temperature for ½–1 hr. is sufficient to increase enormously the permeability of the cuticle to water (Table 11).

Table 11. *Loss of weight in* 4 *hr. at* 22° C. *of* Nematus *larvae with average initial weight of* 80 *mg., and* Blatta *adults with average initial weight of* 850 *mg., and in* 24 *hr. at* 30° C. *of* Rhodnius *nymphs, with average initial weight of* 110 *mg.*

Nematus larvae killed with HCN	1·0 mg.
Nematus larvae exposed to chloroform vapour for 1 hr.	39·3
Blatta adult females, killed with HCN	7·6
Blatta adult females, exposed to chloroform vapour for 1 hr.	49·7
Rhodnius nymphs killed with HCN	1·4
Rhodnius nymphs exposed to chloroform vapour for 1 hr.	3·8

We have seen (p. 105) that after abrasion with alumina dust the insect kept in a moist atmosphere will, to a great extent, recover its impermeability to water, and the points where the layer containing phenols was exposed once more become covered by wax. This recovered area does not resist extraction with chloroform as does the normal cuticle. Thus 5th stage nymphs of *Rhodnius* were rubbed with alumina on one-half of the abdomen only and then allowed to recover for 5 days in moist air. On treatment with ammoniacal silver after this period they show no staining on either side. But after immersion in chloroform for ½ hr. at room temperature they showed on the normal side some staining over the muscle insertions and in the form of scattered points in the depressions between the cuticular folds. On the side that had been rubbed there was in addition extensive staining over the crests of the folds—though this staining was not quite so widespread as in the newly rubbed insect.

The same effect is apparent if the rate of evaporation after extraction with chloroform is observed (Table 12).

Table 12. *Loss of weight in mg. in* 4 *hr. at* 30° C. *in dry air from* 5*th stage nymphs of* Rhodnius 4 *days after feeding* (*average weight* 120 *mg.*)

A. Control	0·7 mg.
B. The same extracted with chloroform for 15 min. at 20° C.	6·9
C. 3 days after rubbing with alumina	1·4
D. The same extracted with chloroform for 15 min. at 20° C.	17·1

From these results it appears that the wax in the layer which overlies the epicuticle of *Rhodnius* is covered with or associated with materials which protect it from extraction. If the protective layer is to be broken down and the insect rendered permeable to water (and perhaps to insecticides) an agent must be used that will attack both the wax and the cement substance and at the same time be sufficiently hydrophil for water to pass readily through it.

With this end in view a long series of wetting agents and detergents and other chemicals have been tested. These have been applied as a thin smear over the dorsal surface of the abdomen of living 4th stage nymphs of *Rhodnius* 24 hr. after feeding. Groups of three or four nymphs have been used for each compound and with many of the compounds the experiments were repeated several times. The anus of the nymphs was occluded with paraffin and they were then exposed for 4 and 24 hr. at 30° C. in dry air, and the percentage loss of weight noted. A selection from the results is shown in Table 13.

The substances used show a very wide range of activity in increasing the rate of loss of water, but certain general principles can be discerned. Simple water-soluble wetting agents such as Turkey-red oil, 'Agral', 'Perminal', 'Aerosol OT', have very little effect. Where such water-soluble substances have definite detergent action, as with 'Lissapol' or sodium cetyl sulphate, their effect is somewhat greater. Refined petroleum oils (P31) have very little effect, and so have olive oil and lanoline. But when oils with polar groups such as ricinoleic acid, linoleic acid, oleic acid, or the naphthenic acids are applied, the effect is much increased.

In general it is the oil-soluble substances with hydrophilic properties that are most effective. This

Table 13. *Loss of weight in 4th stage nymphs of* Rhodnius, *24 hr. after feeding, exposed at* 30° C. *in dry air after smearing the dorsal surface of the abdomen with various chemicals*

Chemical substance	Trade name or code number and maker	Loss of weight % in 24 hr.
Control	—	1·5–2·5
Sodium salt of a sulphonated naphthalene derivative	Perminal BX (I.C.I.)	2·4
Sodium salt of a sulphonated aromatic hydrocarbon	Agral 2 (I.C.I.)	2·9
Refined medium petroleum	P31 (white oil) (Shell Co.)	3·3
Ammonium salt of ricinoleic sulphuric acid ester	Turkey-red oil	3·4
Carboxylic acids from petroleum. Acid value: 230mg. KOH/g.	Naphthenic acid NA9 (Technical Products Ltd.)	3·6
B-naphthyl ether of polyethylene glycol, $R(OC_2H_4)_5OH$ (approx.)	R1660 (I.C.I.)	3·75
B-naphthyl ether of polyethylene glycol, $R(OC_2H_4)_{10}OH$ (approx.)	R1661 (I.C.I.)	3·9
'Lecithin'	Alco Soya Lecithin (American Lecithin Co.)	3·9
Sodium secondary alkyl sulphates (chain length C_{10}–C_{18})	Teepol X (Technical Products Ltd.)	4·2
A phthalic glycerol alkyd resin	Emulsifier B1956 (Röhm and Haas)	4·3
Condensation compound of naphthalene sulphonic acid and formaldehyde	Belloid T.D. (Geigy Colour Co.)	4·5
Polyglycerol ester of coconut oil fatty acids (glycerol: fatty acid = 20 : 4)	Lever Bros.	4·5
Ricinoleic acid	—	4·6
Polyethylene glycol monoiso-octyl tolyl ether	Triton NE (Röhm and Haas)	4·6
A straight chain sodium alkyl sulphate	Lissapol C (I.C.I.)	4·9
Diethylaminoethyloleylamide acetate	Sapamine A (Ciba Co. Inc.)	4·9
Complex alkylated benzene ether of polyethylene glycol, $R(OC_2H_4)_8OH$ (approx.)	R2206 (I.C.I.)	5·2
An alkyl naphthalene sulphonic acid	Belloid NW (Geigy Colour Co.)	5·8
Triethanolamine soap	Belloid F (Geigy Colour Co.)	6·2
Sorbitol laurate	Glyco Products Co. Inc.	6·2
Sorbitol oleate	Glyco Products Co. Inc.	6·3
Linoleic acid	—	6·4
Twitchell's base	Technical Products Ltd.	6·6
Oleic acid	—	6·7
Polyglycerol ester of coconut oil fatty acids (glycerol: fatty acid 20 : 2)	Lever Bros.	6·7
Sodium cetyl sulphate	I.C.I.	6·9
Sodium salt of sulphonated ethylmethyloleylamide	Igepon T (General Dyestuff Corp.)	6·9
Coconut oil fatty acids mono-diglyceride (88 : 12)	Lever Bros.	7·2
Sulphonated fatty esters	Calsolene oil HS (I.C.I.)	7·2
Diglycol oleate	Glyco Products Co. Inc.	7·5
Sorbitan monoleate	Span 80 (Atlas Powder Co.)	7·6
Petroleum oil containing sodium naphthasulphonate or similar compounds	Soluble 'cutting' oils (Shell Co.)	8·7
Complex sulphonate	Foamacrex (Rex Campbell Co.)	9·1
Complex sulphonate	Emulsifier SZ (Rex Campbell Co.)	9·6
Polyglycerol ester of coconut oil fatty acids (glycerol: fatty acid = 20 : 6)	Lever Bros.	9·6
Polyglycerol ester of coconut oil fatty acids (glycerol: fatty acid = 20 : 10)	Lever Bros.	10·5
Carboxylic acids from petroleum. Acid value: 170mg. KOH/g.	Naphthenic acid NA17 (Technical Products)	10·6
Ammonium laurate	Ammonium laurate S (Glyco Co. Inc.)	11·1
Sodium naphthasulphonate 50% in P31	Technical Products Ltd.	12·3
Diglycol laurate	Glyco Products Co. Inc.	12·4
Polyglycerol ester o coconut oil fatty acids (glycerol: fatty acid = 20 : 20)	Lever Bros.	13·3
Propylene glycol laurate	Prolaurin (Glyco Co. Inc.)	14·1
Sodium alkyl sulphate (C_{17} with slightly branched chain)	Technical Products Ltd.	15·2
Triethanolamine ester	Belloid F.R. (Geigy Colour Co.)	15·7
Glyceryl monolaurate.	Glyceryl laurate S (Glyco Co. Inc.)	16·3
Ovolecithin	British Drug Houses	16·7
Cetyl ether of polyethylene glycol, $C_{16}H_{33}(OC_2H_4)_{17}OH$ (approx.)	R2204 (I.C.I.)	24·7
Cetyl ether of polyethylene glycol, $C_{16}H_{33}(OC_2H_4)_4OH$ (approx.)	R2211 (I.C.I.)	39·5
Cetyl ether of polyethylene glycol, $C_{16}H_{33}(OC_2H_4)_8OH$ (approx.)	C09993 (I.C.I.)	48·0

is well seen in the series of polyglycerol esters of the coconut oil fatty acids, in which the percentage loss of water rises as the ratio of glycerol to fatty acid falls and the oil solubility increases. Thus with a glycerol/fatty acid ratio of 20 : 2 the loss of weight is 6·7%; 20 : 4, 4·5%; 20 : 6, 9·6%; 20 : 10, 10·5%; 20 : 20, 13·3%.

But these properties do not provide a full explanation of the differences observed. Materials such as Twitchell's base or sodium naphthasulphonate are readily soluble in oil and lead to its spontaneous emulsification in contact with water, and yet they are only moderately effective in increasing the loss of water through the cuticle. The polyethylene glycol ethers of ring compounds (R1660, R1661 and Triton NE), all of which are water-soluble, have very little action. The cetyl ether of polyethylene glycol (C09993) is the most effective

Table 14. *Percentage loss of weight in 4th stage nymphs of* Rhodnius 1 *day after feeding, exposed at* 30° C. *in dry air for* 24 *hr. after smearing the dorsal surface of the abdomen with cetyl ether of polyethylene glycol* (C09993) *at different concentrations in water*

100%	45·3
50%	23·5
10%	11·9
1%	4·5
Control	1·8

It appears therefore that some other properti[illegible]e involved besides an affinity for both oil and w[illegible]r. It may be that the long cetyl chain of the compounds C09993, R2211, etc., is particularly effective in associating with the long chains of the wax and thus leading to its dispersion.

That there is a relation between the quantity of material applied and the effect produced has been shown by applying C09993, dispersed in distilled water in varying concentrations, as a thin film over the dorsum of the abdomen (Table 14).

It may be that the wide differences between the action of the different materials in Table 13 is determined as much by the cement which covers the wax layer in *Rhodnius* as by the wax itself. If this is so one might expect to find large differences in the relative effectiveness of the materials when applied to different insects—in which differences in the properties of both wax and cement probably occur. A small number of materials showing large differences in their action when applied to *Rhodnius*, have been applied to some other insects and their relative effects compared (Table 15). In all cases the cetyl ethers of polyethylene glycol (R2211 and C09993) were the most efficient substances, but in none was their relative effect so outstanding as in *Rhodnius*. In all these other insects the refined paraffin (P31) was relatively more effective than in *Rhodnius*.

Table 15. *Percentage loss of weight from various insects smeared with different materials* (*see Table* 13) *and exposed for* 24 *hr. in dry air.* Rhodnius *nymphs smeared on the dorsum of the abdomen; the other insects smeared all over;* Rhodnius, Pieris *and* Tenebrio *exposed at* 30° C., Nematus *at* 20° C.

Material	*Rhodnius* nymph	*Pieris* pupa	*Nematus* larva	*Tenebrio* larva
Control	2·0	0·1	7·9	2·5
P31	3·3	4·6	17·7	8·9
Diglycol oleate	7·5	8·6	61·2	16·4
Glyceryl monolaurate	16·3	8·6	54·8	22·9
R2211	39·5	11·6	76·6	29·6
C09993	48·0	11·8	73·8	27·7

Table 16. *Percentage loss of weight from* Calliphora *puparium and pupa during* 4 *hr. at* 30° C. *in dry air*

Object and treatment	Loss of weight %
Normal puparium 24–48 hr. old	1·9
Normal puparium before pupation, thinly smeared with C09993	46·7
Normal puparium after pupation,* thinly smeared with C09993	1·7
Pupa exposed by stripping puparium from posterior half	2·3
Pupa exposed by stripping puparium from posterior half, thinly smeared with C09993	17·2

* As determined by the extrusion of the pupal horns.

of all the compounds (see Pl. 2, fig. 9) and it combines oil solubility with dispersibility in water. But the similar compound (R2204) with the longer polyethylene glycol chain is also a very effective substance although it dissolves readily in water and shows little solubility in oil.

In the puparium of *Calliphora* the effect of C09993, like the effect of abrasive dusts, depends on whether pupation has occurred (Table 16). It is possible, when pupation has taken place, to strip away the puparial shell and smear the exposed pupa with the detergent.

Effect of detergents on the entry of insecticides

It has been shown by Fulton & Howard (1938 1942), Hurst (1940, 1943), Wigglesworth (1942) and others that the solvent used for an insecticide influences greatly its rate of passage through the cuticle. It seems likely that the differences between different emulsifying substances in their ability to break down the waterproof layer of the cuticle will be reflected in differences in facilitating the entry of insecticides. This is a large subject which will require careful study. A few experiments only have been made.

Capsules were secured to the backs of 5th stage nymphs of *Rhodnius* with uniformly thick cuticles, by means of cellulose paint, 1 day after feeding, and left 48 hr. to harden (Wigglesworth, 1942). A suspension of rotenone (Stafford Allen 90%: 0·2 g. in 2 ml. of oil) was then introduced and the capsules closed with cover-glasses sealed down with paraffin. Rotenone in refined paraffin (medicinal paraffin) had produced no effect in 7 days. Rotenone in the cetyl ether of polyethylene glycol (R2211) caused the complete collapse of all the nymphs in 24 hr. Nicotine, 2% in refined paraffin (P31), similarly applied had almost no effect in 2 days; in the cetyl ether of polyethylene glycol (R2211) all were dead in 24 hr.

DISCUSSION

The results described confirm the belief that the layer of the cuticle which is impermeable to water is very superficial and very thin. The effective layer lies in fact over the surface of the epicuticle. The puparium of *Calliphora* forms an apparent exception, for here, as was shown by Hurst (1941), the permeability is not increased if the lipoid layer on the surface is broken down. Hurst attributed this property to the condensed chitin-protein complex of the puparial shell; but it has been shown in the present paper that in the prepupa and young puparium of *Calliphora* the impervious layer is wholly superficial as in other insects and that the hardening of the puparium does not increase its impermeability to water. The greater resistance of the three-day puparium to loss of water is due in fact to the very fragile cuticle of the true pupa. So long as this is unbroken it is far more effective in restricting water loss than the hard puparium that covers it. These observations suggest comparison with other multilayered systems, such as the insect egg (cf. Slifer, 1937).

The waterproof layer is of a waxy nature. Its effectiveness is presumably dependent on the orientation and close packing of the long wax molecules. At a critical temperature, which varies widely in different insects, the packing of the molecules is loosened and water can pass through more readily. As was proved on the cockroach by Ramsay (1935), this will account for the fact that the loss of water by evaporation from insects per unit of saturation deficiency rises with rising temperature—as was found by Koidsumi (1934) in the pupa of *Milionia* and by Evans (1934) in the egg of *Lucilia*. The evidence for these ideas is given in the paper by Beament (1945) on the properties of the isolated waxes.

As shown by Beament (1945) the waxes of the different insects have graded properties; they range from the soft grease with polar characters in the cockroach *Blatta*, to the very hard apolar wax of *Rhodnius* or the pupa of *Pieris*. In the living insect, however, there are further complexities. In the cockroach the greasy layer seems to be freely exposed and to be so mobile that it is largely removed by adsorption on to dusts, and is able to spread slowly back over areas which have been abraded. In other insects the wax is covered and protected to a variable extent by a layer of cement. It will be shown elsewhere (Wigglesworth, 1944*c*) that (at least in *Rhodnius*) this layer is the product of the dermal glands and is poured out over the surface of the orientated wax which is itself the product of the epidermal cells.

The deposition of these layers has not been fully considered in this paper; but the secretion of the long wax molecules by the epidermal cells through the substance of the epicuticle presents an interesting problem. Histologically there appears to be a condensed epicuticular layer of say 1 μ in thickness beyond the limits of the pore canals. According to the estimate of Richards & Anderson (1942) it is unlikely that this is perforated by spaces larger than 0·002 μ in diameter. But the wax molecules are probably something like 0·004 μ in length. The epicuticular pores will not therefore admit the passage of the wax in emulsified form. The wax molecules must either be synthesized *in situ* on the surface of the epicuticle or, associated perhaps with some solubilizing substance, they must pass through the epicuticle singly or in small alined groups. It is conceivable that their discharge in this way may favour the close packing and orientation of the wax molecules needed to form an impermeable film.

This view that impermeability to water is due to a continuous layer of closely packed and orientated wax molecules contrasts with the view of Hurst (1943) who regards the surface of the cuticle (of blowfly larvae) as being not homogeneous but composite and made up of heterogeneous protein/lipoid associations in patches. It is admitted, however, that other materials, often perhaps protein, may be present in the form of a covering layer for the wax. Such materials may even be chemically linked with the wax. In any case it is quite evident that there are great differences in different insects. The permeation of lipoid through the cuticle framework, if it occurs at all, seems to be of no importance so far as the prevention of evaporation is concerned.

The observations described emphasize the living nature of the insect cuticle. The retention of water

seems to be a passive process dependent on the form of the wax layer; but the slightest injury to this layer is reflected in the behaviour of the living cells below. They react as though they had been wounded (Wigglesworth, 1937); they immediately cluster towards the injured site (although the epicuticle has not been visibly damaged) and they then proceed to secrete fresh wax to repair the damage.

The abrasion of this delicate surface layer must be a common occurrence in the life of the insect, and were the insect unable to make good slight injuries to its surface layer it would soon cease to be impermeable to water. In the insect larvae in the soil the abrasion is usually so severe that they have lost their power to retain water. The wireworm, for example, is scratched all over. In spite of its hydrophobe cuticle it loses water by evaporation very rapidly and liquid water passes in and out in accordance with the osmotic equilibrium (Evans, 1943). Thus, although they retain the waterproof cuticle characteristic of their class, the soil insects must remain in moist surroundings.

In discussing the mode of action of fine dusts in causing insects to dry up, Germar (1936) does not altogether rule out the possibility of their sharp edges wounding the cuticle, but he is inclined to doubt this explanation because he could see no injuries under the microscope. The use of ammoniacal silver provides a ready method of demonstrating such injuries to the waxy layer. Mechanical abrasion thus provides a simple explanation of the effect of inert dusts—in the sense that the cuticular surface must be rubbed against the dust if this is to have any action. But, as Alexander *et al.* (1944) and Parkin (1944) have shown, there are very striking differences between different dusts and different insects. Before the true mechanism of their action is understood we need to know more about the physical nature of 'abrasion'. It may be that the attractive forces at the surface of crystalline particles, suggested by Alexander *et al.* as important in the working of the inert dusts, play a part in abrasive action. Parkin (1944) and Alexander *et al.* (1944) have suggested that adsorption of the cuticular lipoid by the dust may be important in interrupting the waterproofing film. This is certainly so in the cockroach in which the 'lipoid' is a soft grease. But in most insects, where the impervious layer is an orientated wax, adsorption is effective only during recovery from abrasion, when new wax is being secreted to repair the interruptions.

The complex nature of the surface layer of the cuticle goes some way to explain the difficulty with which poisons enter by this route. If an insecticide is to pass through the cuticle it must be in a medium which will disrupt both the wax film and its protective layer of cement. As Hurst (1943) says, dispersant action must involve both lipoid and protein components. The possible action of abrasive dusts in facilitating the entry of insecticides will require careful study. It is perhaps the abrasion of the [redacted]-worm cuticle which renders it so permeable to arsenic (Woodworth, 1938)—though the normal cuticle of the cockroach (O'Kane & Glover, 1935) and of the tick *Ixodes* (Burtt, 1944) also allow arsenic to pass through.

The importance of different emulsifying agents and detergents in breaking down the protective layers also needs studying from this point of view. Lennox (1940) has pointed out the toxicity of lipoid solvents, which he attributes to the solution of the epicuticular lipoids; and O'Kane, Glover, Blickle & Parker (1940) have noted that wax solvents pass most readily through the cuticle of the cockroach. Klinger (1936) and Umbach (1934) describe the increased permeability of the cuticle after extraction with chloroform or ether (cf. Bredenkamp, 1942 and Eder, 1940) and Hurst (1943) notes that prolonged immersion of blowfly larvae in fat solvents results in an irreversible increase in cuticle permeability to water, alcohol, etc. Lipoid solvents are efficient carriers of insecticides into the insect because of their dispersive action on the packed lipoids (Hurst, 1943; Wigglesworth, 1942). Hurst (1941) has also described more subtle effects: the desiccation of hard-bodied insects when smeared with lecithin; the excessive evaporation from *Tenebrio* larvae when in contact with larvae of *Calliphora*, which is attributed to the more hydrophilic character of the free lipoid in the cuticle of *Calliphora*. Many further examples of this sort of action have been described in the present paper, and the principles involved are dealt with in the paper by Beament (1945) on the isolated waxes.

SUMMARY

Transpiration through the cuticle of insects is restricted by a thin layer of orientated wax on the outer surface of the epicuticle. In some insects at least this wax layer is covered by a thin layer of cement.

When heated to a certain temperature the wax layer shows an abrupt increase in permeability to water. This 'critical temperature' varies widely in different species and in different stages of the same species. It is highest in those insects which are most resistant to desiccation.

In the newly formed puparium of *Calliphora* the impermeable film of wax is wholly superficial as in other insects. But after pupation the main impermeable layer is on the surface of the true pupa. The critical temperature of the pupa is much higher than that of the puparium.

Abrasion of the wax layer results in a great increase in transpiration through the cuticle. Inert dusts cause the desiccation of insects by getting between the moving surfaces of the cuticle and abrading the wax layer. Such dusts in stationary contact with the cuticle will not remove the wax by adsorption; hence they are without action on dead or motionless insects.

([redacted] cockroach is an exception to this. Here the waterproof layer is a soft grease, freely exposed on the surface; it is largely removed by adsorption on to the dusts.)

The places where the wax has been abraded can be demonstrated by immersing the insect in ammoniacal silver solution. The phenol-containing epicuticle stains deep brown only where the protective layer of wax has been removed.

Although the epicuticle shows no visible injury as the result of abrasion, the underlying cells react as though they had been wounded, and growth processes in the epidermis are affected.

Insect larvae from the soil show great but variable evaporation of water. This is the result of abrasion of the cuticle by soil particles. If the wireworm *Agriotes* is allowed to moult out of contact with the soil it has an impermeable cuticle like other insects.

After abrasion the living insect is able to secrete more wax through the substance of the cuticle and so to restore its impermeability. Adsorption of the wax by dusts while it is being secreted interferes with this process of recovery.

Lipoid solvents remove the wax layer from the surface and so increase transpiration. A long series of wetting agents and detergents has been tested. They show widely different effects on permeability of the cuticle to water.

Removal of the wax layer by means of abrasive dusts or suitable detergents increases the rate of entry of insecticides through the cuticle.

I have received valued help in the supply of insect material from Mr S. G. Jary, Dr C. Potter, Mr A. Roebuck, Dr I. Thomas and Dr I. H. H. Yarrow. I am indebted to the Geigy Colour Co., Imperial Chemical Industries Limited, Messrs Lever Bros., Messrs Rex Campbell and Company, and Technical Products Limited for the supply of chemicals and information. Samples of chemicals of American origin were obtained through the good offices of the British Central Scientific Office in Washington. Finally it is a pleasure to acknowledge the large amount of careful technical assistance given by Miss W. Wall.

REFERENCES

ALEXANDER, P., KITCHENER, J. A. & BRISCOE, H. V. A. (1944). *Ann. Appl. Biol.* **31**, 143–59.
BEAMENT, J. W. L. (1945). *J. exp. Biol.* **21**, 115–31.
BERGMANN, W. (1938). *Ann. Ent. Soc. Amer.* **31**, 315–21.
BREDENKAMP, J. (1942). *Z. angew. Ent.* **28**, 519–49.
BURTT, E. T. (1944). Unpublished work.
EDER, R. (1940). *Zool. Jb., Physiol.*, **60**, 203–40. (Noted from *Biol. Abstr.* **15** (1941), 20358.)
EVANS, A. C. (1934). *Parasitology*, **26**, 366–77.
EVANS, A. C. (1943). *Nature, Lond.*, **152**, 21–2.
FULTON, R. A. & HOWARD, N. F. (1938). *J. Econ. Ent.* **31**, 405–10.
FULTON, R. A. & HOWARD, N. F. (1942). *J. Econ. Ent.* **35**, 867–70.
GERMAR, B. (1936). *Z. angew. Ent.* **22**, 603–30.
HABERLANDT, G. (1914). *Physiological Plant Anatomy*. London.
HOCKENYOS, G. L. (1939). *J. Econ. Ent.* **32**, 843–8.
HURST, H. (1940). *Nature, Lond.*, **145**, 462.
HURST, H. (1941). *Nature, Lond.*, **147**, 388.
HURST, H. (1943). *Trans. Faraday Soc.* **39**, 390–411.
KALMUS, H. (1944). *Nature, Lond.*, **153**, 714–15.
KING, G. (1944). *Nature, Lond.*, **154**, 575–6.
KLINGER, H. (1936). *Arb. Phys. angew. Ent., Berl.*, **3**, 49–69, 115–51.
KOIDSUMI, K. (1934). *Mem. Fac. Sci. Agric. Taihoku*, **12**, 1–380.
KÜHNELT, W. (1928). *Zool. Jb., Anat.*, **50**, 219–78.
KÜHNELT, W. (1939). *VII. Internat. Kongr. Entom. Berlin*, 1938, **2**, 797–807.
LENNOX, F. G. (1940). *Pamphl. Coun. Sci. Industr. Res. Aust.* no. 101, pp. 69–131.
LISON, L. (1936). *Histochimie Animale*. Paris: Gauthier-Villars.
O'KANE, W. C. & GLOVER, L. C. (1935). *Tech. Bull. N.H. Agric. Exp. Sta.* no. 63, pp. 1–8.
O'KANE, W. C., GLOVER, L. C., BLICKLE, R. L. & PARKER, B. M. (1940). *Tech. Bull. N.H. Agric. Exp. Sta.* no. 74, pp. 1–16.
PARKIN, E. A. (1944). *Ann. Appl. Biol.* **31**, 84–8.
PRYOR, M. G. M. (1940). *Proc. Roy. Soc.* B, **128**, 393–407.
RAMSAY, J. A. (1935). *J. Exp. Biol.* **12**, 373–83.
RICHARDS, A. G. & ANDERSON, T. F. (1942). *J. Morph.* **71**, 135–83.
SCHMALLFUSS, H., HEIDER, A. & WINKELMANN, K. (1933). *Biochem. J.* **257**, 188–93.
SLIFER, E. H. (1937). *Quart. J. Micr. Sci.* **79**, 493–506.
TURNER, N. (1943). *J. Econ. Ent.* **36**, 266–72.
UMBACH, W. (1934). *Mitt. Forstwirt. Forstwiss.* **5**, 216–18.
WIGGLESWORTH, V. B. (1933). *Quart. J. Micr. Sci.* **76**, 270–318.
WIGGLESWORTH, V. B. (1937). *J. Exp. Biol.* **14**, 364–81.
WIGGLESWORTH, V. B. (1940). *J. Exp. Biol.* **17**, 180–200.
WIGGLESWORTH, V. B. (1942). *Bull. Ent. Res.* **33**, 205–18.
WIGGLESWORTH, V. B. (1944*a*). *Nature, Lond.*, **153**, 493–4.
WIGGLESWORTH, V. B. (1944*b*). *Nature, Lond.*, **154**, 333–4.
WIGGLESWORTH, V. B. (1944*c*). Unpublished work.
WOODWORTH, C. E. (1938). *J. Agric. Res.* **57**, 229–38.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Surface view of cuticle of *Rhodnius* 5th stage nymph treated with ammoniacal silver. Above: rubbed with alumina; below: normal.

Fig. 2. Section of cuticle of normal *Rhodnius* 5th stage nymph after immersion of the whole insect in ammoniacal silver. No staining.

Fig. 3. Section of cuticle of normal *Rhodnius* 5th stage nymph cut from the fresh insect with the freezing microtome and then immersed in ammoniacal silver. All the epicuticle is stained.

Fig. 4. Section of cuticle of *Rhodnius* 5th stage nymph after rubbing with alumina and immersion of the whole insect in ammoniacal silver. The epicuticle is stained over the crests of the folds.

Fig. 5. Ventral abdominal cuticle of *Rhodnius* 5th stage nymph where it touches the ground. Whole insect immersed in ammoniacal silver after running on fine emery cloth.

Fig. 6. Puparium of *Calliphora*, immersed in ammoniacal silver after rubbing gently on filter paper dusted with alumina.

Fig. 7. Larva of *Tenebrio*, immersed in ammoniacal silver after crawling for some hours on filter paper dusted with alumina. The legs are drawn forwards to show the stained areas where the coxae have rubbed against the thorax. There are abraded and stained areas at some other points, also indicated by arrows.

Fig. 8. Cuticle of 5th stage nymph of *Rhodnius* immersed in ammoniacal silver soon after rubbing with alumina.

Fig. 9. The same, after keeping the insect for 2 days in moist air.

Fig. 10. Cuticle of 5th stage nymph of *Rhodnius* 5 days after rubbing with alumina (the dust being left on), stained with Black Sudan B. The dusted area, which is stained, is above.

Fig. 11. Leg of *Hepialus* larva treated with ammoniacal silver, showing the scratches on the exposed surface.

Fig. 12. Ventral surface of *Agriotes* larva treated with ammoniacal silver, showing abrasion of the ventro-lateral lines of soft cuticle. The scratches in the hard general cuticle are scarcely visible at this magnification (see Pl. 2, figs. 1, 2).

Fig. 13. Side of prothorax of larva of *Aphodius* treated with ammoniacal silver, showing scratches chiefly on the prominent folds.

PLATE 2

Fig. 1. Part of ventral surface of third abdominal segment of wireworm treated with ammoniacal silver. This was a less permeable larva.

Fig. 2. The same, from a highly permeable larva.

Fig. 3. Part of ventral surface of *Pterostichus* larva, untreated, showing sclerites in the soft cuticle.

Fig. 4. The same treated with ammoniacal silver, showing scratches in the sclerites and very extensive abrasion of the soft cuticle.

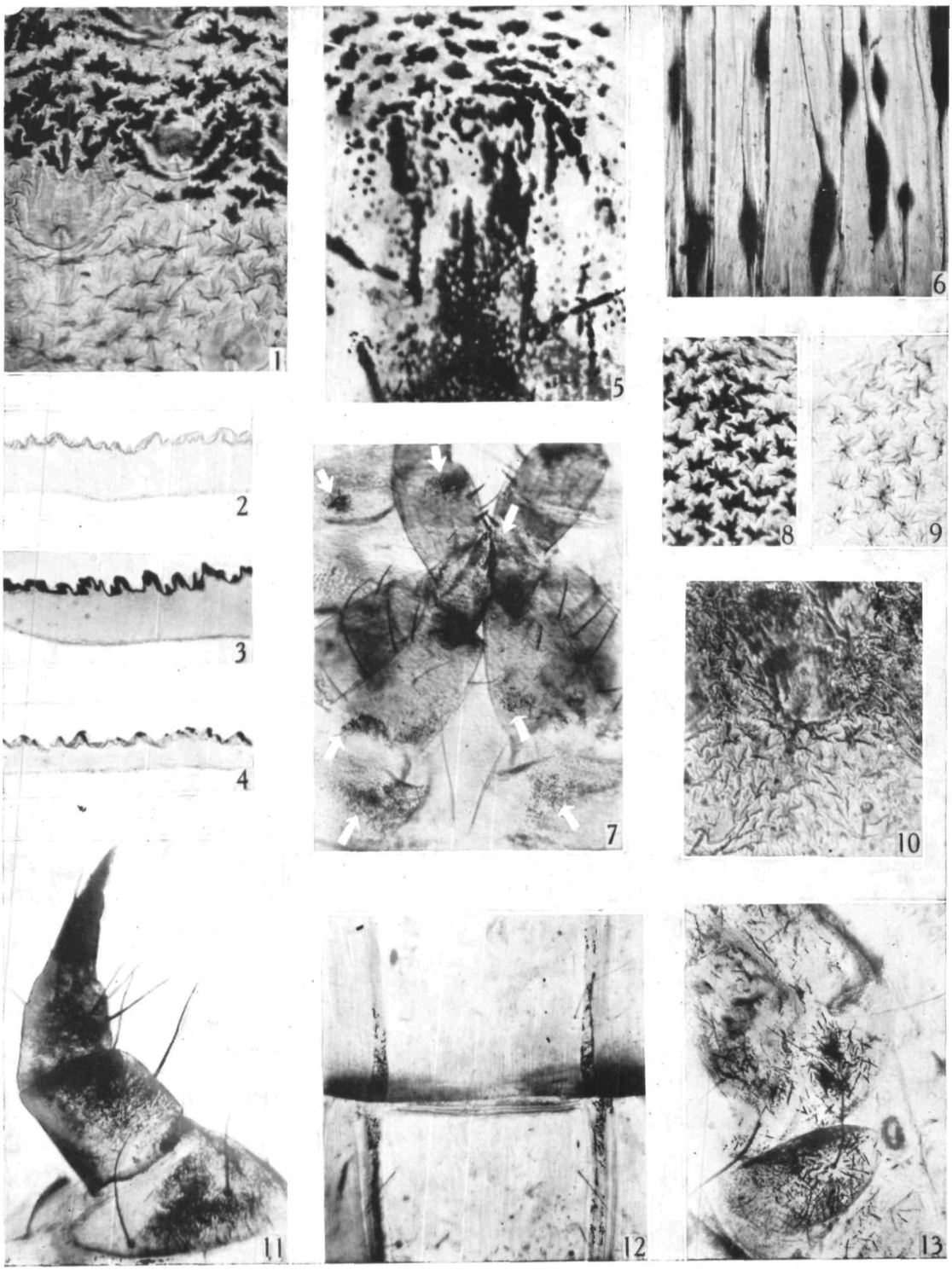
Fig. 5. Cuticle of *Tipula* larva treated with ammoniacal silver.

Fig. 6. Cuticle of *Bibio* larva treated with ammoniacal silver.

Fig. 7 Cuticle of *Rhodnius* 4th stage nymph treated with ammoniacal silver after extraction with chloroform for 15 min. at 20° C. Staining over the muscle insertion and at small scattered points elsewhere (scarcely visible in the photograph).

Fig. 8. The same, after extraction for 15 min. at 50° C. Staining almost universal except over the plaques and here and there over the crests of the folds.

Fig. 9. The same, 24 hr. after smearing with C09993 (see Table 13). Staining chiefly in the depressions between the folds.



WIGGLESWORTH—TRANSPIRATION THROUGH THE CUTICLE OF INSECTS

ort
he r