SHAPE AND MOLECULAR ORIENTATION IN LEPIDOPTERAN SCALES

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[Plates 1 to 3]

The rudiments of all types of scales on the wings of the meal moth, *Ephestia sericarium* (Scott) (=*E. kühniella* Z.), are birefringent from the time of their emergence. Their growth may be regarded as the elongation of a cytoplasm-filled hollow cylinder of oriented protein-polysaccharide. The hair-scales grow as cylinders of approximately constant diameter, but other types of scales dilate progressively towards their distal ends, becoming club-shaped: the rate of volume increase exceeds that of surface-area increase. Dilatation is rapidly followed by flattening of the dilated region; the flattened scale continues to grow in area but undergoes little further change in shape. In spite of these transformations, the mature scale is still largely composed of *oriented* protein-polysaccharide, the orientation of which can be traced back in time to the earliest phase of development. Even in the mature flattened scales on the wing surfaces, sufficient orientation survives for oriented whole wings to yield a rudimentary chitin fibre-diagram when placed in the path of a beam of X-rays. Hair-scales (the 'fur' of moths) give an X-ray diffraction picture showing the principal reflexions of the fibre-diagram of polyacetylglucosamine.

Birefringence studies suggest that the earliest rudiments contain more oriented protein than chitin, while in mature scales the chitin fraction is considerably increased.

The variations in structure observed in different types of lepidopteran scales are compatible with their properties as fibrillar aggregates, and the final shapes of mature scales result from orderly displacements of the fibrillar organization laid down in the rudiment.

The characteristic pattern of longitudinal ridges and transverse rungs may be compared with the patterns that arise in inorganic systems crystallizing out under certain conditions.

It is suggested that the ridges compete *in situ* for the materials of which they are built, and that their regular spacing is an expression of this competition.

1. Introduction

The starting-point of the observations described here was the realization that in many instances where, for one reason or another, the fine structure of elongated, spindle-shaped or cylindrical cells has been examined, it has been found that chain molecules, in the surface membrane or in the substance of the cell, are oriented so that the direction of the molecular axis is related to that of the long axis of the cell. In some cases the molecular axis is parallel to the cell axis, as in smooth muscle cells: the myosin chains run parallel to the fibril axis, and this in turn is usually parallel to the fibre axis (for a recent review of the literature see Astbury 1947); or in cells from the hair cortex: the keratin chains are parallel to the long axis of the cell (Woods 1938); or in the elongated supporting cells in the ectoderm of the starfish, *Marthasterias glacialis* (Smith 1937): a single birefringent sulphur-containing protein fibril (Brown 1947) extends from one end of the cell to the other; or in the bast fibres of ramie: the cellulose chains in the cylindrical cell wall are approximately parallel to the long axis (for references see Meyer 1942); or in nerve: the protein chains in the axon appear to run parallel to the long axis (Schmidt 1937). In other material the molecular axis is inclined to the long axis of the elongated cell, as in the chitinous wall of the sporangiophores of *Phycomyces* (Heyn 1936); or there may be two preferred orientations respectively parallel, and approximately at right angles, to the long axis, as in the wall of *Valonia* (Preston & Astbury 1937) and of *Cladophora* (Astbury & Preston 1940); or both may be inclined to the long axis, as in cotton (Anderson & Kerr 1938).

The biologist cannot but be impressed by this fundamental similarity in structure of materials as diverse as muscle, hair and the bast of *Boehmeria*. Moreover, these three have that in common which separates them from many other natural fibres—from the fibres of elastoidin, collagen and elastin, for example: they are each composed of specialized cells. In each case the embryonic or undifferentiated cells from which the tissue is derived are more or less isometric; in each case differentiation results in the formation of a long, spindle-shaped or cylindrical cell. The fact that in all these instances elongation of the cell body takes place in that direction in which the long axis of the molecules is oriented, or comes to be oriented, or in a direction related to this, has hitherto received little attention. The regularity suggests that there may be some general principle of anisometric cell growth underlying such behaviour, and it was with this in mind that other types of elongated cell have been examined.

The diversity of the examples quoted here is impressive. But it would be premature to suppose that anisometric growth in cells is invariably, though frequently, correlated with molecular orientation. The outer segment of the rods and cones of the retina, of whose structure Schmidt (1938) has made so fascinating an analysis, provide an exception, at least in their fully developed condition. Here, however, we know nothing of the development of the cell itself, and Schmidt's analysis is confined to the outer segment.

Since this work began, the accumulation of evidence has begun to be recognized by other workers, for example, Monné (1940): 'wir müssen annehmen, dass in sehr stark gestreckten Zellen (Muskelzellen, hohe Zylinderepithelzellen) das gesamte Grundzytoplasm eine mehr oder weniger deutlich ausgesprochene submikroskopische fibrilläre Struktur hat'; and more recently Schmidt (1943*b*) has written: 'die feinbauliche Ordnung des Zytoplasmas hängt mit der Zellform zusammen.'

The unicellular scales of Lepidoptera undergo considerable changes in shape during their development, and it has been one of the objects of the present study to determine to what extent the observed changes in shape are correlated with changes in molecular orientation. The work of Stossberg (1938) on the development of the scales in *Ephestia sericarium* had shown that microscopic evidence of orientation of the scale structure—the appearance of faint longitudinal striations—can be detected at an early stage in scale development, when the rudiment is about 30μ long. The work of Schmidt (1934) had shown that the longitudinal ridges (of which the longitudinal striations mark the first appearance) are positively birefringent with respect to their long axis, in mature iridescent scales of the *Urania*-type, while the underside lamella has the structure of a typical high-polymer membrane, showing negative uniaxial birefringence, the optical axis being normal to the plane of the membrane. These observations of Schmidt's together with earlier observations by Zocher and Süffert (Süffert 1924) on the relation between the orientation of the index ellipse and the plane of cleavage in the glass-clear ridges of highly iridescent scales, such as those of *Morpho* and *Chlorippe*, and a few incidental observations made by Mason (1927) in the course of his study of structural colours in insects, were the only published observations on the birefringence of lepidopteran scales available when this work began in 1938.

The first step was to determine at what stage in development molecular orientation with respect to the long axis is initiated: whether from the beginning shape and molecular orientation change simultaneously, or whether the scale rudiment is at first composed of

amorphous material which subsequently becomes oriented. Information concerning the orientation of the scale substance was obtained in the first place from a study of the optical properties of developing scale rudiments and mature scales of *Ephestia sericarium*. Observations were later extended to some of the more striking types of scale in other adult Lepidoptera.

2. Material and methods

The development of the scales was followed in *Ephestia sericarium*, reared at 25° C until shortly before they had finished feeding, transferred at that point to an incubator at 18° C, and allowed to pupate. At that temperature the rate of scale development is conveniently slow. The pupae were fixed in 4 % aqueous formaldehyde (occasionally in aqueous Bouin's fluid) at intervals of 24 hr., cutting off the head and the tip of the abdomen to facilitate penetration of the fixative. Wings were dissected off and either examined whole in water or prepared for sectioning. Both longitudinal and transverse sections were cut, at suitable stages, after clearing in 1 % celloidin in methyl benzoate. After transferring to slides these sections were also examined in water. Adult scales of other Lepidoptera and of members of other orders of insects were also examined; these will be named as occasion arises.

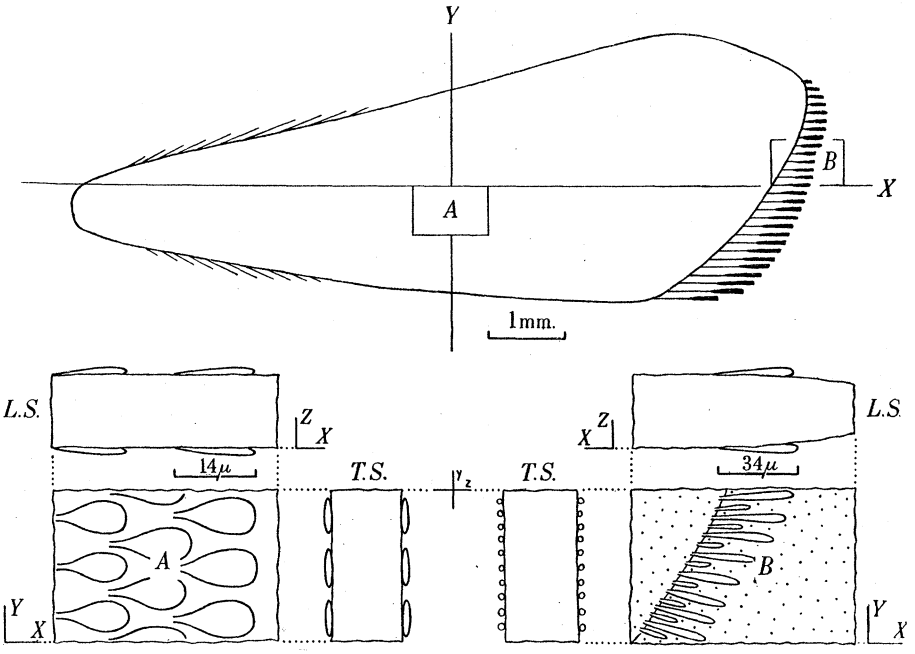

Figure 1. Diagram illustrating the arrangement of the scales on the forewing of *Ephestia* and the different types of scales in different parts of the wing. Explanation in text.

Determination of sign, and measurements, of birefringence were made with the equipment described in Lees & Picken (1945). For observations on birefringence in media of various refractive indices, Merck's 'liquids of special purity for refractive index measurements' and latterly 'liquids for determining the refractive indices of crystals', prepared by the British Drug Houses Ltd., were used.

X-ray photographs were taken using a Philips water-cooled 'Metallix' tube giving filtered Cu radiation.

The relative sizes of the structures examined, their orientation with respect to the wing-surface, and the mutual relations of the various sections are displayed in figure 1. A forewing

is drawn in outline with two small areas *A* and *B* marked, and with axes *X* and *Y* indicated. The *Z* axis is normal to the plane of the drawing. In the same figure, *A* and *B* are drawn on a larger scale to show, again in outline only, the arrangement of the scales on the wing, as seen in a pupa of about 140 hr. The insets, *A* and *B*, also include diagrammatic sections of these two regions of the wing. Defined in terms of the axes, *X*, *Y* and *Z*, the plane of the wing-surface is the *X*, *Y* plane; a transverse section of the wing is a section in the *Y*, *Z* plane; a longitudinal section is a section in the *X*, *Z* plane. The figure also records the fact that the scales are bristle-like on the anterior and posterior margins, paddle-shaped on the lateral margin and typically flattened on the body of the wing. The regions *A* and *B* are viewed along the *Z* axis, normal to the wing-surface. The stippled area to the right of the rudiments in *B* is the marginal zone of the pupal wing.

3. Birefringence of developing wing scales in *Ephestia sericarium* (Scott)

At about 72 hr. after pupation under the conditions described, the lateral marginal scale rudiments on anterior wings of *E. sericarium* are seen to be positively birefringent with respect to the long axis of the rudiment (the slow vibration direction is parallel to the long axis) when formalin-fixed wings are examined in water. The rudiment is, in fact, birefringent as soon as it can be recognized, that is, at a length of 6 to 7μ. Figure 4, plate 1, shows lateral marginal scales, about 7μ long, from a pupa not more than 84 hr. old, photographed in the diagonal position between crossed nicols with a $\frac{1}{16}$th wave-length mica plate in the addition position. In figure 5, plate 1, the stage has been rotated through 90° and compensation is complete; the rudiments are invisible under these conditions. The effect observed corresponds to a path difference of 1 to 5μ. Slightly older rudiments are shown, in addition and in subtraction, in figures 6 and 7, plate 1. By 119 hr. three sizes of lateral marginal rudiments can be seen: markedly club-shaped at *c.* 34μ, more nearly cylindrical at *c.* 10μ and cylindrical at *c.* 3μ (figures 8 and 9, plate 1). In these photographs the microscope is focused on the upper surface of the scale; at a slightly deeper focus it is evident that it is the surface membrane which is birefringent, not the cell contents; the membrane is bright while the contents remain dark. This birefringence is to be distinguished from that of an egg-cell membrane, for example (Runnström 1928), which arises from preferential orientation of anisometric particles in the plane of the surface. Such a membrane is isotropic when viewed along a normal to the surface, but anisotropic when viewed along a tangent to the surface. The surface of the scale, however, is anisotropic when viewed along a normal to the surface. At this early stage, therefore, the material of which the cell membrane is composed is already oriented with respect to the long axis of the rudiment.

From the optical behaviour of a microscopic fibre of glass immersed in water, it is clear that the brightness of the scale rudiments between crossed nicols cannot be due to depolarization by reflexion or diffraction at the boundary between one dielectric and another. Such a fibre is bright at its edges when observed in the diagonal position between crossed nicols, but this brightness cannot be compensated as can that of the scale rudiments.

In sections parallel to the long axis of the wing and normal to the surface (that is, in the *X*, *Z* plane), the birefringence of the lateral marginal rudiments, as of those on the body of

the wing, is evident (figures 16, 17 and 18, plate 1; figures 22, 23 and 24, plate 1). Transverse sections of early, cylindrical rudiments (the plane of section, that is, the *Y*, *Z* plane, is at right angles to the long axis of the wing and normal to the wing-surface) show no trace of birefringence, indicating that the long axis of the cell (the *X* axis) is the optical axis (figures 19, 20 and 21, plate 1; in figures 20 and 21 the bright granules are fat droplets). As soon as flattening becomes appreciable, however, the cell membrane is found to be positively birefringent with respect to the plane of flattening (figures 25, 26 and 27, plate 1). It will be noticed in figures 23 and 24 that the whole wing, and not the scales only, shows signs of optical anisotropy in section, particularly the surface of the epidermis and the basement membrane of the wing. Both these have, presumably, the structure characteristic of many high-polymer membranes; long molecules or micells are distributed with their long axis in the plane of the membrane, but otherwise at random.

The anterior wing margin bears scales that never flatten but continue to grow as cylinders. Two sizes are shown in figures 13 and 14, plate 1. They may be compared with the bristles of other insects, and in particular with the bristles of *Drosophila*, previously examined by Lees & Picken (1945). For the sake of convenience they will be referred to as hair-scales.

Comparable to the hair-scales are the very much larger rudiments which develop into the *frenulum*—a tuft of stout bristles emerging from near the base of the hind wing on its anterior border. The earliest recognizable frenulum rudiments show scarcely elongated processes emerging from the wing margin (figure 28, plate 2). The sunken cell bodies also show traces of birefringence. As elongation continues the birefringence of the frenulum hairs increases (figures 29, 30, 31 and 32, plate 2). This is shown by the fact that in order to compensate the rudiment to blackness, the $\frac{1}{16}$ W.L. plate has to be rotated farther from the null position; if, after compensating to blackness, the rudiment is rotated into the addition position, the field is increasingly bright with successively older rudiments. This may mean that the thickness of oriented material deposited in the cell membrane is increasing, or that the degree of orientation of material already deposited is increasing with age. Both may well occur.

The growth of the hair-scales, and of the bristles composing the frenulum, as elongating cylinders implies that the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$ is constant (Lees & Picken 1945). In contrast, the rudiments on the lateral wing margins soon become club-shaped; their distal ends inflate (figure 8). The process of inflation has certainly begun by the time they reach a length of 20μ. This means that the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$ is no longer constant; the rate of volume increase outpaces that of area increase in the club-shaped rudiment. The period of inflation is followed by the rapid flattening of the rudiment to form a flattened scale, the surface area of which is once more increasing at about the same rate as its volume (figures 10 and 11). This is clear from the figures of Stossberg (1938), which show little change in the thickness of the scale between 168 and 500 hr. Moreover, from about 200 hr. onwards, the growth of the scale is proportional; its dimensions change, but its shape is approximately constant.

By 200 hr. the longitudinal ridges on the upper surface of the flattened scales of *Ephestia* are fully developed. They are separated from each other by areas of the scale which

appear isotropic when viewed along a normal to the plane of the scale (figure 15). They can be seen compensated to blackness in figure 12. In any given type of scale, Stossberg observed that from 168 hr. onwards the number of ridges does not increase (this observation refers to the scales on the body of the wing). My own observations support this observation, at least in strap-shaped scales, and suggest that as the scale increases in width, the parallel ridges become more and more widely separated from each other. In the scales on the body of the wing in *Ephestia*, the ridges on the upper surface of the scale (that is, the 'abwing' surface) run parallel to the long axis of the scale and equidistant from each other throughout their length, save at the distal end of the scale where, on the scale margin, they occasionally approach each other; the lower surface of the scale (the 'adwing' surface) is an isotropic membrane, devoid of ridges, except at the base of the scale or near its edge. The membrane has a structure typical of high-polymer membranes; though isotropic when viewed along a normal to the plane of the surface, it is anisotropic when seen in section, the plane of the section being normal to the plane of the surface. Figures 34 and 35, plate 2, show the birefringence of sections of the membrane forming the lower surface of unpigmented scales from the body of the wing, seen between crossed nicols, in addition and in subtraction.

To complete the picture of the development of scales on the body of the wing in *Ephestia*, one has to add the appearance of regularly spaced cross-connexions between the ridges; the fenestration of the upper surface, so that it is converted to an open lattice-work; and its anchoring to the lower membrane by little pillars of material, the trabeculae. These features are described in detail by Stossberg (1938).

It can readily be seen from successive stages in the development of the lateral marginal scales, that all parts of the scale are growing; in the bristles of *Drosophila* (Lees & Picken 1945), on the other hand, it seems that growth of the rudiment is largely apical. Comparing figures 10 and 11, it is clear that the shaft is increasing in diameter and in length while the flattened blade increases in area. This means that parts of the scale which have already been formed are growing (to use the botanists' term) by intussusception. The distal end of the scale, however, is formed anew, as is shown by the gradual modelling of the terminal processes.

4. Structural and intrinsic birefringence of scale rudiments and mature scales in *Ephestia sericarium* (Scott)

When formalin-fixed fragments of wings bearing scale rudiments from a 90 hr. pupa were soaked in liquids of increasing refractive index, it was found that the birefringence of the scales diminished with increasing refractive index (n_D) of the medium and disappeared completely in *o*-toluidine ($n_D = 1{\cdot}57$). This implies that the birefringence observed in water is due to the parallel orientation of anisometric particles whose refractive index differs from that of water. In *o*-bromiodobenzene ($n_D = 1{\cdot}66$) the birefringence was once more faintly positive and was distinct in methylene iodide ($n_D = 1{\cdot}74$). The path differences of the rudiments in these various media were too small to measure under the conditions of observation, but the changes in path difference showed that the relation between birefringence and refractive index is probably of the parabolic type associated with structural birefringence. The observations also suggest that at this stage birefringence cannot be due

to the presence of 'chitin'. From data collected by Möhring (1922; summarized in Ambronn & Frey 1926) it is clear that for chitin fibrils from the integument of a lobster the path difference in a liquid of $n_D = 1{\cdot}57$ and in green light ($\lambda = 546 m\mu$) is negative and becomes increasingly so as the refractive index of the medium is raised to 1·61. With further increase in refractive index the path difference now becomes less negative and is once more zero at $n_D = 1{\cdot}69$. At the point of inflexion, the path difference is that due to the chitin crystallites, that is, the intrinsic path difference. Reducing the thickness of the specimen might flatten the curve and reduce the intrinsic birefringence to zero, but should not shift the curve along the abscissa. It should not lead, for example, to a positive value for Γ, such as we observe in a medium of $n_D = 1{\cdot}66$; the value might be indistinguishable from zero but could not be positive. On the other hand, the behaviour of the values for path difference with changing refractive index resembles that found in muscle. Muscle fibres and myosin fibrils show both positive structural birefringence and positive intrinsic birefringence (see Fischer 1947). The mean refractive index differs in different muscles, being higher in striated vertebrate muscle (for the rectus abdominis of the mouse the refractive index is 1·57) than in smooth vertebrate muscle (for the retractor penis of the dog the refractive index is 1·55) and higher in this than in unstriated invertebrate muscle (for the retractor proboscis in *Phascolosoma* the refractive index is 1·52). The mean refractive index in these cases is the refractive index at the point of inflexion of the curve: retardation plotted against refractive index of medium. On either side of this value, the retardation increases. Conceivably it is an oriented fibrillar protein at the surface of the scale rudiment which is responsible for the observed birefringence. The virtual absence of chitin is further suggested by the refractive index of the scale rudiment. In *o*-toluidine ($n_D = 1{\cdot}57$) the scales are practically invisible in normal light, indicating that the refractive index of the rudiment is very close to 1·57, whereas the mean refractive index of chitin (Möhring's value, corresponding to the birefringence minimum) is 1·61. In *o*-bromiodobenzene ($n_D = 1{\cdot}66$) the rudiments are again visible. The refractive index of several fibrillar proteins is close to 1·57: human hair keratin, 1·54 to 1·55 (J. Schmid, see W. J. Schmidt 1934); collagen, 1·52 (Küntzel 1929); elastoidin, 1·556 (Schmidt 1934). So far as it goes, the very meagre evidence suggests that the optical properties are determined by a fibrillar protein rather than by chitin at least up to 200 hr., and that the birefringence is largely structural.

Widely differing values for the refractive index of 'chitin' are given in the literature. Sollas (1907), using Becke's method, obtained values between 1·550 and 1·557 (in red light) for crustacean and insect material, treated with 10% hydrochloric acid and boiled for many hours in repeatedly changed 5% aqueous potassium hydroxide, followed by bleaching with chlorine and extraction with alcohol and ether. Becking & Chamberlin (1925) obtained a value of 1·53 for the refractive index of purified material, reprecipitated from solution in hydrochloric acid (2N) by dilution with distilled water, dialyzed against distilled water and dried to a solid of the consistency of gutta percha. They used an immersion method for the determination of refractive index. Möhring's data showing the relation between path difference and refractive index of the medium, for the decalcified inner layer of a lobster carapace, both in blue ($\lambda = 435 m\mu$) and green ($\lambda = 546 m\mu$) light, show clearly that the point of inflexion of the curve, at which the negative intrinsic birefringence is maximal, is at 1·61 in blue light and 1·64 in the green.

On general grounds one would expect the mean refractive index to be less in red light than in blue, and, in fact, Sollas's values are lower than the mean refractive index implicit in Möhring's results. The displacement of Möhring's curves away from the origin along the abscissa with increasing wave-length (at first sight contrary to the rule that the refractive index diminishes with increasing wave-length) may be connected with the fact that a birefringent substance will probably have different absorption spectra for fast and slow directions; according to the position of the absorption bands the *mean* refractive index may increase or decrease with increasing wave-length in certain regions of the visible spectrum.

In a recent paper, Anderson & Richards (1942) found that the transparent vanes composing the ridges of the iridescent scales of *Morpho cypris* Westwood have a refractive index of 1·53 (this refers, presumably, to the untreated vanes). They were led to believe that the scales are not composed of 'chitin', since they disintegrate completely in concentrated aqueous potassium hydroxide after several hours at 160° C, and since electron micrographs showed that the basic structure is attacked when they are treated with 5 % aqueous sodium hydroxide for 12 hr. at 90 to 100° C. In view of the optical properties of the developing *Ephestia* scale rudiment, it seemed desirable to inquire further into the composition of the mature scales.

Entire wings of *Ephestia* adults were heated in saturated aqueous potassium hydroxide at 160° C (saturated at this temperature) for 20 min., following Campbell (1929). After cooling they were transferred to absolute alcohol and hydrated in stages until they could be examined in distilled water. It was found that the scales were all detached from the wings but were still present in the sediment at the bottom of the test-tube in which the wings had been treated with caustic potash. The sediment was poured into a watch-glass; after standing, the supernatant liquid was decanted and the sediment (adherent to the glass) washed with successively more dilute alcohols until the deposit could be examined in water. On flooding with a 0·2 % aqueous solution of iodine in potassium iodide the scales became brown. On withdrawing the iodine solution and replacing with 1% aqueous sulphuric acid, the deposit as a whole became reddish violet in colour; under the microscope the individual scales were seen to be coloured. This positive chitosan reaction was taken as proof of the presence in the scales of polyacetylglucosamine, that is, of chitin in the strict sense.

Though this reaction demonstrated the presence of chitin (*sensu stricto*) in the mature scales, material treated in this way could not easily be used for optical studies because scales become detached from the wing membrane during treatment and are difficult to manipulate in this condition. Accordingly, wings of mature *Ephestia* adults were subjected to milder treatment with caustic alkali. The wings were heated on a water-bath at 90 to 100° C for *c.* 18 hr., in 10 % aqueous potassium hydroxide, until all pigment had disappeared. They were then transferred down the alcohols to distilled water. In most cases the scales were undisturbed and still attached to the wings after this treatment. Material treated in this way did not give a positive chitosan reaction, however. At first it was thought that this must be because de-acetylation had not progressed sufficiently. When, however, the birefringence of the scales was measured in a series of liquids of different refractive index, it was found that the curve obtained resembled that of the scale rudiments, in that the path difference never became negative (figure 2*a*), but had a minimal value in aniline

(n_D=1·5855) in white light. This means that after 18 hr. treatment with 10 % aqueous potassium hydroxide the optical properties of the scales did not resemble those of Möhring's chitin from the lobster carapace.

Since it was possible that this treatment did not suffice to remove all protein from the scales, a further series of wings from *Ephestia* adults was placed in diaphanol (chlorine dioxide in glacial acetic acid) and allowed to remain in contact with this liquid for a fortnight. At the end of this time they were colourless. After washing in absolute alcohol and transferring on a needle through mixtures of absolute alcohol and xylene to pure xylene,

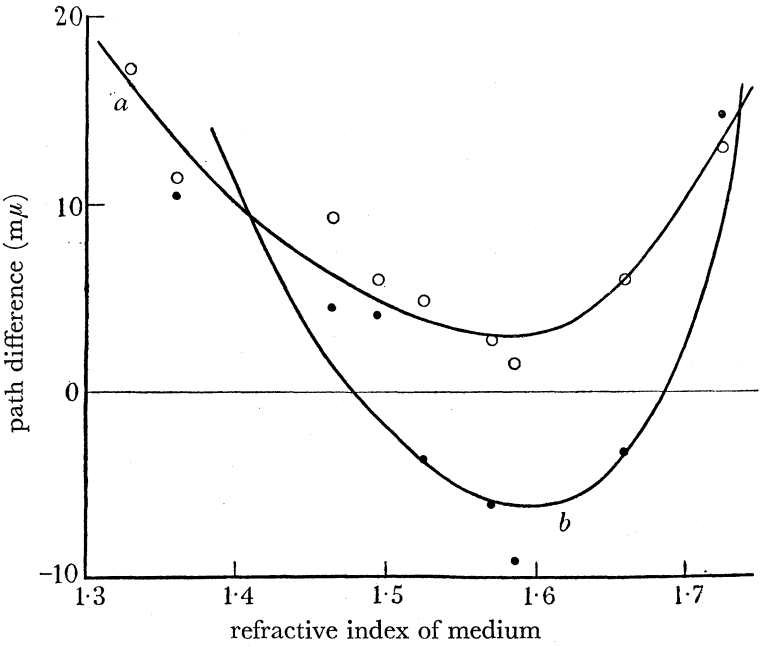

FIGURE 2. Structural and intrinsic birefringence in mature lateral marginal scales of *Ephestia sericarium* (Scott). (*a*) Wings decolorized by heating with 10 % aqueous potassium hydroxide at 90 to 100° C for *c.* 18 hr. (*b*) Wings decolorized by treatment with diaphanol for 14 days. For convenience, smooth curves have been drawn through the two sets of points; no significance is to be attached to their precise form.

a pair of wings was placed in each of a series of corked tubes, each of which contained about 1 ml. of each of the liquids for refractive index measurements, and allowed to stand overnight at room temperature. Next day the two wings from each tube were transferred to a fresh drop of liquid on a cavity slide and covered with a cover-slip; the path difference of the fringe of scales on the distal end of the anterior margin of the forewing was then measured, compensating the fringe as a whole to blackness. From these measurements curve *b* in figure 2 was constructed. It must be emphasized that the measurements of path difference made in this way can only be regarded as a rough, qualitative guide to the change in optical properties in the scales after prolonged treatment with diaphanol; no significance is to be attached to the precise form of the curves drawn, for convenience only, through the two sets of points.

This curve is qualitatively similar to that obtained by Möhring. It is suggested that these observations imply that treatment with 10 % aqueous potassium hydroxide for 18 hr. at 90 to 100° C does not suffice to remove completely the protein component from the protein-

polysaccharide complex, which is 'chitin'. The different values for the refractive index of 'chitin' obtained by different observers may be due in part to their having used preparations of polyacetylglucosamine freed to varying extents from protein. If by 'chitin' we mean the oriented polyacetylglucosamine component, then Möhring's value for the mean refractive index, based on the point of inflexion of the structural birefringence curve, is to be accepted.

Returning to the scale rudiments: wings from formalin-fixed pupae between 80 and 200 hr. old were heated to 100° C on a water-bath in 10 % aqueous potassium hydroxide. They disintegrated at once and completely. Other wings of similar age were exposed to the action of diaphanol for 3 weeks. These, however, did not disintegrate. After this treatment no birefringence could be detected in rudiments up to 200 hr. old.

These observations suggest that the first membrane bounding the developing scale rudiment is a protein-polysaccharide complex, the optical properties of which are determined by the protein, which at this stage may be present in excess of the polysaccharide. Up to 200 hr. the complex is readily dispersed by 10 % aqueous potassium hydroxide, but not by diaphanol after 3 weeks. The mature scale is much more resistant to the action of dilute potash, and even prolonged action fails (as judged by the optical properties) to remove all protein from the complex. This removal is effected in due course (after 2 weeks) by diaphanol. The mature scales survive treatment with saturated potassium hydroxide at 160° C for 20 min. and subsequently give a positive chitosan test. They therefore contain polyacetylglucosamine, or chitin in the strict sense.

Richards (1947) has recently examined scales of a large number of species of Lepidoptera and has shown that chitin may be present or absent, as judged by the behaviour of the scales on treating with strong caustic potash at 160° C, and by the result of the chitosan test. He confirms the presence of chitin in mature scales of *E. sericarium*. In view of the very low percentage of chitin in some insect cuticles, he suggests that the descriptive phrase 'chitinous exoskeleton' is unwarranted, and that the cuticle should be regarded as a protein membrane which may be associated with various other substances: with waxes in the epicuticle, with chitin in the exo- and endocuticle; or it may be impregnated with calcium carbonate and tanned with phenolic substances. Recent reviews of polysaccharide chemistry (Stacey 1943; Haworth 1946) suggest, however, that it would be more natural (and certainly more suggestive from the standpoint of phylogeny) to regard the arthropod cuticle as a mucoprotein, and to recognize that the polysaccharide fraction may be small or large in different groups or species, that changes may occur in the proportion of long-chain polysaccharides present during the individual life history, and that different regions of the cuticle, in one and the same animal, may differ widely in the relative proportions of polysaccharide and protein present.

5. X-RAY DIFFRACTION STUDIES OF MATURE SCALES

While birefringence studies provide a sensitive method of studying changes in orientation in time, their results cannot be directly interpreted in terms of molecular orientation. Such interpretation is only possible if X-ray studies, for example, can be made in order to determine the relation between the molecular axis and the observed optical properties.

Although, since the finished scale is largely composed of chitin, the polyacetylglucosamine chains of which are known to run parallel to the optical axis (in this case, to the slow vibration direction), it was reasonable to regard the positive birefringence of the scales with respect to the morphological axis as evidence of polyacetylglucosamine chains being laid down in the surface membrane, parallel to the long axis of the scale, this could not be proved by birefringence observations alone. Accordingly, the attempt was made to determine directly, by X-ray diffraction studies, whether the mature scales showed any sort of orderly molecular structure and what was the nature of the oriented substance, if present.

(*a*) *Ephestia sericarium*

Some thirty forewings of *Ephestia* adults were carefully superimposed on each other (so that their marginal fringes of scales were parallel) and stuck together at their bases by a trace of Canada balsam. Pressed tightly together by finger pressure between two fragments of paper, the thickness of wings amounted to about 1 mm. For these experiments freshly emerged and perfect adults were used; they were anaesthetized and handled carefully, so as not to disturb the arrangement of the scales. The free ends of the wings, bearing the lateral marginal scales, projected from between the two fragments of paper, and X-ray photographs were taken with the beam traversing (1) the marginal scales only and (2) the whole thickness of the wings; in both the beam was normal to the X, Y plane. In each case the preparation was exposed for 4 hr. at a film-object distance of *c.* 30 mm.

For those unfamiliar with the principles of X-ray analysis, a word of explanation of the photographs under discussion may be helpful. The periodicity of an ordinary linear optical grating can be calculated, if the angle of diffraction and the wave-length of the incident radiation are known. In a crystal, the orderly arrangement of molecules, atoms or ions in space, constitutes a three-dimensional grating far beyond the limits of resolution of the microscope. Just as in a linear optical grating, the distance between the lines can be calculated from the angle of diffraction and the wave-length of the radiation, so, in a crystal, the distances between parallel 'lattice planes' of the structure can be calculated from analogous data. Because of the special properties of a three-dimensional grating, the diffracted rays can be regarded as reflexions from sets of parallel lattice planes; thus the spots on the equator of figure 55, plate 3, can be regarded as reflexions from sets of lattice planes parallel to the long axis of the scales; and those on the meridian as reflexions from planes inclined at an angle of 60 to 90° to this axis. Though it is possible to determine the spacing of the lattice planes, we do not know their relative orientation in the small ordered regions in the scales; indeed, the only definite information that can be obtained from such an X-ray diagram is the periodicity of the molecular pattern in the direction of the long axis of the structure. The interpretation in molecular terms of a diagram such as that in figure 55 is only possible, therefore, if evidence is available from collateral chemical or physical studies, and if the same material occurs elsewhere in samples showing much better orientation.

Polyacetylglucosamine (purified 'chitin') has been studied in well-oriented specimens, and X-ray photographs showing a large number of diffraction spectra have been obtained (Meyer & Pankow 1935). On the basis of these it has been possible, not merely to calculate many periodicities, but to suggest molecular configurations which would account for the

spectra which appear and for their relative intensities. Although it would be impossible to extract very much information about the structure of the molecules of the oriented component present in the scales from the X-ray photographs reproduced in figures 52 to 56, plate 3, the fact that the few spectra visible are the most conspicuous spectra in photographs of well-oriented preparations of polyacetylglucosamine is strong evidence that it is chain molecules of chitin which are oriented parallel to the long axis of the scales, since this axis is parallel to the meridian of the photographs.

The diagram obtained was a fibre-diagram with the fibre-axis parallel to the long axis of the scales (the X axis) in the marginal fringe (figure 53). The scales are arranged on the wings with astonishing regularity and, on the whole, microscopically accurate parallelism. It is probable that imperfect parallelism in the mass of scales is due more to inaccuracy in mounting successive wings than to imperfect alinement in single wings. The meridional dispersion in individual scales may well be less than that shown in the photographs.

It will be recalled that the lateral marginal scales have flattened distal blades (figures 10, 11 and 12, plate 1). The X-ray beam will therefore traverse not only the cylindrical shafts, but also the isotropic underside lamella and the remains of the isotropic upperside lamella (separating the ridges), the transverse rungs joining the ridges, and the trabeculae. The appearance of a considerable powder-component in the photograph is therefore understandable. This is even more marked in photographs obtained when the beam traversed stacked and oriented entire wings (that is, wing membrane together with covering scales and deep scales of both surfaces). The photograph (figure 52) still shows distinct meridional arcs, however, but the equatorial spot is barely visible. Since the wing membrane is isotropic when viewed between crossed nicols along a normal to the wing-surface, it is reasonable to suppose that the scales are responsible for the oriented component. The arcs on the meridian, and the traces of the equatorial reflexion are due, presumably, to the oriented component introduced by the parallel longitudinal ridges of the flattened covering and deep scales.

The reflexions visible in the original photographs are the most conspicuous reflexions of the polyacetylglucosamine diagram obtained by Meyer & Pankow (1935) from an apodeme of the rock lobster. They are, on the equator (following Meyer & Pankow): 200, corresponding to a spacing of 4·65 Å; and on the meridian 020 (5·2 Å) and 031 (3·4 Å) (in each case the principal reflexion only is indexed; according to Meyer & Pankow's indexing the spots often arise from two or three adjacent reflexions). The original of the photograph reproduced in figure 53 also shows faintly the equatorial reflexion 002, corresponding to a spacing of 9·5 Å.

The fibre-diagram given by the marginal scales is not, as might be supposed at first sight, unequivocal evidence of orientation of the chitin chains in the scales parallel to the long axis. Let us consider first the cylindrical shafts: a system of parallel hollow cylinders of the same diameter as the shafts, with walls of typical membrane structure (chains or crystallites lying in the plane of the membrane but otherwise oriented at random) photographed with the X-ray beam normal to the long axis of the cylinders, would give a powder diagram showing a strong preferred orientation of the substance, with the chain-direction parallel to the long axis of the cylinders. On the other hand, if the ridges were merely sharp folds of the amorphous upper-side lamella they would also introduce a preferred orientation

due to the folded membrane structure. To be sufficient in itself as evidence of three-dimensional orientation in the wall of the scale (one crystallographic axis in each crystallite parallel to the long axis of the scale) one needs X-ray evidence from photographs of fragments of a single thickness of the scale membrane—as Preston (1945) has pointed out, for rather different reasons, in the case of wood vessels.

(*b*) *Ourapteryx sambucaria* (Linn.)

The lateral marginal scales of *Ephestia* are flattened distally, so that the X-ray beam perforce traverses both flattened and cylindrical portions of the scales in a preparation composed of many parallel scales. The tegulae of *Ourapteryx sambucaria*, however, bear tufts of cylindrical hair-scales reaching a length of up to 3 mm. and resembling in structure the hair-scales on the anterior margin of the *Ephestia* wing. In cross-section they are approximately circular and are ridged, so that their structure corresponds to that of the shaft in the lateral marginal scales of *Ephestia*.

Six tegulae were removed and their hair-scales caused to adhere together, like the hairs of a small paint brush, by dipping them in xylene. After the xylene had evaporated, the scales remained side by side, approximately parallel to each other. X-ray photographs were taken with the beam at right angles to the long axis of the brush of hair-scales. After 4 hr. exposure at a film-object distance of 30 mm. the principal reflexions of the chitin fibre-diagram were visible, namely: on the equator, 002 (9·5 Å) and 200 (4·65 Å); on the meridian, 020 (5·2 Å) and 031 (3·4 Å). The orientation is better than that revealed by the photographs of the marginal scales of *Ephestia*, and it is perhaps legitimate to conclude that this is correlated with the cylindrical, pronouncedly anisometric, non-flattened shape of the hair-scales (figures 54 and 55).

(*c*) *Euproctis phaeorrhea* (Donovan)

For comparison with *Ephestia*, fragments of the lateral wing margin of *Euproctis phaeorrhea* (a moth, considerably larger than *Ephestia*) were superimposed, so that the fringing scales overlapped to a thickness of about 1 mm. X-ray photographs were then taken with the beam at right angles to the long axis of the strap-shaped fringing scales. The fibre-diagram showed the four reflexions present in that from the hair-scales of *Ourapteryx sambucaria*, but with more general scattering and poorer definition (figure 56, plate 3).

The X-ray photographs obtained from these three different types of untreated lepidopteran scales may be compared with that from another protein-polyacetylglucosamine fibrillar aggregate, reproduced in figure 57, plate 3. This was obtained from a small bundle of the large untreated bristles (chaetae) of the polychaete worm, *Aphrodite*, the sea mouse. These are extracellular structures, secreted in cylindrical pits. On freeing from protein by treatment with KOH or diaphanol, the X-ray diagram approximates to the chitin (polyacetylglucosamine) diagram, from a *Palinurus* apodeme, obtained by Meyer & Pankow (1935), that is to say, the supernumerary meridional and other reflexions, some of which are visible in figure 57, disappear when the protein is removed. It is clear that figures 52 to 56 are simplified versions of figure 57. The differences are due, presumably, not only to poorer orientation, but to the smaller bulk of material in unit volume in the air-filled scale preparations, as compared with the solid bristles of *Aphrodite*. Although, as

we have seen, the results of X-ray studies of the scales do not alone suffice to establish the fibre-structure of a small fragment of the wall of individual scales, they accord with the conclusions reached from a study of the optical properties of developing and mature scales.

6. Scales and ridges as fibrillar aggregates of higher and lower order

The ridges are detectable by ordinary staining methods, and by their birefringence, at 120 hr. after pupation and at 200 hr. have become conspicuous. Without pursuing the question of their origin, we observe in dilatation and flattening that the optically anisotropic ridges come to be separated from each other by an isotropic membrane—isotropic when viewed along a normal to the plane of the scale surface. The dilatation of the scale by the formation of a membrane separating the oriented ridges may be compared with the splitting of a fibrillar aggregate, parallel to the direction in which the component fibrils are oriented. Several different types of scale offer instances of structural details compatible with the inferred properties of the scale as a fibrillar aggregate. In the androconia or scent scales of *Pieris brassicae* (Linn.), for example, figure 51, plate 2, it is clear that the distal fringe of filaments is continuous with the ridges, and it may be supposed that the fringe arises as a result of the breakdown of the membrane between the ridges in the course of development.

Again, the ridges themselves show a tendency to split like other natural fibres. But in most cases the splitting is not parallel to the long axis of the ridge. This is seen very clearly in the spine-like prolongations of the lateral marginal scales of *Thais polyxena* Schiff. & Den. (figure 36, plate 2), where the surface is broken into minute, overlapping, scale-like structures making an angle of *c.* 20° with the long axis of the spine. It will be recalled that Zocher & Süffert (Süffert 1924) showed that the ridges of *Morpho* scales cleave along planes inclined to the long axis of the ridge. More recently, Anderson & Richards (1942), Gentil (1942) and Kinder & Süffert (1943), using the electron microscope, have shown that the ridges of iridescent *Morpho* scales are built up from structures inclined to the long axis of the ridges at an angle of 12° in the glass-clear ridged scales of *M. achilles* Linn.; Süffert (1924) had calculated an angle of 17°. The discrepancy between observed and calculated angles may be due to distortion of the scale in the electron beam. In some scales, such as those of *M. aega* Hubner, the direction of cleavage of the ridges suggests that the axis of orientation in the ridges is practically parallel to the long axis of the ridge; in others (for example, *Chloripppe seraphina* Hubner), the axis of orientation may be inclined to the long axis at an angle of as much as 45° (Süffert 1924). Anderson & Richards showed that the ridge structure in non-iridescent scales of *Morpho cypris* Westwood is essentially similar to that in the iridescent scales, and Kühn & An (1946) have published superb electronmicrographs demonstrating the similar structure of the ridges in the non-iridescent scales of *Ephestia* and *Ptychopoda*.

The hair-like processes which run off from the longitudinal ridges on the spatulate blades of the scales from the 'fur' patch on the hind wing of the male of *Trepsichrois mulciber* (Cramer) (figures 37, 38 and 39, plate 2), also suggest fibrillar splitting of the ridges and, perhaps, that the 'cleavage plane' is somewhat inclined to the long axis of the ridge. The Christmas-tree-like early rudiment of *Drosophila* may be compared with this scale (Lees & Picken 1945).

On the basis of studies using polarizing microscope and electron microscope, Gentil (1941, 1942) came to the conclusion that the ridge of the iridescent scales of *Morpho* is a fibrillar structure—a *Stäbchenmischkörper*—but supposed that the ridge of the glass-clear covering scales was built up from a linear series of overlapping, half-conical lamellae. Kinder & Süffert (1943), however, point out that this latter suggestion is untenable, since the ridge would then show much stronger light absorption along the line of its attachment to the scale than at its free edge; this in fact is not observed.

Regarding the detailed interpretation of ridge structure, Kinder & Süffert differ from Anderson & Richards. The former suppose that each ridge is built up by the superposition of ten lamellae (in the *X*, *Y* plane) (*Morpho achilles*), each of which is composed of two parallel rods (lying side by side in the *X*, *Y* plane) which may or may not be united by a membrane. Anderson & Richards, on the other hand, conclude from their photographs that each ridge is built up from about five vertical vanes (in the *X*, *Z* plane) whose plane is normal to the surface of the scale; each vane is composed of about twelve horizontal mullions (lying in the *X*, *Y* plane) united by a thin membrane which is thickened at intervals to form vertical mullions (in the *X*, *Z* plane). The horizontal mullions, lying side by side in the ridge, form the reflecting planes, though not united laterally. Both sets of workers agree that the ridges of the *Morpho* iridescent scales are composed of fibrils, parallel to the *X* axis, united in a plane normal to the scale surface (the *X*, *Z* plane) by regularly spaced slender pillars, and that the spaces between the fibrils and pillars are filled by a thin membrane. The structure of the ridge seems to repeat that of the scale itself at an order of magnitude about ten times as small.

After examining a wide range of scales, it is difficult to escape the conclusion that the ridges themselves are capable of independent growth, that they have to accommodate themselves at the surface of a limited bulk of material, and that in some cases their increase in length may considerably affect the shape of the scale.

The first example is taken from the hair-scales of the scent-brush of *Danais septentrionalis* Btlr. If a single hair-scale is examined from base to tip, it is found to be of constant diameter throughout the greater part of its length (the tip itself is often missing). Under oil immersion it can be seen that whereas at the base of the scale the ridges are running parallel to the long axis (figure 40, plate 2), they become 'wavy' as they pass along distally (figures 41 and 42, plate 2). The amplitude of the waves increases distally, until the ridges appear to break up into short segments, jumbled together more or less at random (figure 43, plate 2). These changes suggest that the ridges have to be accommodated in a surface of limited area, and that their meandering course is the expression of the fact that they are continuing to grow in length in a surface already limited by the volume which it bounds; that is to say, they continued to grow in length after the cylindrical bristle was laid down.

A second example is provided by the androconium of *Pieris brassicae*, already considered in another connexion. It is clear from figure 58, plate 3, that the course of the ridges is intimately connected with the shape of the scale. Assuming that the rudiment of this scale does not differ in organization from that of other scales, the formation of the posteriorly directed horns at the base of the androconium, on either side of the stalk, can be regarded as an exaggeration of the tendency—observable in other scales (for example, the wing-

surface scales of *Hesperia comma* (Linn.)—for the base of the scale to be square cut, not tapering into the stalk; or even for it to develop, on either side of the stalk, a somewhat backwardly directed lobe. It is possible that, after the flattening of the club-shaped rudiment, the ridges continue to grow not merely at their distal ends, as the scale elongates, but also in their middle region and particularly near the base of the scale. The shape of the scale might then be imposed by the necessity for accommodating parallel ridges, elongating particularly in their middle region, at the surface of a limited volume. In any case, the close relation between ridge pattern and the shape of the scale is nowhere shown more clearly than in this type of scale. The scale of the thysanuran, *Lepisma* shown in figure 46, plate 2, again illustrates the posterior extension of the surface of the flattened scale as two horns into which the ridges on the upper surface are prolonged. The fan-like course of the few ridges on the lower surface can be seen in this type of scale also.

7. Ridge patterns

Reference has already been made (p. 6) to the last stages in the completion of scale development: the anchoring of the ridges to the underside-lamella by slender pillars of material (the trabeculae); the lateral linking of the ridges with each other by fine rungs developed in the upperside-lamella; and lastly, the fenestration, to a greater or lesser extent, of the membrane between the rungs. All these features are well shown in the photographs published by Kühn & An. In *Ephestia* the rungs are spaced at regular intervals of about $0{\cdot}2\mu$ and are approximately at right angles to the ridges; occasionally they branch. Figure 3 has been redrawn from Kühn & An to complete the picture of *Ephestia* deep-scale structure presented here.

This arrangement of the ridges on the flattened scales is not found in all types of *Ephestia* scales, still less in all genera of Lepidoptera (Süffert 1924). Nor is the rung pattern or the degree of fenestration the same in all genera. In contrast to the scales on the body of the wing (the covering and deep scales), the lateral marginal scales of *Ephestia* have ridges on both surfaces of their flattened blades. Sections of mature scales show that the spacing of the ridges increases somewhat in passing from the shaft to the flattened scale-like blade. It seems legitimate to conclude that in the course of development, of marginal as of covering and deep scales, the dilatation and flattening of the original cylindrical rudiment is associated with the development of a membrane separating the ridges.

Again, in the fan-like blades of the lateral marginal scales of *Plusia gamma* (Linn.), upper and lower surfaces of the blade alike bear ridges. In the mature scale these are equally spaced in spite of their divergence from the shaft. This is made possible by the intercalation of new ridges as soon as the divergence of any two is greater than the average spacing (figure 47, plate 2). This demonstrates that ridges can be formed *de novo* and at quite a late stage in scale development, if the perimeter of the cross-section of the scale is increasing. Whether new ridges arise as branches from existing ridges, or whether they may appear quite independently, has not been determined.

In the deep scales on the body of the wing of *Plusia gamma*, it can be seen that the ridges nearest the edges of the scale pass to the undersurface as they run backwards towards the base of the scale (figure 44, plate 2). In *Eilema lurideola* (Zincken) the lower surface is

covered by a fan-like system of diverging ridges, while the upper surface bears parallel ridges, as in *Plusia* and *Ephestia* (figures 48, 49 and 50, plate 2). Towards the base of the scale the outer ridges of the upper surface can be seen to pass over the edge on to the under surface and so into the stalk. Ridge systems of upper and lower surfaces unite at the distal end of the scale to form a series of loops, as is shown clearly in the electronmicrographs of Kühn & An, and was described by Süffert (1924).

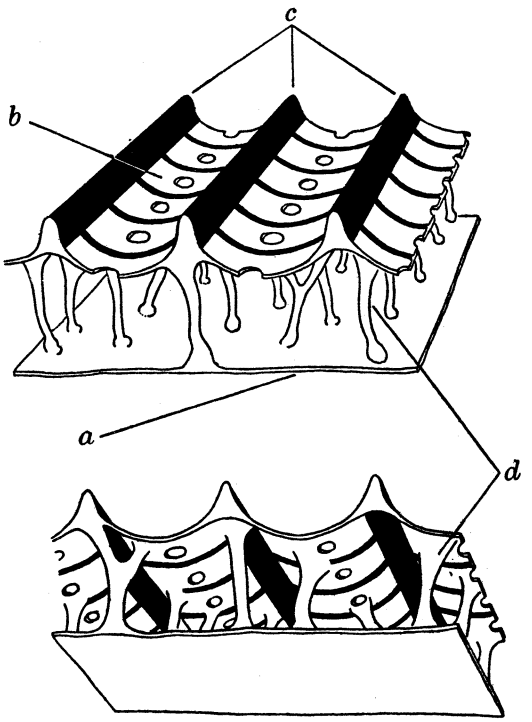

FIGURE 3. Structure of a deep scale from the body of the wing of *Ephestia*; no details of ridge structure are shown. Redrawn from Kühn & An (1946). *a* = underside lamella; *b* = perforated upperside lamella; *c* = ridges; *d* = trabeculae.

The dilatation of the membrane separating the ridges can be seen in other types of scale Such are the lateral marginal scales of *Thais polyxena* (figure 45, plate 2), in which the scale is prolonged distally into three bristle-like processes, separated at the base by a region in which the interval between the ridges is noticeably greater than elsewhere on the scale.

In the spatulate scales of the male *Trepsichrois mulciber* already referred to (p. 14), the separation of the ridges follows the breadth of the strap-shaped scale, being least in the narrowest and greatest in the widest parts, as shown in figures 37, 38 and 39, plate 2.

Together with the androconium of *Pieris brassicae* the scales mentioned in this section may serve as instances of the correlation between ridge patterns and scale shape observable in many different types of lepidopteran scales.

8. DISCUSSION

Many of the observations recorded here were first made between 1938 and 1940. *Drosophila* material prepared by Dr A. D. Lees subsequently provided an opportunity for testing out ideas on scale and bristle development already proposed in relation to lepidopteran material, and collaboration made possible the publication in 1945 of an analysis

of bristle development in *Drosophila* (Lees & Picken 1945). The mutant types of *Drosophila* bristles were natural experiments; their occurrence was predictable in the light of an hypothesis based on lepidopteran material; they could be analyzed though not imitated. In connexion with the analysis we then proposed, attention is directed to the parallel between our ideas and those of Young (1944), on the role of cytoplasmic pressure in maintaining normal orientation of the axon proteins, and to the relation established by Lewis & Modlibowska (1944) between pollen tube length and diameter at constant volume.

(i) *Shape and orientation*

Before proceeding to consider in greater detail the implications of the observations on scale development and structure recorded here, it is proposed to discuss a matter of more general interest. While the existence of a correlation between anisometric cell shape and orientation of chain molecules at the surface of the cell or in its interior seems to be established for the instances quoted in the introduction and for the example described at length in these pages, the complexity of the changes of orientation in time, revealed by Preston's careful analytical studies of plant cell walls, for example, cautions against assuming too hastily a causal relation between the onset of orientation and changes in cell shape. The chemical diversity of examples of anisometric, hollow-tubular or solid-cylindrical, 'growths' in inorganic systems—silicate gardens, the *arbor Dianae*, sodium carbonate crystals in calcium chloride, zinc in potassium ferricyanide, Pharaoh's Serpents' Eggs—suggests that the generation of such forms is independent of the chemical nature of the components, and that they are determined by certain general conditions obtaining in systems undergoing, for various reasons, an increase in volume. A study of such *simulacra vitae* might reveal the essential factors at work in their production and so, perhaps, indicate the way to a more profound analysis of the nature of anisometric cell growth.

If the onset of orientation is to cause changes in cell shape, whether at constant, or with increasing, volume, it would seem that orientation must be initiated at but few (or even at only one) centres in the cell or at the cell surface. In setting, a solution of gelatin becomes microcrystalline, but it does not happen that collagen fibrils separate out from a supersaturated solution of gelatin; the formation of a fibril implies that it is easier to add to an existing nucleus of orientation rather than to start a new nucleus. Alternatively, we may suppose that orientation is initiated at many points simultaneously, but that because of restraints imposed by the spatial limits of the system or, as Preston & Astbury (1937) suggest, because of the active intervention of a secreting protoplasmic surface, the crystallites have to adopt a single (or double) preferred orientation.

If orientation is to lead to an anisometric deformation of the cell (whether at constant, or with increasing, volume), work must be done by the growing ordered aggregate on the rest of the cell. The energy for this work may be expected to be derived from the heat of crystallization of the material. If it were possible to measure the heat of crystallization of a substance crystallizing under mechanical restraint in a calorimeter, we should find it to be less than the theoretical amount by a quantity equivalent to the work done. Though the orientation of highly hydrated molecules (in some sort of fibril, for example) cannot be expected to occur with the evolution of heat of crystallization comparable in magnitude to that evolved during the formation of true crystals, this need not necessarily mean that the

energy available is insufficient for the work of deformation. The values for the surface tension of various types of cell-surface imply that the work of deformation will be very small.

It is perhaps worth emphasizing that, although crystallization is improbable, in the sense that order is less probable than disorder, it occurs because the orderly association of molecules in the process of crystallization takes place with a diminution in their internal energy. The entropy decrease during crystallization is due to the limitation on rotational and translational movements of the molecules which their arrangement in an orderly aggregate entails.

The onset of orientation could only be expected to bring about an anisometric change in shape if the crystallites themselves grow anisometrically. While long chain molecules generally seem to give rise to crystallites elongated in the direction of the long axis of the molecules, it is conceivable that, under certain conditions, growth of the crystallites in a direction at right angles to the molecular axis might be as rapid as in that parallel to the molecular axis; that is, orientation might arise without the development of a pronouncedly anisometric shape.

The converse case, of anisometric shape developing without molecular orientation with respect to the long axis of the cell, might be realized by a mechanism such as that proposed by Holtfreter (1946) to account for the elongation and motility of entire and fragmentary embryonic cells. He compares the autonomous expansions and contractions of the plasmalemma with the movements of the external membranes of vesicles of lecithin or cephalin in water and concludes that both 'are due to the presence of oriented layers of phosphatide molecules which periodically change their state of hydration'. As yet, however, it is not known whether molecular orientation with respect to the long axis of the cell or cell fragment is present or absent in Holtfreter's material.

(ii) *Scale development*

(*a*) *Dilatation and flattening*

It is not intended to recapitulate here the general picture of scale and bristle development sketched by Lees & Picken (1945); certain points only will be discussed in detail.

Whereas typical scales (lateral marginal, covering and deep) show signs of passing through one period only in which the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$ rapidly increases, the androconia of *Pieris brassicae* show signs of a second period of this kind. Figures 58 and 59, plate 3, show that the stalk of the scale ends in an almost spherical bladder by which it is attached to the wing-surface. It is not possible to say at what point in time during development this is produced; but the whole scale may perhaps be compared with those mutant bristles of *Drosophila*, such as forked or Bristle (Lees & Picken 1945) which show signs of two or more inflations. It would be interesting to examine the development of the androconia of *Pieris*, as also of the astonishing segmented bristles of beetles such as *Anthrenus*. A lepidopteran scale whose shape may perhaps be interpreted as a record of periodic changes in secretory activity is the scent scale of *Hesperia comma*, which breaks into segments at a touch.

Of great interest are the abnormalities in *Ephestia* scales which Kühn (1941) has described in individuals exposed as pupae between the ages of 102 and 156 hr. to a temperature of 45° C for 45 min. Up to 144 hr., the most common type of disturbance takes the form of

a pointed process growing out of the scale at an angle to its long axis; on entering the process the ridges undergo a sharp change in direction and then run parallel to the long axis of the process, precisely as in the branches of forked mutant bristles of *Drosophila*. In the same period, other abnormal types occur which Kühn regards as 'twinning'; the process bears a close resemblance to a scale of inverted polarity, attached by its broad end to the parent scale and with its 'stalk' freely projecting. As Kühn observes, however, the 'stalk' 'ist doch nie so scharf vom Schuppenkörper abgesetzt und quer abgestuzt wie ein normaler Schuppenstiel'. This type he contrasts with a third (which occurs with increasing frequency as heat stimulation is postponed to a later stage in development) in which a scale-like process emerges from the scale in normal orientation, that is, attached to the parent scale by its narrow end.

I am inclined to regard these types not as indicating reversal in polarity, but as types of disturbance of the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$, as with *Drosophila* mutants (Lees & Picken 1945).

The fact that, in scales, flattening always follows dilatation of an originally cylindrical rudiment to a club, suggests that the sudden change in shape of the cross-section of the scale is due to the fact that the rudiment with a circular cross-section becomes mechanically unstable when its diameter exceeds a certain limiting value. It is possible that the scale collapses like a thin-walled cone in which, at the base, the ratio of diameter to wall thickness has exceeded a limiting value determined by the stiffness of the material. The collapse of the scale, however, can scarcely be under its own weight. It is observed that the scale rudiments are always applied to the wing-surface, and it is suggested that there is a tendency for the scale to adhere to this surface (figures 16, 19, 22, 25, plate 1); flattening in the plane of the surface may follow as a consequence of this adhesion when the diameter of the rudiment exceeds a limit set by the stiffness of the surface membrane.

A further possibility is that the increase in perimeter and the intercalation of new ridges lead to such an increase in the rate of surface growth that the volume increment no longer suffices to distend the newly formed envelope to a cylinder. It has already been pointed out that, once the scale has flattened, the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$ is again constant until the growth of the scale is practically complete; the final shape of the scale, therefore, like that of the *Drosophila* macrochaeta, can be regarded as a record of changes in time of the ratio $\frac{\text{rate of volume increase}}{\text{rate of area increase}}$. This alternative explanation of flattening does not explain why flattening occurs in the plane of the wing surface. On either hypothesis some adhesional affinity between the rudiment and the epidermal surface must be postulated.

Until it is possible to observe the development in time of individual scales and bristles, it is probably not profitable to speculate further on the factors operative in their formation.

(*b*) *Ridge patterns*

It is perhaps significant that the ridges of the upper surface are continuous with those of the lower, at least in certain flattened scales, and that, in forms such as the deep scales of *Eilema*, the scale can be regarded as a thin bag covered by a series of loops (the continuous

ridges of upper and lower surfaces) of approximately equal length (ignoring the distal processes), crossing the edge of the scale obliquely, to run from lower to upper surface and eventually back to the lower surface (figures 48, 49 and 50, plate 2). On the upper surface the ridges are parallel, save at the base (where they diverge from the stalk) and at the tip of the distal processes (where they approach each other); on the lower surface they diverge from the stalk more gradually and only become parallel to each other about halfway along the scale. The maximum spacing of the ridges seems to be the same on upper and lower surfaces. The ridge pattern might then be the outcome of the tendency of fibrillar units, constrained to space themselves as far as possible equidistantly at the surface of a flattened bag, to grow to approximately equal lengths.

In some types of scale, the manner in which the ridges cross from the lower to the upper surface, radiating from the stalk over the lower surface to become parallel on the upper surface (figures 44 and 46, plate 2) suggested, at first, that dilatation and flattening could be regarded as a controlled burst of the original, approximately cylindrical rudiment. If we imagine a club-shaped rudiment splitting longitudinally along the adwing surface, ridges will be transferred to the abwing surface of the flattened portion of the scale. If the widening split were covered by an amorphous membrane, we should have a scale structure not unlike that of the scales on the body of the wing of *Ephestia*. The existence of scales with complete ridge patterns on upper and lower surfaces (such as the deep scales of *Eilema*, figures 48, 49 and 50, plate 2) rules out, however, the possibility of interpreting all cases of flattening as a consequence of longitudinal splitting of the dilated rudiment.

It is interesting to note that in giant scales (up to ten times the normal size) recorded by Kühn & Piepho (1940) in epidermal implants from the skin of the last-stage caterpillar of *Galleria mellonella* (Linn.) into the fatbody of a caterpillar of the same age, the increase in length and breadth of the scales is not associated with coarsening of the structure of the membrane, ridges or trabeculae.

(*c*) *The structure and growth of the ridges*

The electronmicrographs of various workers amplify, and provide additional evidence for, the picture of the scale as a fibrillar aggregate which emerges from optical and X-ray diffraction studies. In one respect they add considerably to the picture in revealing the structure of the ridges. The growth of the ridges in length by intussusception is difficult to imagine if they are microscopic aggregates of parallel fibrils of length comparable to the length of the ridge. If, however, they are composed (as has been shown to be the case, by Anderson & Richards 1942; Kinder & Süffert 1943; Kühn & An 1946) of much shorter, overlapping fibrils inclined at a small angle to the long axis of the ridge, it is not difficult to imagine the separation of these units by dilatation of the plastic rudiment, and the intercalation of new units as soon as the spacing of the old units permits—as is observed to happen when the ridges themselves are separated laterally.

This intercalation of new ridges and the parallel spacing of the ridges may be of considerable importance. They point to an interpretation of the ridges and of their formation which may have far-reaching consequences.

Chance led to the observation, under the polarizing microscope, of thin films of a saturated solution of potassium mercuric iodide on a microscope slide, losing water by evaporation.

It was observed that at a certain moment, a sheet of acicular crystals grew simultaneously, spaced at regular intervals, and accurately parallel to each other, through the liquid. A few moments later, 'rungs' developed between them, also spaced at regular intervals, but with a different periodicity from that of the parallel needles. The axes of the 'rungs' were oriented at right angles to those of the needles. Though the order of magnitude of this pattern of 'ridges' and 'rungs' was perhaps a hundred times larger than the scale pattern, its impressive regularity and similarity to the latter prompted the question whether the factors at work in producing the ridge pattern might not be similar to those concerned in the production of the needles and 'rungs' in the film of potassium mercuric iodide.

The regularity of the crystal pattern arises from competition between adjacent nuclei of crystallization in a medium undisturbed by convection (the great surface/volume ratio of the film and the microscopic dimensions of the system both contribute to this condition). Each nucleus is removing molecules from solution so that within a certain distance of a given nucleus it is impossible for a second nucleus to be established; if it arises it will soon regress, because it cannot compete with the nucleus already established and rapidly augmenting in size. The fact that each nucleus gives rise to an acicular crystal depends on the relative rates of growth of the different crystal surfaces, which in turn depend on the nature of the substance. The equidistant spacing and parallelism of the needles are both due to competition.

When the system of parallel needles has been established, however, the solution continues to lose water by evaporation, and the concentration of the mother liquor between the needles rises until the probability of new nuclei forming is once more significant. A new set of acicular crystals forms, regularly spaced, though at a different spacing because of changed conditions, and at right angles to the original needles. Their regular spacing is again due to competition with each other; their orientation at right angles to the original needles, to competition with these.

It may be objected that, however striking, this is but an analogy, and the history of theories of morphogenesis is strewn with abandoned crystal analogies. It was perhaps premature in Schwann (1839) to envisage the growth of cells and their nuclei as a process analogous to crystallization. But the time has come when it must be accepted that crystal 'growth' offers much more than an analogy to organic growth. Hinshelwood (1946) suggests that 'the synthesis of a protein partakes of the nature both of a polycondensation reaction and of crystal growth' and has discussed, in the same work (chapters I, X, XI, XII), the nature of cell organization in the light of this picture of the nature of synthesis.

Lees & Picken (1945) suggested that the materials of which the bristle surface and the ridges in particular are built are synthesized in the bulk of the cytoplasm and subsequently transferred to the surface. It seems now, however, that whether or not some precursor is elaborated elsewhere, the spacing of the ridges implies that they are competing *in situ* for material for their growth. It is suggested that Hinshelwood's picture of the autoreproduction of a globular protein as a process of crystallization combined with synthesis may be extended in these unicellular scales to growing aggregates of a fibrillar protein-polysaccharide. Not, perhaps, that fibrillar molecules are themselves autoreproducing, but that the growing fibrillar aggregates, with their associated synthetic enzymes, compete *in situ*, like crystal nuclei, for the residues of which they are constructed.

As we see from the scales, the ridges are autoreproductive in the sense that they increase in length after they have been formed, and they appear to be able to grow independently of the cytoplasmic bulk, distorting the scale as they grow, as in the androconium of *Pieris*. The cytoplasm increases in bulk, but the length of the ridges seems to determine to some extent how the surface is shaped that bounds it.

Schmidt (1943*a*) has described a new type of structural-colour scale in the 'mosaic' scales of *Teinopalpus imperialis* Hope. The space between upper and lower surfaces of these scales is entirely filled with vertical lamellae, built up from parallel fibrils running obliquely between upper and lower surfaces. Sets of underlying lamellae, oriented in different ways with respect to the long axis of the scale, cause the surface to appear characteristically divided into fields. While the interpretation of this remarkable structure given by Schmidt must be regarded as provisional, the existence of such a type could be explained by an enormous development of the trabeculae, which like the ridges are also presumably fibrils of protein-polysaccharide. If the growth of the scale comes to an end while chitin synthesis is still vigorous, the chitin last produced can only be put into trabeculae and these, that their length may be maximal, might run obliquely instead of spanning the shortest distance between upper and lower surface. Their regularity of arrangement might then be determined by the necessity for stowing away a maximum amount of their substance in a minimal volume. This type of scale structure may be closely related to that of *Entimus* described by Mason (1927).

(iii) *Subcellular ontogeny*

The development of the scale provides an example of an ontogenetic process, at a cellular level of organization, in which a microscopic and submicroscopic order, established at the beginning of development, persists, though modified by the displacement and distortion of parts, to the end of development, so that the mature scale bears the stamp of its early history. Moreover the scale can only come into being, seemingly, as a modification of what is initially a bristle-like structure; it is, like the organism of which it is a part, a record in three dimensions of orderly material movements in time. In terms of its history it is a logical development, even though when viewed as a finished product it appears to be an arbitrary fantasy. The development of scales may reveal inherent morphogenetic tendencies set free from the operation of gross selection, restrained only by the conditions of competition between microscopic or submicroscopic units in the scale rudiment. The parts of a scale are less than cells, yet they grow and multiply; they seem to compete and as certainly they develop in harmony. The sum of their activities results at last in the production of a 'dead' structure which may serve as a pigment holder, a splash of iridescence, a scent bearer, a tactile or auditory baton, the details of whose execution are beyond the resolution of insect eyes. These details, it is suggested, are to be explained as the result of the interaction of parts; they indicate the operation of some principle of mutual harmonious regulation even below the level of cellular organization.

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Description of plates 1 to 3

Plate 1

All photographs on this plate are of formalin-fixed material examined unstained in water; with the exception of figure 11, all figures are at the same magnification. They were taken with a Leitz 6L (polarizing) $\frac{1}{6}$th objective and a $\times 10$ eyepiece with the draw-tube extended to 170 mm.

Figure 4. Scale rudiments on lateral margin of forewing of *Ephestia sericarium* (Scott) 81 ± 3 hr. after pupation; length of rudiment *c.* 7μ; nicols crossed; brightness slightly enhanced by rotating substage compensator.

Figure 5. The same preparation rotated through 90°; the birefringence of the rudiments is compensated to zero and they are invisible. For ease of comparison with figure 3 the orientation of the photograph is the same as in the previous figure.

Figure 6. Slightly older rudiments of the same scale type; pupa fixed 90 hr. after pupation; length of rudiment *c.* 11μ; nicols crossed; compensator in addition.

Figure 7. The same preparation rotated through 90°. The brightness of the rudiments is considerably reduced by compensation.

Figure 8. Three sizes of rudiments of the same type on the lateral wing margin of the forewing from a pupa fixed 118 ± 1 hr. after pupation. The oldest rudiments are obviously club-shaped. Three sizes visible: *c.* 34, 10 and 3μ.

Figure 9. The same rotated through 90°; compensation is incomplete.

Figure 10. Older rudiments of the same type on the forewing of a pupa fixed 181 ± 3 hr. after pupation. In the two larger sizes flattening is advanced. The smallest rudiments are still club-shaped. Crossed nicols; compensator in the addition position.

Figure 11. Flattened blades and shafts of lateral marginal scales in a pupa fixed 194 ± 5 hr. after pupation. Crossed nicols; compensator in the addition position.

Figure 12. The same preparation under oil immersion achromatic $\frac{1}{12}$th. The longitudinal ridges are visible, compensated to blackness; they are spaced at *c.* 1μ.

Figure 13. Hair-scale rudiments on the anterior margin of the forewing of a pupa fixed 88 ± 2 hr. after pupation; length of rudiments *c.* 14μ. The rudiments are cylindrical.

Figure 14. Cylindrical hair-scale rudiments on the anterior wing margin of a forewing from a pupa fixed 124 ± 2 hr. after pupation. The sizes of rudiment shown are *c.* 40, 30, 15 and 6μ.

Figure 15. Flattened scales from the body of a wing from a pupa fixed *c.* 200 hr. after pupation. The ridges are seen bright in the addition position; the interridge material is isotropic. Breadth of scale *c.* 50μ.

Figure 16. Club-shaped lateral marginal rudiments seen in a longitudinal section of a forewing from a pupa fixed 110 hr. after pupation. The clubs are *c.* 14μ long. Analyzer out.

Figure 17. The same with nicols crossed and the compensator in the addition position. Rudiments (and outer edge of epidermis) bright.

Figure 18. The same rotated through 90°. The rudiments are now in the subtraction position and are invisible.

Figure 19. Lateral marginal scales seen in transverse section in a section from a pupa fixed 110 hr. after pupation. Diameter of rudiments *c.* 3μ. Analyzer out.

Figure 20. The same; nicols crossed; rudiments practically invisible.

FIGURE 21. The same rotated through 90°.

FIGURE 22. Longitudinal section of a hind wing from a pupa fixed 150 hr. after pupation showing rudiments of the wing-body scales. Rudiments *c.* 14μ long; maximum diameter *c.* 4μ. Analyzer out.

FIGURE 23. The same with nicols crossed and compensator in the addition position. Epidermis and basement membrane, as well as the scale rudiments, show birefringence.

FIGURE 24. The same rotated through 90°. Compensation to blackness is almost complete.

FIGURE 25. Transverse sections of flattened rudiments in a section of the hind wing from a pupa fixed 140 hr. after pupation. On the upper left hand edge of the section, two fragments of flattened scales are seen in surface view. Analyzer out.

FIGURE 26. The same with nicols crossed and compensator in the addition position. The most flattened rudiments visible in figure 24 are slightly birefringent in transverse section.

FIGURE 27. The same rotated through 90°. Birefringence completely compensated to blackness.

PLATE 2

Figures 28 to 35 were taken under polarizing $\frac{1}{6}$th (Leitz 6L) with a × 10 eyepiece. All others on this plate were taken under oil immersion apochromatic $\frac{1}{12}$th (Leitz) with compensating ocular (× 10) and immersion condenser of N.A. 1·40.

FIGURE 28. Frenulum; earliest rudiments from pupa fixed at 107 ± 5 hr. Nicols crossed; compensator in addition. Rudiments *c.* 5μ long.

FIGURE 29. The same; pupa fixed at 124 ± 2 hr. Longest rudiments *c.* 12μ long.

FIGURE 30. The same; pupa fixed at 144 hr. Longest rudiments *c.* 21μ long.

FIGURE 31. The same; pupa fixed at 184 ± 3 hr. Longest rudiments *c.* 65μ long.

FIGURE 32. The same; pupa fixed at 199 ± 5 hr. Longest rudiments *c.* 250μ long.

FIGURE 33. Transverse section of forewing of adult of *Ephestia sericarium*. The section passes through wing membrane and scales on both surfaces. The single conspicuous scale section is *c.* 56μ broad. Analyzer out.

FIGURE 34. The same. Nicols crossed and compensator rotated to give slight enhancement of brightness. The underside lamella of each scale is birefringent. Occasional large ridges are also bright as seen in transverse section, indicating that the optical axis is not parallel to the long axis of the ridge.

FIGURE 35. The same rotated through 90°. Compensation to blackness is almost complete.

FIGURE 36. A ridge on a distal, bristle-like process of a lateral marginal scale of *Thais polyxena* seen in profile; the ridge shows an obliquely laminated structure. Height of ridge *c.* 1μ.

FIGURE 37. The base of a spatulate, strap-shaped scale of *Trepsichrois mulciber*. The ridges are *c.* 2μ apart, and the space between them is bridged by rungs spaced at *c.* 0·6μ.

FIGURE 38. Shaft and base of spatulate end of the same scale. In the narrowest part of the shaft the spacing of the ridges is reduced to *c.* 0·5μ. At the base of the spatulate end, fine, tapering hairs are seen to run off obliquely from the ridges. The spacing of the ridges and their number are increased in the spatulate end.

FIGURE 39. Spatulate end of same scale showing spacing of ridges; hairs out of focus.

FIGURE 40. The base of a hair-scale from a scent brush of *Danais septentrionalis*. The ridges are parallel to each other and to the long axis of the scale. They are spaced at *c.* 0·7μ. The magnification in this photograph is slightly less than in the following three figures.

FIGURE 41. The same scale; a portion distal to that seen in figure 39. The parallel ridges are becoming wavy.

FIGURE 42. The same scale; a portion distal to that seen in figure 40. The ridges are still more wavy.

FIGURE 43. The same scale; a portion distal to that seen in figure 41. Fragments of the ridges are still parallel to each other, but the main pattern is disrupted.

FIGURE 44. A deep scale from the body of the wing of *Plusia gamma*. On the upper surface the ridges are parallel to the long axis of the stalk and to each other; on the lower surface they radiate fan-wise from the stalk. Those on the upper surface are *c.* 2μ apart.

FIGURE 45. The distal end of a lateral marginal scale of *Thais polyxena*. The bristle-like processes are separated from each other at their bases by regions of the scale in which the spacing of the ridges is increased. On the body of the scale the spacing of the ridges is *c.* 2μ.

FIGURE 46. A scale of *Lepisma* showing the pattern of the ridges on the upper and lower surfaces of the scale. The scale extends into backwardly directed horns on either side of the stalk.

FIGURE 47. The base of the blade of a lateral marginal scale of *Plusia gamma*. New ridges are intercalated at the points marked by arrows. The spacing of the ridges is *c.* 2μ.

FIGURE 48. A deep scale from the wing of *Eilema lurideola*. The ridges on the upper surface are in focus. Except at the base of the scale where they emerge from the stalk they are parallel to each other and to the axis of the stalk. In parts the rungs joining the ridges are visible, as also are the pillars (trabeculae) uniting upper and lower surfaces and seen as dots in section. The spacing of the ridges is *c.* 2μ.

FIGURE 49. The same. The radiating ridges on the lower surface are in focus. At the sides of the scale the connexion between ridges of upper and lower surfaces is visible in some instances.

FIGURE 50. The same scale; the distal end is now in the field. The ridges of upper and lower surfaces are simultaneously in focus in parts. The continuity between the ridge systems of upper and lower surfaces at the distal end and on the lateral margins of the scale is suggested in several places.

FIGURE 51. The distal end of an androconium or scent scale from the male of *Pieris brassicae*. The fringe of 'hairs' is continuous with the ridges. At the point where they separate as 'hairs' the ridges are spaced at *c.* 1μ.

PLATE 3

Figures 52 to 56 inclusive are X-ray diffraction photographs. All were taken at the same film-object distance of *c.* 30 mm. with filtered Cu radiation. The reflexions visible are those of poly-acetylglucosamine (chitin *sensu stricto*).

FIGURE 52. The X-ray beam was normal to the surface of a stack of oriented forewings of adult *Ephestia sericarium*. The reflexions visible on the equator and meridian are parts of practically continuous circles. The beam traversed wing membrane and flattened body scales on upper and lower surfaces; the photograph is that of a powdered chitin with traces of fibrillar orientation.

FIGURE 53. Another preparation of the same. The X-ray beam was normal to the fringe of oriented lateral marginal scales. Meridional and equatorial reflexions are sharper and show less angular dispersion than in figure 52.

FIGURE 54. The X-ray beam was normal to the long axis of a bundle of hair-scales on the tegulae of *Ourapteryx sambucaria*. Both meridional and equatorial reflexions are sharper than in the preceding photographs.

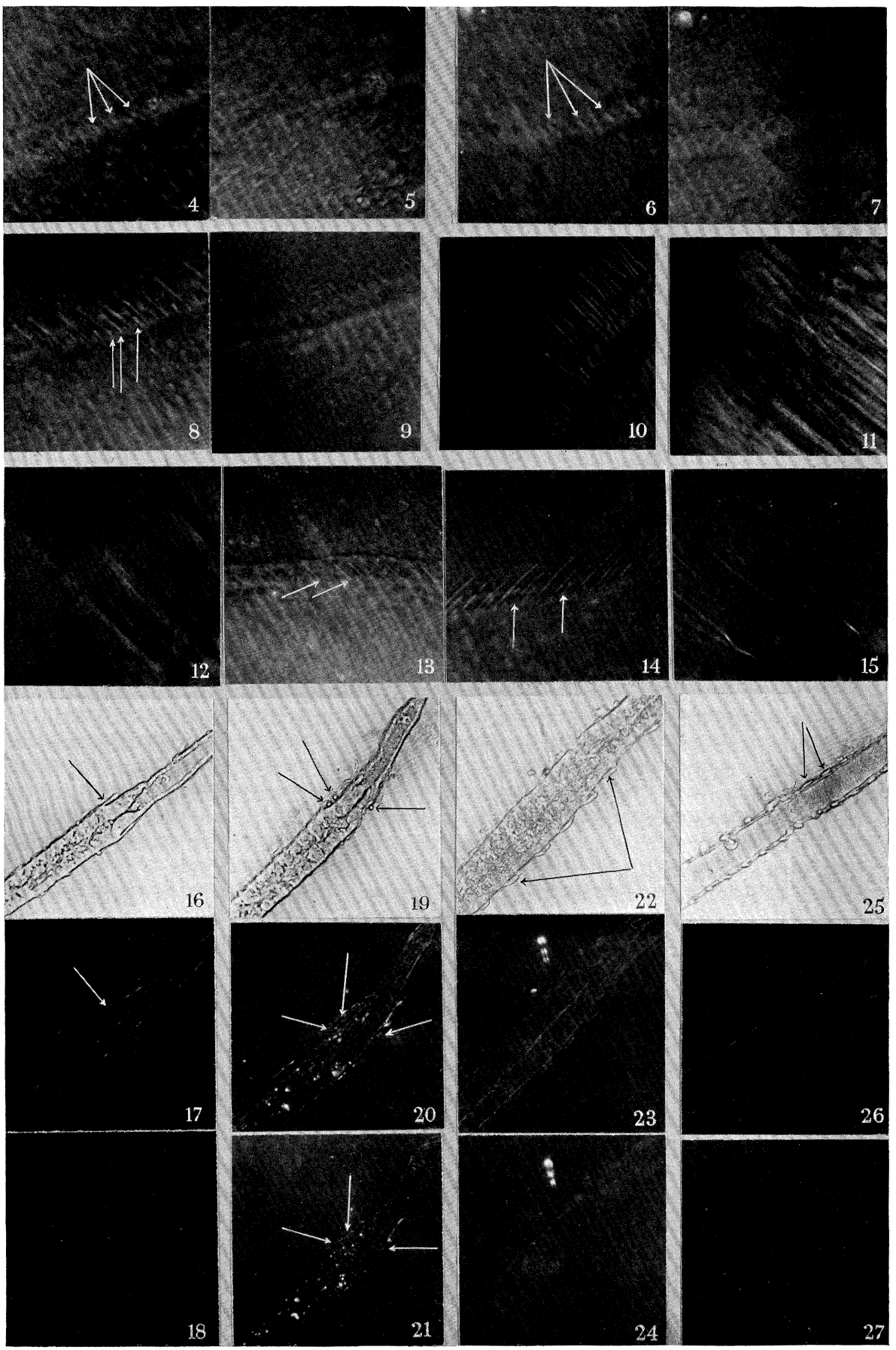
FIGURE 55. Another preparation of the same showing better orientation. On the meridian two reflexions are visible: 020 (5·2 Å) and 031 (3·4 Å). In the original, two reflexions are visible on the equator: 002 (9·5 Å) and 200 (4·65 Å).

FIGURE 56. The X-ray beam was normal to the fringe of strap-shaped, lateral marginal scales in stacked and oriented forewings of *Euproctis phaeorrhoea*. The fibre diagram is sharper than that given by the lateral marginal scales of *Ephestia*, but not as sharp as that from the hair-scales on the tegulae of *Ourapteryx*.

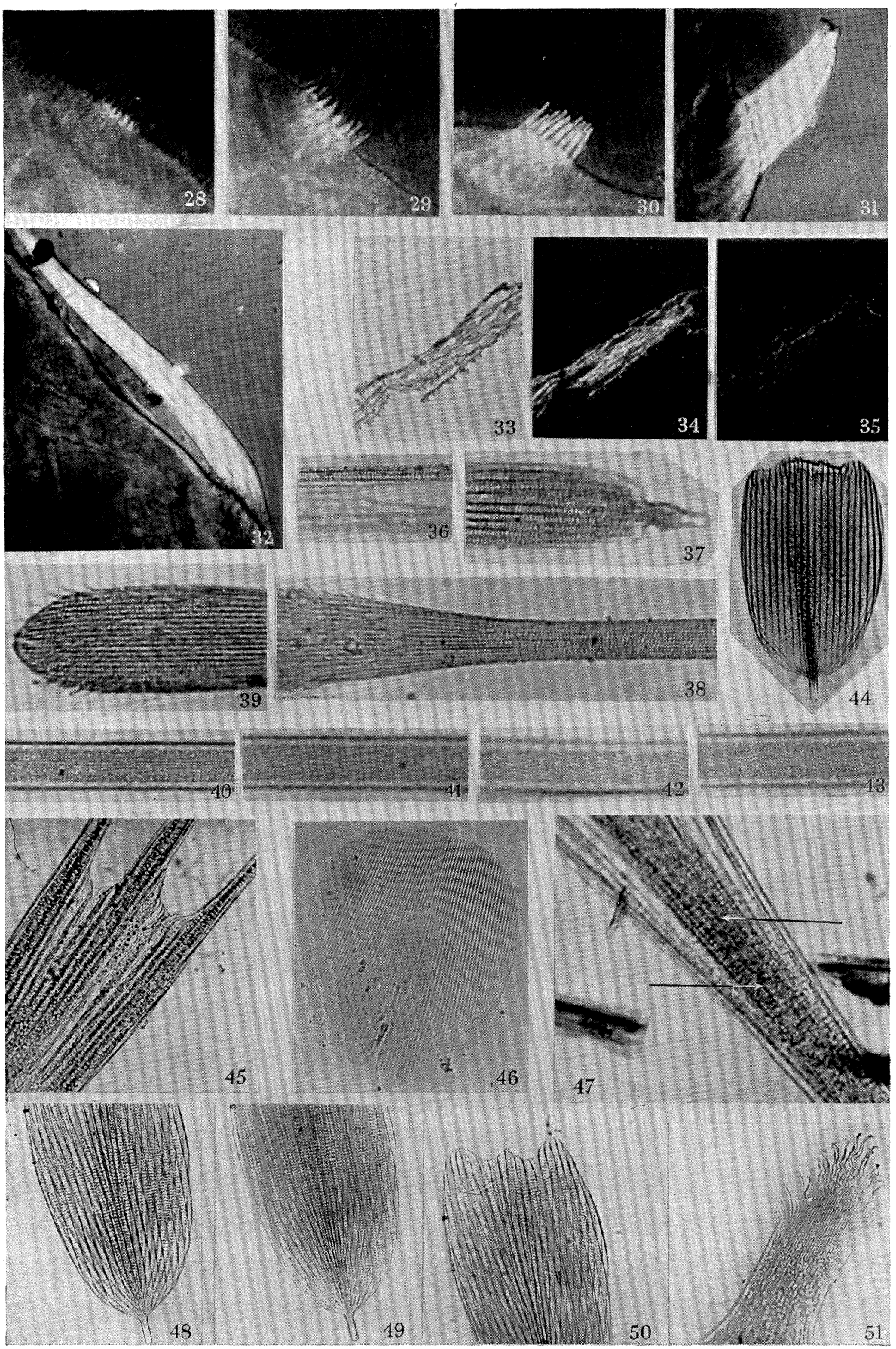
FIGURE 57. X-ray photograph obtained from a bundle of chitinous bristles of *Aphrodite*, the sea mouse, a polychaete worm. The original shows the complete set of reflexions due to polyacetylglucosamine (chitin) together with extra reflexions (some of which are to be seen here on the meridian), which disappear when the bristles are treated with aqueous potassium hydroxide or diaphanol. It represents a degree of orientation superior to that in any of the scales examined, but shows that the X-ray photographs of the scales are simplified versions of the chitin photograph.

FIGURE 58. An entire androconium mounted in water and photographed under an oil immersion apochromatic $\frac{1}{12}$th. The ridge pattern and the posteriorly directed horns on either side of the stalk are shown.

FIGURE 59. Another specimen of the same, photographed with the same equipment but in air, to show the stalk and the balloon-like basal enlargement (seen collapsed in figure 58).



(*Facing p.* 28)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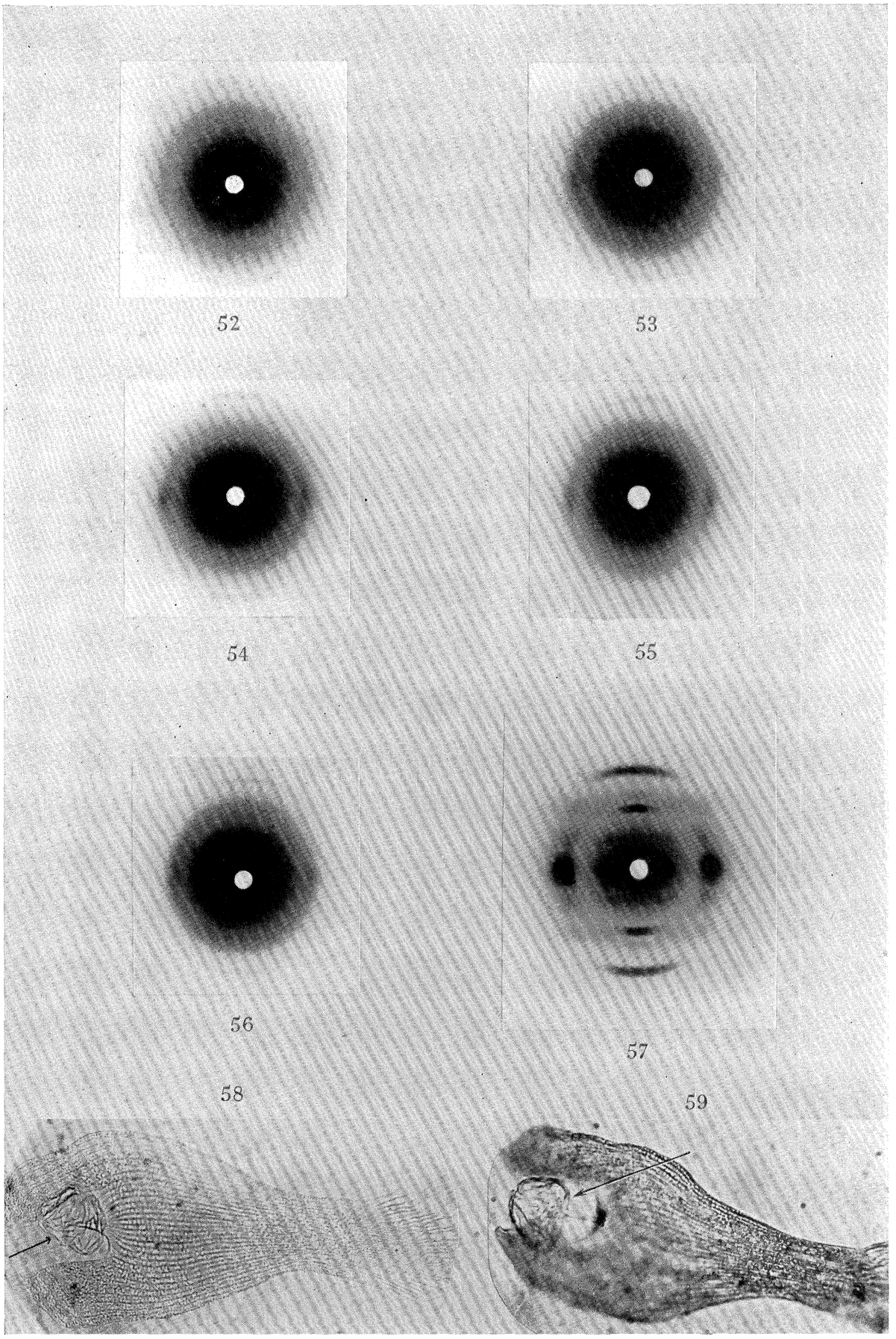
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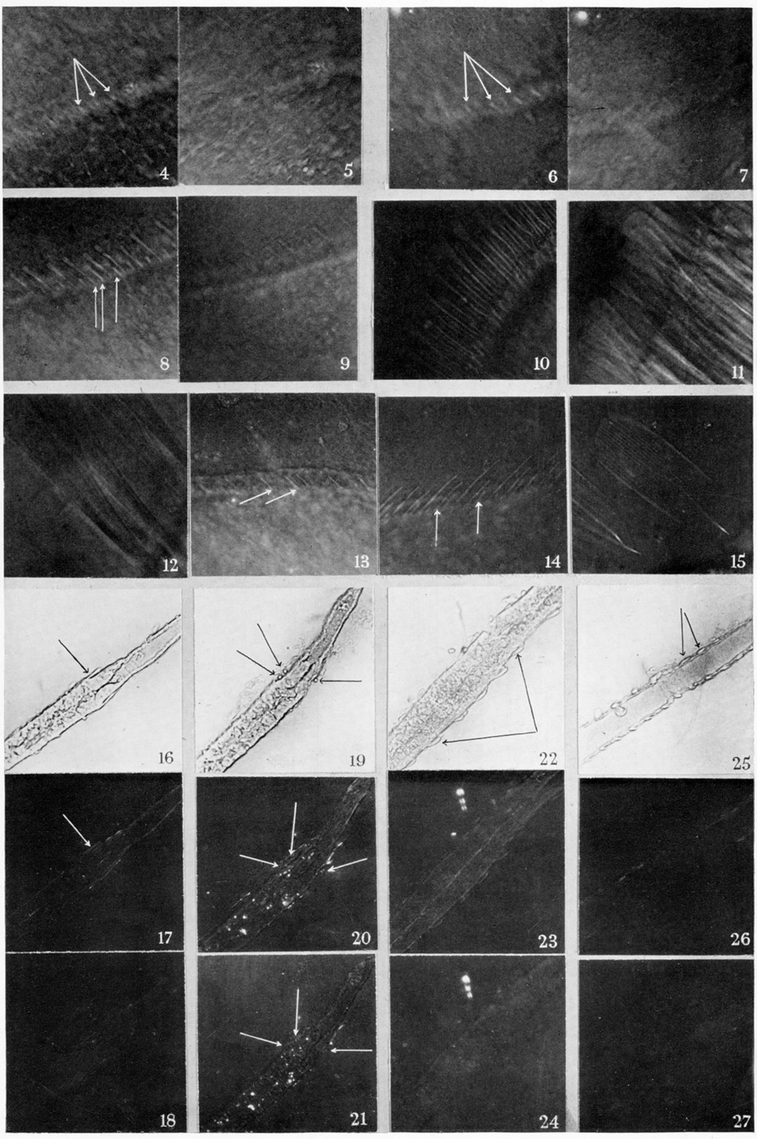

PLATE 1

All photographs on this plate are of formalin-fixed material examined unstained in water; with the exception of figure 11, all figures are at the same magnification. They were taken with a Leitz 6L (polarizing) $\frac{1}{6}$th objective and a $\times 10$ eyepiece with the draw-tube extended to 170 mm.

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FIGURE 7. The same preparation rotated through 90°. The brightness of the rudiments is considerably reduced by compensation.

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FIGURE 9. The same rotated through 90°; compensation is incomplete.

FIGURE 10. Older rudiments of the same type on the forewing of a pupa fixed 181 ± 3 hr. after pupation. In the two larger sizes flattening is advanced. The smallest rudiments are still club-shaped. Crossed nicols; compensator in the addition position.

FIGURE 11. Flattened blades and shafts of lateral marginal scales in a pupa fixed 194 ± 5 hr. after pupation. Crossed nicols; compensator in the addition position.

FIGURE 12. The same preparation under oil immersion achromatic $\frac{1}{12}$th. The longitudinal ridges are visible, compensated to blackness; they are spaced at *c.* 1μ.

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FIGURE 16. Club-shaped lateral marginal rudiments seen in a longitudinal section of a forewing from a pupa fixed 110 hr. after pupation. The clubs are *c.* 14μ long. Analyzer out.

FIGURE 17. The same with nicols crossed and the compensator in the addition position. Rudiments (and outer edge of epidermis) bright.

FIGURE 18. The same rotated through 90°. The rudiments are now in the subtraction position and are invisible.

FIGURE 19. Lateral marginal scales seen in transverse section in a section from a pupa fixed 110 hr. after pupation. Diameter of rudiments *c.* 3μ. Analyzer out.

FIGURE 20. The same; nicols crossed; rudiments practically invisible.

FIGURE 21. The same rotated through 90°.

FIGURE 22. Longitudinal section of a hind wing from a pupa fixed 150 hr. after pupation showing rudiments of the wing-body scales. Rudiments *c.* 14μ long; maximum diameter *c.* 4μ. Analyzer out.

FIGURE 23. The same with nicols crossed and compensator in the addition position. Epidermis and basement membrane, as well as the scale rudiments, show birefringence.

FIGURE 24. The same rotated through 90°. Compensation to blackness is almost complete.

FIGURE 25. Transverse sections of flattened rudiments in a section of the hind wing from a pupa fixed 140 hr. after pupation. On the upper left hand edge of the section, two fragments of flattened scales are seen in surface view. Analyzer out.

FIGURE 26. The same with nicols crossed and compensator in the addition position. The most flattened rudiments visible in figure 24 are slightly birefringent in transverse section.

FIGURE 27. The same rotated through 90°. Birefringence completely compensated to blackness.

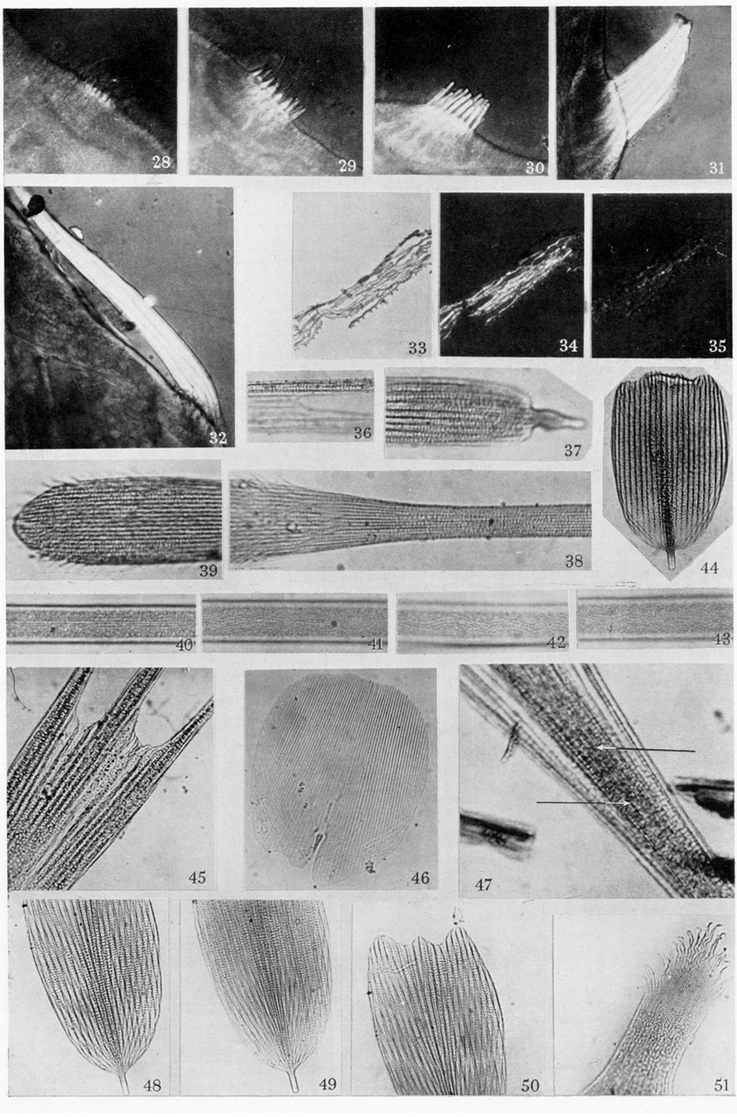

Plate 2

Figures 28 to 35 were taken under polarizing $\frac{1}{4}$th (Leitz 6L) with a ×10 eyepiece. All others on this plate were taken under oil immersion apochromatic $\frac{1}{12}$th (Leitz) with compensating ocular (×10) and immersion condenser of N.A. 1·40.

Figure 28. Frenulum; earliest rudiments from pupa fixed at 107 ± 5 hr. Nicols crossed; compensator in addition. Rudiments *c.* 5 μ long.

Figure 29. The same; pupa fixed at 124 ± 2 hr. Longest rudiments *c.* 12 μ long.

Figure 30. The same; pupa fixed at 144 hr. Longest rudiments *c.* 21 μ long.

Figure 31. The same; pupa fixed at 184 ± 3 hr. Longest rudiments *c.* 65 μ long.

Figure 32. The same; pupa fixed at 199 ± 5 hr. Longest rudiments *c.* 250 μ long.

Figure 33. Transverse section of forewing of adult of *Ephestia sericarium*. The section passes through wing membrane and scales on both surfaces. The single conspicuous scale section is *c.* 56 μ broad. Analyzer out.

Figure 34. The same. Nicols crossed and compensator rotated to give slight enhancement of brightness. The underside lamella of each scale is birefringent. Occasional large ridges are also bright as seen in transverse section, indicating that the optical axis is not parallel to the long axis of the ridge.

Figure 35. The same rotated through 90°. Compensation to blackness is almost complete.

Figure 36. A ridge on a distal, bristle-like process of a lateral marginal scale of *Thais polyxena* seen in profile; the ridge shows an obliquely laminated structure. Height of ridge *c.* 1 μ.

Figure 37. The base of a spatulate, strap-shaped scale of *Trepsichrois mulciber*. The ridges are *c.* 2 μ apart, and the space between them is bridged by rungs spaced at *c.* 0·6 μ.

Figure 38. Shaft and base of spatulate end of the same scale. In the narrowest part of the shaft the spacing of the ridges is reduced to *c.* 0·5 μ. At the base of the spatulate end, fine, tapering hairs are seen to run off obliquely from the ridges. The spacing of the ridges and their number are increased in the spatulate end.

Figure 39. Spatulate end of same scale showing spacing of ridges; hairs out of focus.

Figure 40. The base of a hair-scale from a scent brush of *Danais septentrionalis*. The ridges are parallel to each other and to the long axis of the scale. They are spaced at *c.* 0·7 μ. The magnification in this photograph is slightly less than in the following three figures.

Figure 41. The same scale; a portion distal to that seen in figure 39. The parallel ridges are becoming wavy.

Figure 42. The same scale; a portion distal to that seen in figure 40. The ridges are still more wavy.

Figure 43. The same scale; a portion distal to that seen in figure 41. Fragments of the ridges are still parallel to each other, but the main pattern is disrupted.

Figure 44. A deep scale from the body of the wing of *Plusia gamma*. On the upper surface the ridges are parallel to the long axis of the stalk and to each other; on the lower surface they radiate fan-wise from the stalk. Those on the upper surface are *c.* 2 μ apart.

Figure 45. The distal end of a lateral marginal scale of *Thais polyxena*. The bristle-like processes are separated from each other at their bases by regions of the scale in which the spacing of the ridges is increased. On the body of the scale the spacing of the ridges is *c.* 2 μ.

Figure 46. A scale of *Lepisma* showing the pattern of the ridges on the upper and lower surfaces of the scale. The scale extends into backwardly directed horns on either side of the stalk.

Figure 47. The base of the blade of a lateral marginal scale of *Plusia gamma*. New ridges are intercalated at the points marked by arrows. The spacing of the ridges is *c.* 2 μ.

Figure 48. A deep scale from the wing of *Eilema lurideola*. The ridges on the upper surface are in focus. Except at the base of the scale where they emerge from the stalk they are parallel to each other and to the axis of the stalk. In parts the rungs joining the ridges are visible, as also are the pillars (trabeculae) uniting upper and lower surfaces and seen as dots in section. The spacing of the ridges is *c.* 2 μ.

Figure 49. The same. The radiating ridges on the lower surface are in focus. At the sides of the scale the connexion between ridges of upper and lower surfaces is visible in some instances.

Figure 50. The same scale; the distal end is now in the field. The ridges of upper and lower surfaces are simultaneously in focus in parts. The continuity between the ridge systems of upper and lower surfaces at the distal end and on the lateral margins of the scale is suggested in several places.

Figure 51. The distal end of an androconium or scent scale from the male of *Pieris brassicae*. The fringe of 'hairs' is continuous with the ridges. At the point where they separate as 'hairs' the ridges are spaced at *c.* 1 μ.

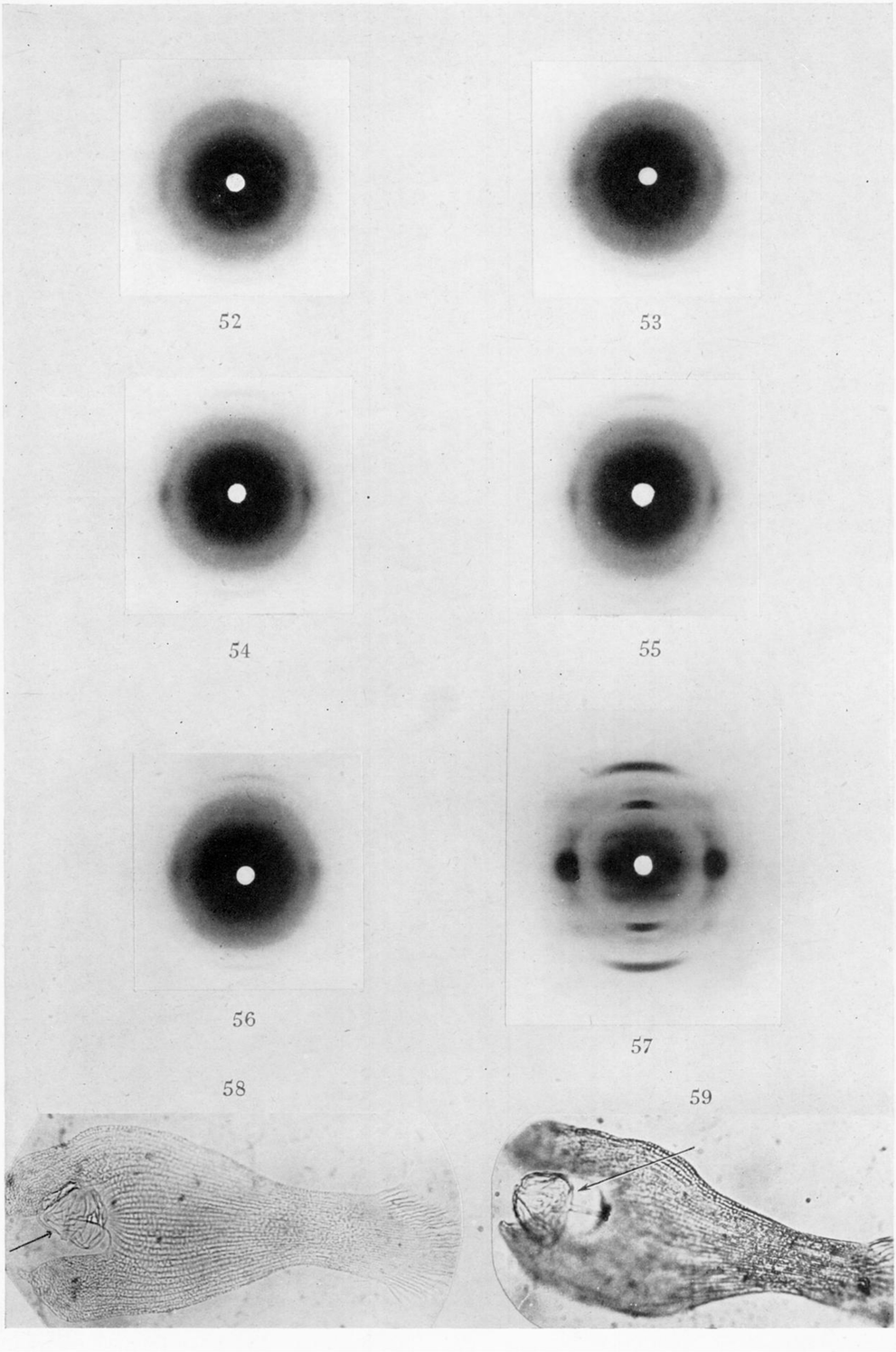

PLATE 3

Figures 52 to 56 inclusive are X-ray diffraction photographs. All were taken at the same film-object distance of *c.* 30 mm. with filtered Cu radiation. The reflexions visible are those of polyacetylglucosamine (chitin *sensu stricto*).

FIGURE 52. The X-ray beam was normal to the surface of a stack of oriented forewings of adult *Ephestia sericarium*. The reflexions visible on the equator and meridian are parts of practically continuous circles. The beam traversed wing membrane and flattened body scales on upper and lower surfaces; the photograph is that of a powdered chitin with traces of fibrillar orientation.

FIGURE 53. Another preparation of the same. The X-ray beam was normal to the fringe of oriented lateral marginal scales. Meridional and equatorial reflexions are sharper and show less angular dispersion than in figure 52.

FIGURE 54. The X-ray beam was normal to the long axis of a bundle of hair-scales on the tegulae of *Ourapteryx sambucaria*. Both meridional and equatorial reflexions are sharper than in the preceding photographs.

FIGURE 55. Another preparation of the same showing better orientation. On the meridian two reflexions are visible: 020 (5·2 Å) and 031 (3·4 Å). In the original, two reflexions are visible on the equator: 002 (9·5 Å) and 200 (4·65 Å).

FIGURE 56. The X-ray beam was normal to the fringe of strap-shaped, lateral marginal scales in stacked and oriented forewings of *Euproctis phaeorrhoea*. The fibre diagram is sharper than that given by the lateral marginal scales of *Ephestia*, but not as sharp as that from the hair-scales on the tegulae of *Ourapteryx*.

FIGURE 57. X-ray photograph obtained from a bundle of chitinous bristles of *Aphrodite*, the sea mouse, a polychaete worm. The original shows the complete set of reflexions due to polyacetylglucosamine (chitin) together with extra reflexions (some of which are to be seen here on the meridian), which disappear when the bristles are treated with aqueous potassium hydroxide or diaphanol. It represents a degree of orientation superior to that in any of the scales examined, but shows that the X-ray photographs of the scales are simplified versions of the chitin photograph.

FIGURE 58. An entire androconium mounted in water and photographed under an oil immersion apochromatic $\frac{1}{12}$th. The ridge pattern and the posteriorly directed horns on either side of the stalk are shown.

FIGURE 59. Another specimen of the same, photographed with the same equipment but in air, to show the stalk and the balloon-like basal enlargement (seen collapsed in figure 58).