THE EARLY ESTABLISHMENT OF DIMORPHISM IN THE FEMALE HONEYBEE, *APIS MELLIFERA* L.

by

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Introduction.

Polymorphism and its social expression, caste, are widespread in the order Hymenoptera. Because of economic interest in the honeybee, *Apis mellifera*, variation in form in this species has received much attention. The present discussion will be restricted to the honeybee; various areas of research will be reviewed. Among the excellent general discussions of polymorphism available are: Brian (1957), Light (1943), Mitchener and Mitchener (1951), and Wheeler (1928). There are, as well, specific treatises on ants: Bier (1956), Brian (1954-56), Wilson (1953); bumblebees: Free and Butler (1959); honeybees: Gontarski (1941), Haydak (1943), Lukoschus (1956), Smith (1959); termites: Buchli (1958), Lüscher (1956); wasps: Blackith (1958); and parasitic hymenoptera: Flanders (1950), Salt (1937, 1952), and Schmieder (1939).

Caste characteristics in honeybees.

Structural and physiological differences between worker and queen have been described in detail by Ribbands (1953), Snodgrass (1956), and Lukoschus (1956). In addition to her greater size, the queen differs from the worker in a number of external features. The differences in reproductive development are the aspect which is most pertinent to this discussion. The queen is capable of mating and laying a large number of fertilized and unfertilized eggs over a period of several years (Ribbands). Each of her ovaries consists of 160-180 ovarioles, and the spermatheca is well developed (Snodgrass). By contrast, the spermatheca of the worker is rudimentary and the ovaries are reduced to 2-12 ovarioles each (Snodgrass). The ovarioles, though reduced in number, are not rudimentary, but produce oocytes, which are normally resorbed (Wigglesworth, 1954). Under certain conditions, the ovaries may be activated, resulting in the production of a small number of unfertilized eggs (Ribbands). The ability to mate and lay fertilized eggs is the ultimate criterion of a queen.

Comparative data on salivary gland development in the queen, worker, and drone honeybee have been presented in detail elsewhere (Ribbands, Snodgrass). Of the 6 sets of glands comprising the salivary system, the hypopharyngeal and mandibular glands are in the light of present know-

ledge of most interest in relation to caste differences. The hypopharyngeal glands are vestigial in the queen and developed in the worker to a degree varying with her physiological state, being small and empty in newly emerged bees and fully developed only in nurse bees (Kratky, 1931), and "winter" bees (Maurizio, 1954). Protein (pollen) ingestion causes rapid development (Kratky, Maurizio). Bees restricted to a carbohydrate diet rear brood for only a short period; under these conditions the hypopharyngeal glands do not develop (Kratky). The hypopharyngeal glands elaborate secretions fed to all larvae and to the adult queen (Snodgrass). The mandibular glands are well developed in both female castes, but are larger in the queen. Like the hypopharyngeal glands they require protein for full development (Kratky). Ribbands' suggestion that the mandibular glands of workers may contribute to the larval diets has recently been confirmed (Butler and Simpson, 1958; Callow, Johnston and Simpson, 1958).

The endocrine glands of adult bees appear to be similar in position and appearance to those found in other insects (Nelson, 1924; Rehm, 1939; Lukoschus, 1952; L'Hélias, 1950, 1952; Pflugfelder, 1948; Schaller, 1950; Thomsen, 1954; Weyer, 1935). Less is known of the physiology of the endocrine glands of the bee because bees are not as susceptible to surgical techniques as some other insects.

Larval development in queens and workers.

Growth. — At 35° C and 100 per cent relative humidity eggs were observed to hatch about 75 hours after laying (Dixon and Shuel, 1959). The duration of post-embryonic development is normally about 13 days for queens and 17 or 18 days for workers (Bertholf, 1925), subject to a modifying influence of temperature (Milum, 1930). According to Bertholf the first 4 moults occur at approximately the same times in both castes between the fourth and seventh days after oviposition. Subsequent development is faster in the queen. The queen larva is sealed in its cell on the seventh day, the worker at the end of the eighth day. The fifth (prepupal) moult of the queen comes on the tenth day and the sixth (emergence) at the end of the fifteenth. The corresponding worker moults occur at the end of the eleventh and twentieth days, respectively.

Larval growth follow the familiar sigmoid curve, with a rapid increase in weight after 60 hours (Nelson and Sturtevant, 1924; Stabe, 1930). Stabe's measurements, made at 6 hour intervals, showed an almost identical growth rate in queen and worker larvae for the first 48 hours, followed by a more rapid increase in weight of workers which lasted for about a day. After 96 hours queen larvae surpassed the workers in weight and continued to grow faster, achieving on the average a maximal weight of 322 mg. at 132 hours, compared with 152 mg. at 120 hours for the workers. The queen larva unlike the worker is supplied with food when sealed, and continues to gain in weight.

DEVELOPMENT OF THE REPRODUCTIVE ORGANS. — Development of the reproductive organs in the honeybee was treated comprehensively by Zander, Löschel, and Meier (1916). Sexual development was similar in queen and worker larvae for the first day. By the end of the second day, however, a disparity in ovary growth had appeared, the queen ovary measuring 0.55 mm., the worker ovary only 0.38 mm. By the end of the third day the queen ovary had 130 ovarioles as compared with 90 for the worker. As the number of individuals measured was not stated, one cannot be certain of the exact significance of the quantitative differences. However, a qualitative difference appeared on the second day. Whereas the ovarioles of the queen extended almost the entire length of the ovarian tissue, those of the worker were limited to the proximal half. The same authors described the reduction in number of ovarioles in the worker during the prepupal stage and the retrogression of the spermatheca in the pupal stadium. Here, then, is evidence of an early dichotomy in queen and worker development. Further evidence has been afforded by the cytological studies of Mickey and Melampy (1941). Nuclear fragmentation in fat cells begins during the third larval day in the queen and a day later in the worker.

Biochemical and metabolic studies.

The chemical analyses of Melampy et al (1940) show marked differences between castes with respect to tissue composition of 3 or 4 day old larvae. The tissues of queen larvae contain a higher percentage of lipids and a lower percentage of nitrogen and reducing substances. During the next two or three days there is a substantial percentage increase in reducing substances in the queen but little change in nitrogen or lipids; in the worker there is a marked reduction in nitrogen accompanied by a moderate increase in lipids and a large percentage increase in reducing substances.

Differences in respiratory activity between castes are also manifested early in larval life. Melampy and Willis (1939), using the direct Warburg method, found a much higher rate of gas exchange in 2 to 3 day old queen larvae. The respiratory quotient was 1.16 for queens and 1.42 for workers. Cartesian diver measurements of gas exchange in larvae during the first 24 hours (Shuel and Dixon, 1959) have revealed a much higher net carbon dioxide evolution on a substrate of royal jelly than on the diet supplied to worker larvae during the first three days. Evidently metabolic differences are established very early in the larval period and are a reflection of nutritional differences.

Quantity and composition of larval diets.

Queen larvae receive a surplus of food throughout the larval period, whereas worker larvae are supplied with an excess only for the first 3 days (Nelson and Sturtevant, 1924).

Comprehensive reviews of the chemistry of royal jelly have been written by Johannson (1955) and Johannson and Johannson (1958). Sufficient royal jelly for macrochemical analysis is fairly easily obtained. A macrochemical analysis by Townsend and Lucas (1940) showed the solids in royal jelly to include 30-35 per cent protein, 28 per cent sugars, and 10-15 per cent ether-soluble materials. A free fatty acid, later identified as 10-hydroxy-$\triangle^2$-decenoic acid (Butenandt and Rembold, 1957; Barker, Foster, and Lamb, 1959) constituted 80 to 85 per cent of the ether-soluble fraction. Although reliable comparative and serial analyses have had to await the advent of microanalytical techniques, differences in the chemical composition of larval diets have long been recognized (Planta, 1888-89; Kestner and Plaut, 1924). Planta found royal jelly to be higher in fat than the diet of young worker larvae. After the fourth day the percentage of protein in worker diet decreased and the percentage of reducing substances increased. Kestner and Plaut reported similar changes. The change in the composition of the worker diet around the third or fourth day can be attributed to the addition of honey and pollen by nurse bees (Ribbands, 1953). Apparently the addition of honey is the more significant; Simpson (1955) has shown that the amount of nitrogen added as pollen to the diet of older worker larvae is relatively minor. Recently Shuel and Dixon (1959) compared the diets of queen and worker larvae in the age groups 0-30 and 72-96 hours. No appreciable change with larval age was noted in the major constituents of royal jelly. Statistical comparison revealed, in addition to the expected change in worker diet after the third day, significant differences between the respective secretions fed to young larvae of the queen and worker castes. The diet of the worker was very high in protein and very low in sugars, that of the queen intermediate in respect to both constituents. Differences were found also in the lipid content and the titratable acidity. Butenandt and Rembold (1957), however, reported similar concentrations of 10-hydroxy-$\triangle^2$-decenoic acid in royal jelly and worker diet. They did not specify the age of the larvae in the cells from which the worker diet was obtained.

The first known qualitative difference between the diets of royal and worker larvae has recently been reported. Butenandt and Rembold (1958) have isolated biopterin (2-amino-4-hydroxy-6-(1,2 dihydroxyl-propyl) pteridine from royal jelly, and have also established its presence in queen larval tissue, but not in either the diet or the body tissue of worker larvae (1).

(1) Low levels of biopterin have recently been found in worker diet (HANSEN and REMBOLD, 1960).

Brown and Freure (1959) have found sebacic acid and 2-decendioic acid in royal jelly. Comparable data for worker diets are not yet available.

Analyses have been made of the free and combined amino acids in royal jelly (Pratt and House, 1949; Weaver and Kuicken, 1951; Ammon and Zoch, 1957), but precise quantitative data for worker diets have not as yet been reported. The protein electrophoretic patterns of royal jelly and the food of worker larvae under 3 days of age are qualitatively similar (Habowsky and Shuel, 1959; Patel, Haydak and Gochnauer, 1960).

Vitamin assays by Haydak and Vivino (1950) indicated similar concentrations of B vitamins in royal jelly from cells containing larvae 1-5 days of age, and worker jelly from cells with 1-2 day larvae. The vitamin concentration was greatly reduced in the diet of older worker larvae. Lingens and Rembold (1959) found a considerable reduction in the pantothenic acid content of royal jelly fed to 5-day larvae as compared with royal jelly fed to 3-day larvae.

Other differences in larval diets may arise from physical and chemical changes on standing. Abbott (1955) noted a slow evolution of carbon dioxide in stored royal jelly. Goillot (1957) found a rapid increase in the electrical conductivity of royal jelly after 30 hours' storage at temperatures between 0° and 30° C. Dixon and Shuel (1958) noted a rapid oxygen uptake in freshly collected royal jelly at 35° C. Whether such changes are of biological significance is not yet known.

The term "worker jelly" has been proposed for the secretion fed to young worker larvae, and the term "modified jelly" for the diet altered by the nurse bees after the third day (Shuel and Dixon, 1959). Recently Haydak (1959) has suggested that the latter term be amended to the more specific "modified worker jelly". The terms "worker jelly" and "modified worker jelly" will be used in the remainder of this review. The general term "worker diet" will include both worker jelly and modified worker jelly.

The existence of two distinct types of royal jelly has been postulated by v. Rhein (1950-51, 1956). Although present knowledge of its chemical composition is far from complete, changes in the major constituents of royal jelly with larval age appear to be negligible (Jacoli and Poggioli, 1956; Shuel and Dixon, 1959). The changes in pantothenate found by Lingens and Rembold (1959), though striking, do not necessarily indicate the provision of a unique secretion to older royal larvae.

Rearing experiments.

The experimental rearing of honeybee larvae has received much attention. Criteria of dimorphism have included: size of ovaries (v. Rhein, 1933; Weaver, 1955, 1957, 1957*a*), number of ovarioles (v. Rhein, 1933; Smith, 1959; Weaver, 1955, 1957, 1957*a*), size and shape of the spermatheca (v. Rhein, 1933; Weaver, 1957; Vagt, 1955), size and shape of the

abdomen (Weaver, 1955, 1957), characteristics of the metathoracic legs, the sting, and mandibles (v. Rhein, 1933; Smith, 1959; Weaver, 1955, 1957), tongue length (Weaver, 1955, 1957; Smith, 1959), size of the mandibular glands (v. Rhein, 1933; Weaver, 1955, 1957), and the duration of the developmental period (Weaver, 1957).

The quantitative nature of caste determination has been demonstrated in various experiments (Klein, Zander and Becker, cited by Ribbands, 1953; Smith, 1959; Vagt, 1955; Weaver, 1957). When larvae varying in age from one-half day to 3 days are transferred from worker cells to queen cells, certain morphological characters in the resulting imagoes are intermediate between queen and worker means (Vagt, 1955; Weaver, 1957). Evidently differentiation begins on the first day and is progressive. As the larval age at the time of transfer is increased, a higher percentage of workers and intermediate forms is obtained. Transitional forms are especially common among laboratory-reared bees (Weaver, 1955; Smith, 1959). Weaver (1957) has suggested that the potential for polymorphism exists in the female honeybee, but that certain controls in the natural milieu limit its expression to two forms. Eckert (1934) found only one intermediate adult among 281 queens produced from larval transfers within the colony.

The influence of larval age on the effectiveness of a given diet is illustrated by the work of Weaver (1957) and v. Rhein (1933, 1950-51, 1956). Weaver found that 1-day old larvae transferred to queen cells for 24 hours and then returned to worker cells became normal workers, whereas 2- or 3-day old larvae similarly treated exhibited some queen characteristics. One-day old larvae transferred for 2 days and then returned to worker cells also developed some queen-like features. In Rhein's experiments, larvae transferred from royal jelly to modified worker jelly developed into adults resembling workers, intermediates, or queens depending on their weight when the diet was changed. Those weighing less than 20 mg. became workers, those weighing more than 46 mg. developed into queens. According to Stabe's growth data (1930), this weight range would comprise larvae from about 72 to 85 hours of age.

A significant feature of v.Rhein's experiments was the inadequacy of worker jelly for pupation, a finding confirmed by Smith (1959).

Results of laboratory feeding experiments suggest that storage of royal jelly may affect its queen-determining properties. Weaver (1956) found that the majority of larvae fed on royal jelly which had been stored for a year at 5° C developed into worker or worker-like imagoes, and postulated a labile determinant. Smith (1959) obtained adult queens on royal jelly which had been freshly collected; stored at 4° C for 6 months; stored at—15° C for a year or longer; or lyophilized, stored at—15° C for 18 months and rehydrated. Survival to the imago stadium varied from 9 per cent (on royal jelly which had been lyophilized and reconstituted) to 56 per cent (on royal jelly kept at 4° C). Survivors included worker and intermediate forms as well as queens. Distribution comparisons based on Chi-squared tests show by far the highest ratio of

queens to other forms among larvae fed with royal jelly from 1,2, and 3—day queen cells as they reached the appropriate age. Prolonged storage greatly increased the relative frequency of worker and transitional forms. Jay (1959) compared weights at defecation or larvae taken from queen and worker cells and reared on royal jelly freshly collected or stored at — 15° C for 2 years. The fresh material was in general superior to the stored, the superiority being more pronounced among larvae taken from queen cells. Reducing the intervals between transfers of larvae to fresh food from 24 to 6 hours resulted in an appreciable increase in weights at defecation.

High mortality rates are common in laboratory rearing experiments (Weaver, 1956; Smith, 1959; Hoffmann, 1956). Although these might be due in part to mechanical injury, other factors are probably involved. A technique by which a majority of young larvae could be reared to the imago stage would be invaluable.

Although the physical environment, particularly with respect to temperature and humidity, may be critical in the laboratory rearing of honeybees (Smith, 1959; Jay, 1959), there is at present no convincing evidence that physical factors are of major significance in caste determination under natural conditions.

Laying workers and queen substance.

Under certain conditions, usually when colonies are both queenless and broodless, ovaries of worker bees may be activated and a limited number of eggs produced. Although ovary activation occurs in the adult stadium long after caste determination, it may be regarded as a partial reversal of the direction of worker development, and treated in the same context as caste establishment.

In 1942, Hess postulated the production by the queen of a substance capable of inhibiting ovary ripening in the workers. Since then Butler and his colleagues (1954-59), de Groot and Voogd (1954), Müssbichler (1952) and Pain (1954, 1955, 1956, 1956*a*) have confirmed Hess' theory and have further elucidated the nature of the inhibitory substance. In addition to suppressing ovarian function it inhibits the production of emergency queen cells (Butler, 1956, 1957; Butler and Gibbs, 1958). The substance has been named "ectohormone" by Pain and "queen substance" by Butler. Its effects both on the ovaries (Pain, 1956*a*) and on queen cell production (Butler, 1957) are quantitative. Queen substance is elaborated in the mandibular glands of the queen (Butler, Collaw, and Johnston, 1959; Butler and Simpson, 1958) and it appears to be closely related chemically to 10-hydroxy-$\triangle^2$-decenoic acid (Butler, Callow, and Johnston) (1). Its source and structural affinity are particularly interesting, as 10-hydroxy-

(1) The structure of queen substance has recently been established as 9-oxodec-2-enoic acid Callow and Johnston, 1960).

$\triangle^2$- decenoic acid is produced in the mandibular glands of worker bees (Barker et al, 1959; Callow et əl, 1959).

Inhibition phenomena similar to those caused by queen substance have been described for termites (Castle, 1934; Lüscher, 1956) and for ants (Gregg, 1942).

The mode of action of queen substance is still unknown. Considered in the light of (1) known protein needs for both egg production (Wigglesworth, 1954) and the development and functioning of the pharyngeal glands (Kratky, 1931) and (2) the appearance of laying workers in colonies which are both queenless and broodless and in which one would expect a surplus of protein, a likely role for queen substance is in the mobilization of protein in the worker body.

Queen substance is distributed throughout the colony in regurgitated food (Butler, 1956). As modified worker jelly contains an admixture of honey, it is likely that queen substance is present in the diet of older worker larvae. It is possible, moreover, that it may contribute to the retrogressive changes in the reproduction organs of the worker during the prepupal and pupal stadia. Its presence in modified worker jelly might be demonstrable by the use of isotopic tracer techniques.

In contrast to the action of queen substance in suppressing ovarian development, Altmann (1950, 1952) produced a stimulation of the ovaries of the adult workers with injections of extracts of queen adults or larvae, 2-day old worker larvae, royal jelly from cells of 1-2 day queen larvae, or the heads and thoraces of worker bees. The source of the active principle appeared to be the corpora allata.

Nutritional and humoral factors.

In summary of the evidence of many histological, nutritional, and biochemical investigations, it may be stated that development in the worker larva appears to comprise two phases, defined by the addition of honey to the diet by nurse bees. Comparisons between workers and queens may conveniently be related to these periods. In the first phase both groups are supplied with unrestricted quantities of highly nutritious diets, but the compositions of the diets differ with respect both to the major constituents (Shuel and Dixon, 1959), and trace substances (Butenandt and Rembold, 1958; Lingens and Rembold, 1959). Intercaste differences in respiration, tissue composition, and the state of development of the reproductive organs already exist. Instars are of approximately equal duration in both castes, but the growth rate of the worker exceeds that of the queen. The worker diet will not, however, promote metamorphosis.

In the second phase the queen larva continues to be fed on a high nutritional plane, while the worker receives a low protein, high carbohydrate diet restricted in quantity. Protein synthesis in worker tissue is diminished, and the content of reducing substances is greatly increased (Melampy

et al, 1940). The growth rate of the queen greatly exceeds that of the worker and the interval between moults is much shorter in the queen. This situation is characteristic of animals reared on a high plane of nutrition (Waddington, 1956). Some of the endocrine organs are more highly developed in the older queen larva (Lukoschus 1956*a*). Reproductive development in the worker is now reversed, the ovaries and spermatheca being reduced in size (Zander et al, 1916).

From the evidence of studies of larval metabolism, chemical composition, and nutrition, it appears that the dichotomy between castes is initiated in the first phase, and consummated in the second.

The evidence of numerous rearing experiments rules out the possibility of a genetic causation for dimorphism. It must be epigenetic, and there can be little doubt that nutrition is the major extrinsic factor controlling caste expression in the honeybee, as it appears to be in bumblebees (Free and Butler, 1959), wasps (Brian, 1957; Light, 1943), ants (Brian 1954-56, 1957; Light, 1943), and termites (Buchli, 1958).

Two questions mays now be asked: (1) what dietary factor initiates the series of events culminating in female dimorphism? and (2) what is the mode of action of this factor? The second question will be considered first.

The role of hormone balance in metamorphosis has been thoroughly reviewed by Wigglesworth (1954). Wigglesworth regards metamorphosis as a special case of polymorphism. Present information indicates that the general concept of hormone balance in relation to metamorphosis is applicable to the honeybee (Fyg, 1956, 1959; Schaller, 1951, 1952; Lukoschus, 1955, 1955*a*, 1956, 1956*a*). The evidence for the extension of this concept to caste differentiation will now be examined.

During adult life differing states of activity of the various endocrine glands are operative. Scharrer and Scharrer (1945) noted that the neurosecretory cells are most active in foraging bees when they are collecting pollen and nectar. More recently Formogoni (1956) has given a quantitative expression to these changes in secretory activity in relation to behaviour and the division of labour. Pflugfelder (1948) showed that the corpora allata were larger in the worker adult than in the queen. Lukoschus (1956) showed that in the immature forms the endocrine organs were considerably larger in the queen than in the worker after the fourth or fifth day of larval life, and correlated corpora allata volume with the oxygen uptake data of Melampy and Willis (1939). The lower blood densities in queen larvae (Smith, 1959) may be a consequence of the larger corpora allata; Altmann (1953, 1956) found that the injection of corpora allata extract into adult bees lowered blood density. In the light of these facts, and of the known metabolic, biochemical, and anatomical differences between castes in the early larval period, it is reasonable to surmise that hormonal differences are established early in larval life. A relationship between nutritional and hormonal factors should therefore be sought in the early larval instars. There is as yet little histological evidence of differences in endocrine activity between queen and worker larvae at this time.

Both worker and queen larvae one day old have well developed corpora allata (Pflugfelder, 1948; Thomsen, 1954; Lukoschus, 1955*a*). The corpora cardiaca, however, are more or less loose strings of cells (Schaller, 1950; L'Hélias, 1950), and it is difficult to consider them as significant organs for either storage or release of hormone. Moreover the young larvae do not as yet have typical neurosecretory cells in the brain (Dixon and Shuel, 1959). The protocerebrum which later contains the neurosecretory cells shows, in the very young larvae, large undifferentiated cells with nuclei containing disorganized clumps of nuclear material. These cells were first described by Nelson (1924) as "degenerating cells".

Initially, dietary factors might be expected to cause hormonal differences: subsequently the hormonal differences might in their turn affect the utilization of nutrients. There are known examples of nutrition causing morphological effects which resemble changes induced by experimental interference with the hormone balance of insects. In *Ephestia*, for example, a protein deficiency can cause a supernumary larval moult instead of a pupal moult (Kühn, 1955). A protein deficiency may also check development of the corpora allata and the prothoracic glands of the honeybee (Mussbichler, 1952).

Nutrition could conceivably influence hormonal balance by imposing limitations either on the supply of essential nutrients or the synthesis and functioning of the enzyme systems involved in the development of the endocrine organs. Pfeiffer (1945) for example demonstrated the role of the corpora allata in the mobilization of both proteinaceous and fatty materials in female grasshoppers, and Wang and Dixon (1960) showed that extirpation of the corpora allata of the female cockroach reduced transaminase activity. Another possible dietary factor is a deficiency in the worker jelly of a vitamin or other substance functioning as a co-enzyme, as for example pantothenate or biopterin. L'Hélias (1955) has adduced evidence for the presence of folic acid, chemically related to biopterin, in the pars intercerebralis of *Carausius* and *Clitumnus*, and has postulated an uncoupling effect of folic acid on cell division (L'Hélias, 1957).

The hypothesis that a difference in hormone balance established during early larval life is the intermediary factor linking nutrition to dimorphism is consistent with present evidence and is amenable to experimental rest. Comparative studies of the endocrine organs might reveal the potential for the establishment of hormonal differences. Following this, it would be logical to attempt to simulate patterns of development on natural diets by altering hormone balance experimentally, as for example by the extirpation of endocrine organs or the implantation of extra organs. An early criterion of differentiation towards queen or worker would be helpful. Two possible criteria are respiration and tissue composition. Experiments could be performed using both worker larvae on worker jelly and queen larvae on royal jelly.

Finding the dietary factor which initiates the series of events culminating in a dichotomy of form might be more difficult. The most direct

approach would involve the development of a chemically defined diet. The results of rearing experiments by Weaver (1956), Smith (1959), and Jay (1959) strongly suggest the involvement of a critical substance which is either volatile or unstable. Obviously the total quantity of food available to queen and worker larvae respectively during the first 3 days is not a factor in the initiation of caste differences. After this period, however, the food supply may contribute to the enhancement of these differences.

Precise data on the interrelationships of quantity of food ingested, length of the developmental period, size attained, and form, would be of great value. In nature the queen develops more rapidly and is much larger. Haydak (1943) suggested that partial inanition of the worker might influence internal secretion which in term would affect growth and development. Jay (1959) found that the size attained by larvae at defecation was inversely correlated with the duration of the larval stadium, a situation reminiscent of the ants (Brian, 1954-56) and the termites (Buchli, 1958). Smith (1959), however, obtained some queens from comparatively small larvae, and Fyg (1959) has stated that dwarf queens may appear in periods of food scarcity. Eckert (1934) found no correlation between body weight and the number of ovarioles in adult queens.

In view of the marked biochemical differences between castes found by Melampy et al (1940), information on nitrogen and lipid contents of experimental larvae obtained in conjunction with quantitative data on food ingestion, might be enlightening.

Perhaps not of primary importance in determining caste, but nonetheless striking, are the "retrogressive" changes occurring in the reproductive organs of the worker following modification of the diet. The degree of regulation to which the process is subjected once initiated, would provide an interesting study. The possible influence of queen substance could be evaluated by adding it to an artificially modified worker jelly.

For the ultimate factors in the causal sequence leading to female dimorphism one must look beyond the physiology of the developing larvae to the physiology and behaviour of the nurse bees of the previous generation. What is the stimulus that causes nurse bees to elaborate worker jelly rather than royal jelly, and to add honey to the secretion supplied to the older worker larvae? If the distribution of nursing activities is essentially random and not correlated with the age of nurse bees or larvae, as Lindauer (1953) and Free (1960) have found, how is qualitative variation of the salivary secretion accomplished?

The evolutionary aspects of female dimorphism are also intriguing: Rhein (1933, 1950-51, 1956) regards the worker as the normal form, whereas Lukoschus (1956*a*) considers the queen to be the normal form. The functional deficiencies in the two forms are complementary, the worker lacking the ability to perpetuate the species, and the queen the ability to nourish the brood, although she possesses vestigial hypopharyngeal glands. It would therefore seem more reasonable to regard the two forms as end-products of two lines of divergence from the original form (Gontarski, 1941).

Finally, there is the relationship of caste differentiation in *Apis mellifera* to caste differentiation in other genera and species. In the honeybee a complex of events has been noted. In wasps and bumblebees, by contrast, a higher ratio of nurses to brood, resulting in more abundant feeding, appears to achieve a similar result (Light, 1943; Free and Butler, 1959).

Summary.

1. Histological, nutritional, and biochemical studies relating to female caste differentiation in *Apis mellifera*, L. are reviewed. Development of the worker larva evidently comprises two distinct phases, delimited by the addition of honey to the diet by nurse bees around the third or fourth day of the larval stadium. Comparisons between workers and queens may conveniently be related to these phases.

2. The dichotomy between castes appears to be initiated during the first phase and consummated in the second. There can be little doubt that nutrition is the major extrinsic factor in caste establishment. The identity of the dietary factor initiating the series of events culminating in female dimorphism has not been established. The results of many rearing experiments strongly suggest the involvement of a substance which is either volatile or unstable.

3. The mode of action of the dietary factor likewise is unknown. It is suggested that a difference in hormonal balance between castes is established in early larval life and is the intermediary factor linking nutrition to dimorphism. Caste differences in the endocrine system are known to exist by the fourth or fifth day of larval life; the earlier instars should now be examined for a relationship between nutritional and hormonal factors.

Zusammenfassung.

1. Histologische, ernährungsmässige und biochemische Studien in Verbindung mit weiblichen Kastenmerkmalen in *Apis mellifera* L., werden besprochen.

Die Entwicklung der Arbeiterinnen-Larve umfasst zwei sichtlich verschiedene Phasen, deren Abgrenzung durch Zugabe von Honig zur Nahrung durch Ammenbienen, ungefähr am 3. oder 4. Tag des Larvenstadiums erfolgt. Vergleiche zwischen Arbeiterinnen und Königinnen können zweckmässiger Weise mit diesen Phasen verbunden werden.

2. Die Unterteilung zwischen den Kasten beginnt augenscheinlich während der ersten Phase und wird in der zweiten vollendet. Zweifellos ist die Ernährung der Hauptfaktor in der Kastenbestimmung. Die Identität des Ernährungsfaktors, der diese Vorgänge, welche in der weiblichen Dimorphose enden, veranlasst, ist noch nicht bekannt. Die Ergebnisse

vieler Aufzuchtversuche weisen stark darauf hin, dass eine Substanz, flüchtiger oder instabiler Art, daran beteiligt ist.

3. Die Art und Weise des Ernährungsfaktors ist ebenfalls unbekannt. Es wird vermutet, dass eine Veränderung im hormonalen Gleichgewicht der Kasten im frühen Larvenstadium stattfindet. Dies ist der Zwischenfaktor, der die Ernährung mit der Dimorphose verbindet. Es ist bekannt, dass Kastenunterschiede im innersekretorischen System am 4. oder 5. Tage des Larvenstadium vorhanden sind. Die vorangegangenen Larvenstadien müssten nun untersucht werden, um festzustellen, ob eine Verbindung zwischen Ernährungs- und Hormonfaktoren besteht.

Résumé.

1. Une revue est faite des études histologiques, nutritionelles et biochimiques concernant la différention des castes de la femelle *Apis mellifera*, L. Évidemment le développement de la larve ouvrière comprend deux phases distinctes délimitées par l'addition du miel au régime par l'abeille nourricière durant la troisième ou quatrième journée du stade larvaire. Des comparaisons entre l'ouvrière et la reine peuvent aussi être faites en relation avec ces phases.

2. La dichotomie entre les castes semble commencer durant la première phase et se terminer durant la seconde. Il ne fait pas de doute que la nutrition est un facteur extrinsèque majeur dans l'établissement d'une caste. On n'a pas établi la nature du facteur diététique commençant la série d'événements dont l'aboutissement est le dimorphisme de la femelle. Le résultat de plusieurs expériences d'élevage suggère fortement l'implication d'une substance qui est soit volatile soit instable.

3. On ne connaît pas non plus le mode d'action du facteur diététique. Il a été suggéré qu'il existe une différence entre les castes dans la balance des hormones au début de la vie larvaire et que cette balance est le facteur intermédiaire reliant la nutrition au dimorphisme.

Il est reconnu qu'à la quatrième ou cinquième journée de la vie de la larve, il existe dans le système endocrine des différences de caste ; les stades plus jeunes devraient être examinés maintenant en vue de découvrir une relation entre les facteurs nutritifs et hormonaux.

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