and one observes that the vapor pressure is lowered correspondingly. If water is added, the matrix swells by the same pressure. The fact that water can be pressed out of a gel, and that the swelling pressure has been found to be equivalent to the lowering of its vapor pressure, suggests that the mechanism is the same as in the capillary system, i.e., that the hydrostatic pressure is equivalent but opposite (negative) to the swelling (matrix) pressure. When a solution is forced against a semipermeable membrane, only the solute molecules are held back by the barrier, and hence their osmotic pressure can be measured. However, when the barrier is simply the free surface of the solvent, the solute molecules must exert their force upon it, thereby causing the solvent pressure to drop 24 atm per mole, with an equivalent drop in the vapor pressure. Viewed in this manner, not only the activity but indeed the hydrostatic pressure of the solvent is the same in all compartments of an osmotic system at equilibrium. Seeing solute and matrix pressure as analogues gives, therefore, a uniform explanation for the water relations in capillary, colloidal, and solute systems. It explains quantitatively the osmotic pressure and the lowering of the vapor pressure, and does away with the contradictory hydrostatic solvent gradients in the membrane at equilibrium.

* This investigation was supported by U.S. Public Health Service grant no. GM-10521, from U.S. Department of Health, Education and Welfare.

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*THE FINE STRUCTURE OF THE HYPOPHARYNGEAL GLAND CELL OF THE HONEY BEE DURING DEVELOPMENT AND SECRETION**

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Communicated April 11, 1966

The hypopharyngeal glands, located in the head of the honey bee, synthesize and secrete for a short period of the adult worker's life the so-called royal jelly. The cells of these glands begin to differentiate in very young pupae and are still quite undeveloped at the time the imago emerges. The young worker, after feeding very heavily on beebread (stored pollen) and honey, begins to secrete the royal jelly

about 5 or 6 days after emergence and continues through about the eleventh day of hive life. Then the worker ceases to act as a nursemaid to larvae, drones, and queen, and becomes a waxworker and acts as a guard for the hive. During this period, it eats less and less beebread and royal jelly, and secretory activity wanes. When the worker is about 3 weeks old, it becomes a forager and eats almost nothing but honey. Under normal summer temperatures and hive conditions, a worker lives for about 36 days.

Numerous analyses have been made of the content of the royal jelly, and in a recent paper by Rembold (1964),[1] it is reported that in addition to a number of different kinds of proteins there are both lipids and eight vitamins. Since the royal jelly along with honey constitutes the food of queen bees both in development and in reproduction, the former must contain all the materials needed for growth and the production of eggs, which in very favorable conditions may approach in weight that of the queen in 24 hr. Many years ago one of us[2] described the development of the hypopharyngeal glands and morphological changes cell organelles undergo in preparation for secretion, as these are seen with a light microscope.

Our study of the fine structure of the royal jelly gland cells begins with pupae about a week before they would have emerged as adults and ends with details of structure of quiescent gland cells of old bees. A number of facts of general interest have come to light. In very young pupae the outer membrane of the nuclear envelope is thrown up into amoeboidlike processes, or blebs. In a single thin section of the nucleus we have counted a dozen or more blebs, some very elaborate in their ramifications, so the number present at this period in a nucleus may number hundreds. These blebs are cut off and form the anlage of the endoplasmic tubules to which fully formed polysomes eventually become attached.

Most of the polysomes seen in secreting cells are derived from the cyclical breakdown of numerous nucleoli. As gland cells differentiate, they undergo endomitosis. In interphase nuclei intact nucleoli appear, but as the endomitotic cycle approaches a prophase condition, these nucleoli fragment, releasing into the nuclear sap both single ribosomes and large quantities of nuclear-synthesized proteins. Eventually, both derivatives pass into the cytoplasm through the nuclear pores. As ribosomes enter the nuclear pores they become organized into polysomes and emerge into the cytoplasm as such. They lie free in the cytoplasm until the ER tubules can give them a lodging space in their outer walls.

In the late prophase of the endomitotic cycle, one finds in the nuclear sap bundles of chromonemata, much like those described for the nurse cells of the *Drosophila* ovary.[3] A new crop of nucleoli appears in the next interphase period and the whole process is repeated over again.

Material and Methods.—Living honey bees and various stages in larval development were taken directly from the hive. Under these conditions it is not possible to determine exactly just how old a given individual is, but our experience indicates that there is a good deal of variation; for example, in the extent to which the endoplasmic reticular tubules have developed in bees taken as they were cutting their way out of the comb.

The hypopharyngeal glands were dissected from severed heads of worker bees in Ringer's insect solution and fixed either in 1 per cent osmic acid (buffered for various

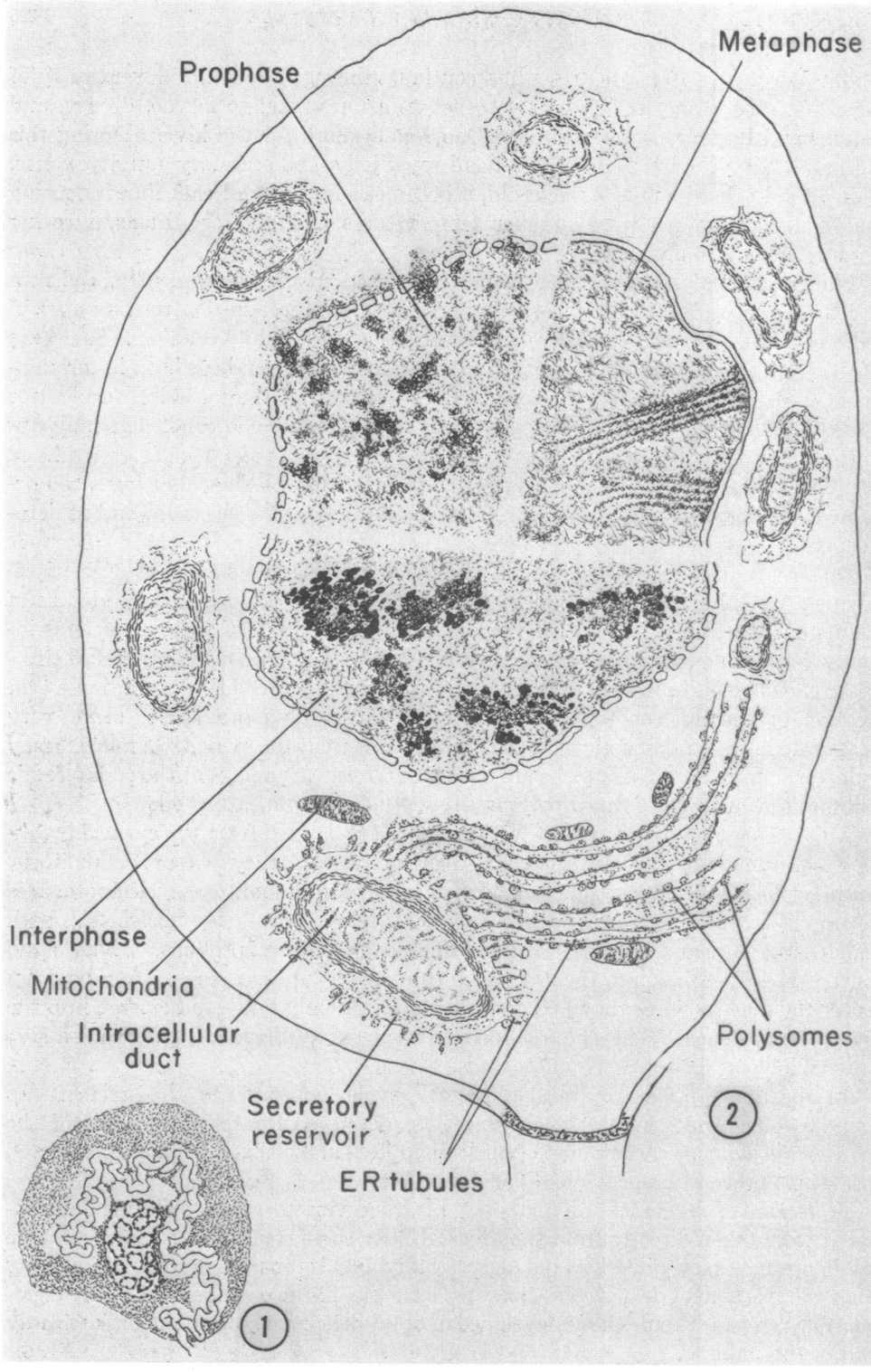

FIG. 1.—Gross morphological structures seen in a light microscope, including the intracellular duct and the secretion reservoirs in the cytoplasm and nucleoli within the nucleus.

FIG. 2.—A semidiagrammatic representation of the organelles seen at electron magnifications of an actively secreting gland cell. Three phases of the endomitotic division cycle are represented for nuclei.

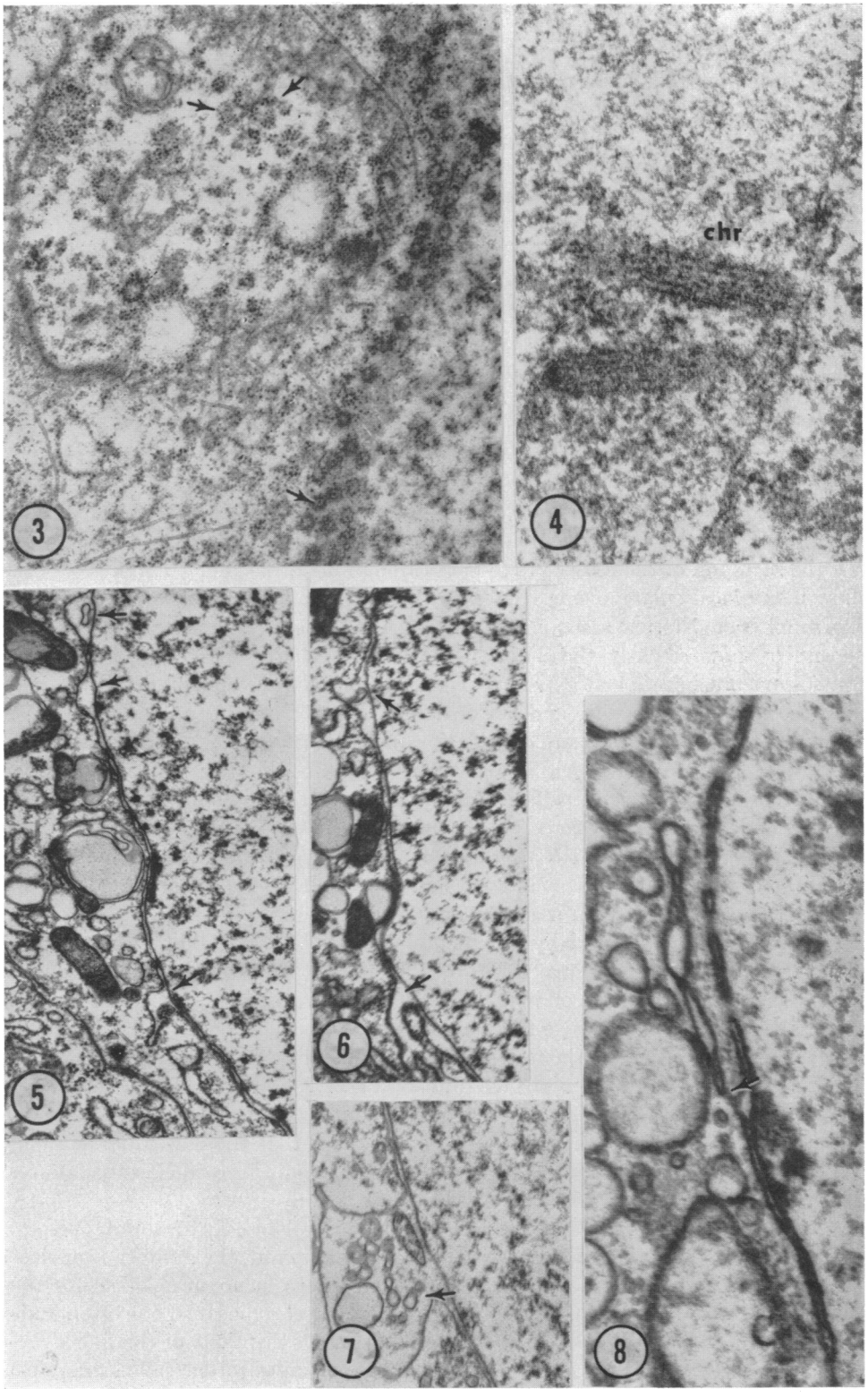

FIG. 3.—Nuclear pores with polysomes; osmic acid followed by strontium permanganate. ×29,000. Fig. 4 shows chromosome bundles; osmic acid and permanganate. ×52,600. Figs. 5–8 show nuclear blebs—see arrows; glutaraldehyde fixation. Figs. 5–7, ×22,500. Fig. 8, ×69,000.

pH's), or in glutaraldehyde. After initial fixation, the tissue was treated in various ways, as indicated in the legends of the figures. We have found that ribosomes and polysomes show most clearly in osmium-fixed material at either pH 7.2 or pH 6.0. Chromosomes show most clearly when the fixative is buffered for pH 6.0. Glutaraldehyde shows the two membranes of the nuclear envelope and the nuclear pores best, perhaps, as well as feltlike protein passing through the pores, but ribosomes are often only faintly visible. Thus, the two fixatives complement each other. Electron photographs were made with a Siemens-Elmiskop I.

Results.—First, Figure 1 shows the structures seen in an actively secreting gland cell in a light microscope; and second, Figure 2, diagrammatically, shows the cell organelles seen in electron photographs and their interrelations. The voluminous cytoplasm is crowded with more or less laminated endoplasmic tubules as found in exocrine pancreas cells, many of which are interconnected, and all of which open into secretion reservoirs through which the intracellular duct passes in a tortuous course about the nucleus. This duct is always conspicuous in thin section because of the longitudinal staves, or lamellae, which are quite dense to electrons. It is assumed that these lamellae act as supports to keep the duct open and yet allow the secretion of the reservoirs to pass through unobstructed. The ER tubules show many local enlargements (cisternae) in which there is a fibrous precipitate. The number of cisternae seen and the prominence of the secretion reservoirs seem to depend on how recently the individual concerned has discharged its secretions. The fibrous precipitate first appears in cisternae during development of the ER system, then in the reservoirs, and last in the lumen of the intracellular duct. We assume that the fibers seen are coagulation products of the protein compounds in the royal jelly. Mitochondria lie everywhere scattered between the ER tubules, but we have not as yet identified Golgi apparatus, although many dictyosomes are seen in developmental stages.

In actively secreting cells the appearance of nuclei is quite variable, depending on the phase of the endomitotic cycle. In Figure 2, we have shown three characteristic conditions. The bottom sector corresponds to the interphase stage of normal mitosis and is characterized by large numbers of nucleoli. Ordinarily, chromosome filaments cannot be seen at this time. To the left, an early prophase condition is represented with the nucleoli in fragments and the nuclear pores very prominent. This has proved the best stage for the study of the nuclear pores and the organization of polysomes within them. To the right in Figure 2, there is a stage corresponding to a late prophase in normal mitosis (Fig. 4). No intact nucleoli are seen in such stages and, at most, a few scattered nucleolar fragments may be present. The chromosomes appear in bundles and one can see that the individual chromonemata consist of small granules attached to axes which lie in parallel array. Pores in the nuclear wall are not prominent at this time.

Nucleoli, ribosomes, and polysomes: In presumed diploid cells of worker bees, up to eight nucleoli may be seen. As endomitosis goes forward, the number of nucleolar organizers is doubled in each cycle, and since there is no mechanism for the separation of the chromonemata derived from a single chromosome, one often finds clusters of nucleoli. In common with all the recent descriptions of the fine structure of nucleoli,[4–6] we find a nucleolus made up of units (often called granules) about 150 Å in diameter. Some of these units contain ribosomes lying in a felt-

like mass of protein, which is removed on pepsin digestion.[4] When nucleoli begin to fragment in the early prophase of a cycle, one or a small group of units breaks away from the main mass and begins to pass toward the nuclear envelope. During this process, the ribosomes usually (always?) separate from the protein and enter the nuclear pores, where they are organized into polysomes (Fig. 3). They remain free in the cytoplasm until the endoplasmic ducts are ready to receive them attached to the outer walls of the ducts. Endomitosis continues until late in the secretory activity, and its termination seems correlated with a change in the diet of the worker.

Origin of the endoplasmic tubules: It was not until extremely young pupae were studied that evidence was found of the way in which ER tubules are formed. Figures 5–8 illustrate this process (see arrows). In pupae which would not have emerged as adults for perhaps a week, the outer membrane of the nuclear envelope shows hundreds of outpocketings, or amoeboid processes, some of which are very elaborate. Since one finds surrounding the envelope many rudiments of the ER tubules, this process must have set in much earlier. These evaginations of the outer membrane are cut off but the actual attachment of polysomes to the outer wall of the ER tubules frequently does not occur until the adult worker is several days old.

The gland cells in old forager workers show no nucleoli, the secretory reservoirs have disappeared and the ER tubules, while still intact, show no evidence of secretion products.

The authors are indebted to Dr. William R. Lee, of our Department, for the bee material and to Mr. Robert W. Riess for electron microscope assistance.

In a paper now in press, the authors show the many electron photographs upon which our conclusions given above are based, and discuss in detail nucleolar structure, the behavior of the chromosomes and their structure, and a number of questions raised by our findings.

* This investigation was supported in part by U.S. Public Health Service research grant HD-01016 from the National Institute for Child Health and Human Development and by U.S. Public Health Service Research Career Award 5-K6-CA-18,366 from the National Cancer Institute.

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