

THE ORIGIN OF THE NUCLEIC ACID BASES FOUND IN THE ROYAL JELLY OF THE HONEYBEE

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Abstract.—The discovery that the royal jelly of the honeybee contains large quantities of nucleic acids raises the question, "Where do these nucleic acids come from?" A review of pertinent literature strongly suggests that the same mechanism is involved as in the case of the extrachromosomal DNA of amphibian oocytes.

From cytological studies of the several mechanisms of providing ova with the foodstuffs needed for the rapid development of embryos¹⁻³—proteins, nucleic acid bases, and many other substances, the writer has, for many years, been convinced that the royal jelly of the honeybee must contain similar substances. The basis for this conclusion rests on the fact that in the summertime the queen bee, which feeds on royal jelly and honey day after day, lays up to an estimated 1200 eggs per day, and it seems unlikely that there is time enough for the cells of the ovary to synthesize *de novo* the complex substances needed for the development of an embryo. It follows that the royal jelly must contain the proteins, the purines and pyrimidines, and many other preformed substances. Royal jelly has long been known to contain some 15 per cent of protein (wet weight), and an analysis made in 1964 by Rembold⁴ showed a great variety of other substances, but no mention was made of the nucleic acid bases. The ovary of the queen bee, like that of many other insects, consists of a great number of ovarioles each of which contains an egg string in all stages of oocyte formation. A follicular membrane encloses nurse cells and the definitive oocyte.

The first evidence, so far as I am aware, that the royal jelly contains nucleic acid bases was given by Marko and Pechan.⁵ Using lyophilized royal jelly treated with cold perchloric acid, these authors were able to isolate, by paper chromatography, four nucleic acid bases. The amounts of these bases are astonishingly large. In 1 gm of lyophilized jelly they found 47.0 mg of RNA associated with phosphorus and an equal amount of phosphorus-associated DNA. Two of my associates, Drs. C. D. Laird and J. M. Lagowski, have recently repeated such an analysis, using an initial protease treatment, and they isolated the four bases reported by Marko and Pechan.

The question arises, "What is the source of the nucleic acid bases found in royal jelly?" In this communication, this question will be discussed.

In a recent symposium⁶ I pointed out that there is a very interesting parallel between the functions of nurse cells in oogenesis, the behavior of the germinal vesicle in amphibian oocytes, and the functions of the royal jelly in the honeybee. While the mechanisms involved are very different, the end result is the same, that is, to provide the mature egg with enough materials—proteins, nucleic bases, and many other things—so that the fertilized egg may develop rapidly into a normal embryo.

In the enrichment of the oocyte cytoplasm the high polyploid nurse cells, which are directly connected by intracellular ducts play an important role. The contents of the nurse cells are directly or indirectly absorbed into the oocyte and are the source of the needed materials, including myriads of polyribosomes,⁷ proteins, and the degradation products of many thousands of chromosomes.

In the amphibian germinal vesicle, an amplification of the DNA of the genes complementary in part to rRNA⁸ occurs in secondary oogonia, or possibly earlier, and such DNA appears in the leptotene stage as extrachromosomal DNA granules (frogs)² or rings (urodeles).⁹ At pachytene small nucleoli develop in association with one or more granules or rings. Such nucleoli are extremely rich in RNA, due, as Kemp¹⁰ showed in electron microscope photographs, to ribosome precursors. Later these small nucleoli, which lie just under the nuclear envelope, fade from view along with their DNA and RNA. As this occurs, a halo of RNA appears in the cytoplasm; it is densest near the nuclear membrane and, as electron photographs show, is made up of ribosome precursors. It may be concluded that with the breakdown of the nucleoli, the contained ribosome components pass through the pores of the nuclear envelope, just as has been observed in electron photographs of developing royal jelly gland cells.⁷ What happens to the DNA granules must be inferred, as no one has seen their passage through the nuclear pores, but electron photographs have recently shown¹¹ that if the cytoplasm in *Xenopus* (a toad) is first treated with protease, there appear in the cytoplasm two types of DNA molecules. One is ring-shaped and is derived from mitochondrial DNA and the other type, ten times as numerous, consists of long double-helix molecules. It appears then that DNA is present in the cytoplasm but is complexed with protein.

In the case of the honeybee, ready-synthesized materials needed for the development of the queen and later for the support of her egg-laying activity are furnished by the royal jelly, which is secreted for a few days by young worker bees. In the development of royal jelly gland cells the first step is the absorption of a polyploid nurse cell¹² followed by a series of endomitotic division cycles. During each cycle at a period which corresponds to the prophase of normal mitosis, the numerous nucleoli disintegrate, releasing into the nuclear sap a myriad of ribosome precursors as well as other proteins which pass into the cytoplasm via the nuclear pores.

If one assumes that the rDNA associated with nucleolar formation in royal jelly gland cells behaves as the rDNA granules, or rings, in the amphibian egg, then such DNA, probably complexed with proteins, would pass out of the gland cell along with the other substances found in the royal jelly. This seems to be a reasonable way to explain the presence of nucleic acid bases in the royal jelly.

If the above proposed explanation is correct, it may be expected that other gland cells which secrete copious quantities of protein would also show the presence of nucleic acid bases, and there is some evidence that this is true. It has been known that avidin—a biotin-protein complex—which may also be associated with quantities of nucleic acid occurs in the white of the hen's egg. There are also a number of papers reporting the presence of nucleic acid bases in both human and cow's milk.^{13, 14} But in the latter cases it has been shown that cow's

milk contains appreciable numbers of polymorphonuclear leukocytes, which might also contribute nucleic acid bases.¹⁵

From a general biological standpoint it is interesting to note that while the typical nurse cell or germinal vesicle mechanism for supplying the cytoplasm of ova with essential precursor materials is a function of the ovary of the female involved, the delegation of this function in the honeybee to a separate caste of sterile females (worker bees) makes the formation of large colonies possible. It makes the queen bee an egg-laying machine in that the queen takes ready-synthesized products and deposits these in the egg cytoplasm.

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