


Beginning in the 1950s, researchers started to investigate interactions between different tissues within embryos to understand how cells and tissues differentiate. John M. Cairns and John W. Saunders [13] worked with chick [14] embryos, respectively at the University of Texas in Austin, Texas, and Marquette University [15] in Milwaukee, Wisconsin. In 1954 they presented evidence that mesoderm [16] stimulates interactions between mesoderm [16] and epithelium [12], and that it contributes to the structural specificity of the structures that developed from those interactions. Other researchers conducted studies that led to similar conclusions about the properties of mesoderm [16]. However, mesoderm [16] forms several different types of tissues, including mesenchyme [7], and it contributes to many embryonic characters, including some epithelial structures. Thus, scientists worked to narrow the signaling for tissue differentiation [10] to a more specific source.

By the 1960s, researchers who studied tissue interactions highlighted the importance of both the epithelium [12] and the mesenchyme [7], but they could not clarify the roles of the respective tissues or discern their inductive capacities. In 1963, Mary E. Rawles, then at the Carnegie Institute of Washington in Baltimore Maryland, published "Tissue Interactions in Scale and Feather Development as Studied in Dermal-epidermal Recombinations," in which she studied the development of feathers in bird embryos. Her work influenced many biologists to study how structures like scales and feathers differentiated from embryonic tissues. Despite recognizing the importance of epithelium [12] and mesenchyme [7] for development, scientists still lacked insight into the nature of their interactions; especially how epithelium [12] and mesenchyme [7] worked during differentiation [10] and how they conferred structural specificity.

to see how epithelio-mesenchymal interactions produce differentiated tissues, Kollar and Baird designed an experiment in 1969 to test the tissues' induction [11] potentials in vitro [7]. They wanted to understand the influence of the dental papilla, a mesenchymal embryonic structure seen as teeth develop, on the emergence of tooth shapes in the developing mouse [9] tooth germ. Kollar and Baird isolated incisor and molar tooth germs from the mandibles of embryonic mice and soaked them in a solution to break the protein bonds between the mesenchyme [7] and epithelium [12], a process called trypsinization. Once the mesenchyme [7] from the dental papilla and the epithelium [12] separated, Kollar and Baird joined the tissues into four sets of recombinants that, following Rawles' results in 1963, tested induction [11] potentials between different developmental stages [18] and between tissues derived from molars and incisors. The recombinants were then moved to Petri dishes and allowed to develop for several days, after which Kollar and Baird sectioned the recombinant structures to study their cells and tissues. The structures that developed in vitro [7] always took the form of the teeth from which the mesenchyme [7] derived—even when the two tissues were from different developmental stages [18]. The results of these experiments showed that the mesenchyme [7] directs tissue differentiation [10] and structural specificity.

Kollar and Baird's 1969 results helped establish the role of the mesenchyme [7] in tissue differentiation [10] and structural specificity. However, their evidence didn't resolve all of the questions about epithelio-mesenchymal induction [11]. Allowing the tissues to grow in vitro [7] constrained the amount of time that they could develop, and consequently, the structures which formed were not completely like molars or incisors. Questions remained about the range over which mesenchyme [7] taken from the mouth could induce specified and differentiated structures in epithelium [12] from other parts of the embryo.

Following their 1969 experiments, Kollar and Baird set out to accomplish two things: first, to revise their methodology to allow a longer developmental window; and second, to understand the inductive powers of mesenchyme [7]. In pursuit of the first goal, the two used experimental techniques developed out of research testing the effects of environmental changes on growth, which transplanted tooth germs into new sites in the same and different animals. In 1952, Harold S. Fleming, from the Department of
Pathology at Yale University [19] in New Haven, Connecticut, published "Homologous and Heterologous Intraocular Growth of Transplanted Tooth Germs" in which he detailed the transplant of tooth germs from mouse [9], guinea pig [9], cat, and rabbit [9] embryos or fetuses into the novel environment of the anterior chamber of the eyes of anesthetized mice, rabbits, and guinea pigs. These intraocular environments had several advantages over in vitro [7] methods for the growth and development of tooth germs. First, the nutritional level achieved through intraocular growth far surpassed what any culture medium [22] could provide. Second, the setting allowed direct observation of advanced stages of growth. And third, the aqueous environment produced less strain on the developing structure. Kollar and Baird recognized these advantages and adapted their next series of experiments to this new technique.

Kollar and Baird wanted to clarify the extent of oral mesenchyme [7]’s ability to induce tooth formation and to specify the structure formed by the epithelium [12]. In one set of experiments published in 1970, Kollar and Baird examined whether oral mesenchyme [7] could induce tooth formation in any part of the dental epithelium [12], regardless of its distance from the mesenchymal source. They first tested the induction [11] potential of mesenchyme [7] from the dental papilla isolated from molars and incisors of embryonic mouse [9] mandibles on sections of epithelium [7] derived from a structure near the papilla, known as the enamel organ. They sectioned the epithelium [12] taken from the enamel organ into three parts corresponding to increasing distance from the dental papilla in vivo [23].

Similar to the 1969 experiments, Kollar and Baird excised tooth germs and subjected them to trypsinization. Once the mesenchyme [7] and epithelium [12] developed in the culture medium [22] for two days, to let the components cohere, Kollar and Baird transplanted the recombinants into the anterior chamber of a mouse [9]’s eye. The technique worked. The resulting structures resembled normal teeth in all respects, including the presence of advanced tissues like enamel. The results confirmed the 1969 results and showed that the oral mesenchyme [7] induced teeth to form and specified the developing structure regardless of the epithelial section.

Following these results, Kollar and Baird used the same methods, but they substituted epithelium [12] harvested from the lip-furrow of mice in place of the enamel organ epithelium [13]. Lip-furrow epithelium [12] arises with dental epithelium [13], but it becomes surface epithelium [12] in the adult animal. Thus, lip-furrow epithelium [12] represents a border area between dental and non-dental epithelium [13]. This second part of their experiments again confirmed that oral mesenchyme [7] both induces tooth formation and controls structural specificity.

The results of the second set of experiments that Kollar and Baird published in 1970 extended the range of the inductive powers of oral mesenchyme [7] beyond dental epithelium [12]. Instead of associating the mesenchyme [7] from the dental papilla with dental epithelium [12], Kollar and Baird used epithelial sections taken from increasing distances from the mouth. The two used the same methodology established in the first 1970 paper to create recombinants of oral mesenchyme [7] with snout and foot pad epithelium [13]. Once again, they allowed the recombinants to cohere in culture for two days and then transplanted them into the anterior chamber of a mouse [9]’s eye. In each case, the oral mesenchyme [7] proved capable of inducing the epithelium [12] to form teeth specific to the tooth germ from which the mesenchyme [7] was harvested.

Sources

effects.

**Subject**
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- Embryos
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**Topic**
- Experiments

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- The Embryo Project at Arizona State University, 1711 South Rural Road, Tempe Arizona 85287, United States


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