

histocompatibility antigens on cell surface is extremely variable within a rapidly dividing cell population^{19,20} as the PHA-induced blast population is at the peak of the response.

Note added in proof. Since this manuscript was submitted for publication, J. J. T. QWEN, P. HUNTER and M. C. RAFF (Transplantation 12, 231 (1971)) have reported

¹⁹ B. BJARING, G. KLEIN and I. POPP, Transplantation 8, 38 (1969).

²⁰ M. CIKES, Nature 225, 645 (1970).

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that the \emptyset -antigen is present in most blast cells resulting from stimulation with PHA in diffusion chambers in vivo.

Zusammenfassung. Lymphozyten, die in vitro auf das Phytohämagglutinin reagieren, tragen den \emptyset -isoantigenen Markierer auf ihrer Oberfläche. Die Abkömmlinge dieser Zellen sind zum allergrössten Teil ebenfalls \emptyset -positiv.

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'Halo Formation' as a Characteristic of the Interaction Between Ragweed Pollen and Saliva

For the primary purpose of discerning differences in the handling or treatment of ragweed pollen between normal and allergic individuals, I initiated a study of the interaction between ragweed pollen and body constituents (fluids or tissues).

Initially, I chose to use saliva from both allergic and nonallergic individuals in our investigations because saliva is the only secretory fluid that could be interacting with airborne pollen and that could be obtained in large quantity without stimulation.

Saliva was collected without stimulation in a beaker that was immersed in ice and water, centrifuged cold, and stored at 0°C, but not frozen. The saliva donors consisted of 6 individuals who were allergic to the pollen and of 21 individuals who were not allergic to the pollen. No donor was showing allergic manifestations at the time of the saliva collections. Evidence of allergy consisted of clinical history of ragweed pollinosis and history of a positive skin test.

The ragweed pollen (*Ambrosia trifida*) was collected from cut plants by allowing it to fall onto aluminium foil.

The pollen was left untreated and was stored in a vacuum desiccator to keep it dry and to avoid air oxidation.

In order to study any possible interactions between the pollen and the saliva, a drop of saliva was mixed with about 20 μ g of fresh, mature ragweed pollen (about 800–1000 grains) on a glass slide; a coverslip was added and sealed on with Permout®. The slides were then incubated at room temperature for 3–14 days. These incubations were prepared with the 6 allergic and the 21 nonallergic saliva samples. As control media, we used distilled water, saline, and isotonic potassium chloride solution in place of the saliva. The incubation slides were examined intermittently under the microscope.

There were visible events common to all media. However, some of the pollen grains in the allergic saliva demonstrated a change that was manifested in the form of a halo of white, mat-like appearance around the pollen grain (Figure). Halos have been seen to grow in diameter as though emanating from the grain. If the slide is not disturbed, the halo will remain; if currents are developed, it washes away. The earliest appearance of halo formation has been noted at 18 h, but usually halos develop at about 24 h after the onset of incubation.

In 57 cultures of pollen and nonallergic saliva from 21 nonallergic individuals, only one culture showed any indication of the presence of halos; whereas halos were seen in 24 out of 29 cultures of pollen and allergic saliva from the 6 allergic individuals. Of the 5 negative results, 2 were obtained with the saliva of an individual who was on medication at the time of saliva collection. Usually, if one halo is seen on a slide, more can be found by careful examination of the slide. The incidence of halos on a slide has varied from several up to 30%.

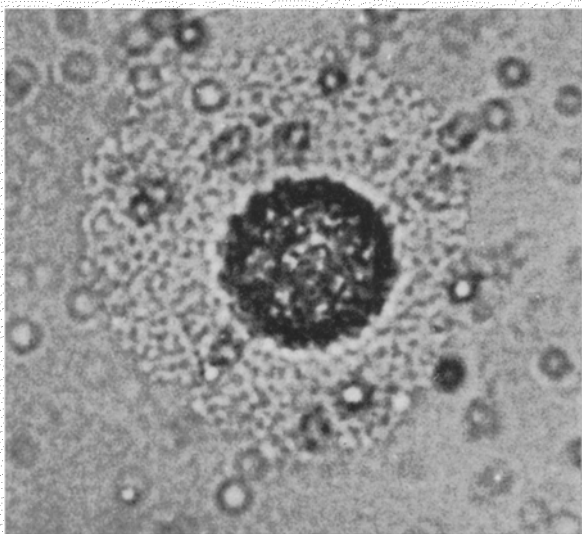
Zusammenfassung. Wenn Pollen von *Ambrosia trifida* mit dem Speichel von mit Allergie belasteten Personen zusammen kultiviert wird, so entsteht um die Pollenkörner ein Hof («halo»), was in Kulturen von nicht allergischen Personen nicht der Fall ist.

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¹ L. W. MAYRON and R. J. LOISELLE, Clin. Sci. 42, 1 (1972).

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Halo formation on a ragweed pollen grain that had been incubated in the saliva of an allergic individual. $\times 1300$.