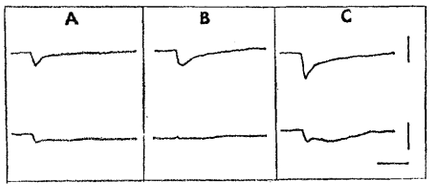

Fig 2. Successive responses of the same glomerulus to three odorous stimuli: citral (*A*), beta-ionone (*B*), and *n*-butanol (*C*). Upper tracing, EOG; Lower, glomerular response. Time bar, 1 second. Vertical lines: (both with positive in the upward direction), (upper) 5 mv, (lower) 0.5 mv.

ume of a glomerulus, and it was lowered perpendicular to a monolayer of glomeruli; accordingly the electrical response (if any) recorded under these conditions was taken as the response of a single glomerulus. The electrodes and the head of the animal were held in a stereotaxic apparatus. Records were displayed on a two-channel d-c pen recorder having high sensitivity and high input impedance.

The olfactory mucosa was stimulated by a puff of odorized air blown into one nostril through a glass canula; the stimulus lasted about 0.2 second. The volume of the puff and the concentration of odorous material were adjusted so that the EOG was infra-maximal. Nine different odorous stimuli were arbitrarily selected (Fig. 1) and applied successively while the bipolar microelectrode remained in each glomerulus tested.

The responses of 47 glomeruli in 23 young rabbits were finally selected. In such experimental conditions the glomerular response appeared to be a slow monophasic potential, a few tenths of 1 mv in amplitude and lasting for a few seconds. In shape this potential was strongly reminiscent of an EOG; more-precise analysis showed, however, that the shapes of these two potentials varied quite independently in response to different odorous stimuli. The EOG preceded the glomerular potential by about 40 to 50 msec (*6*).

Moreover, successive responses of the same glomerulus to many different stimuli often varied in an all-or-none way: a given glomerulus remained completely silent with some odorous stimuli while giving full response to others (Fig. 2). Conversely, other glomeruli responded equally to all nine stimuli tested.

A glomerulus remaining silent during stimulation raised the question of whether olfactory stimulation was effective or not in the mucosal field of this glomerulus. The presence of an EOG, the constancy of aerodynamic conditions, and the possibility of re-elicitation of a glomerular response by turning back to an effective stimulus led us to assume that we were recording the same healthy glomerulus throughout (Fig. 2). During this preliminary investigation every degree of selectivity was encountered: 12 of 47 glomeruli responded to all nine stimuli. Among the remainder, selectivity varied from 1/9 to 8/9.

The experimental data are detailed in Fig. 1. One may note that of 35 selective glomeruli only six could be paired according to identical response spectra. One could consider that under such experimental conditions a given glomerulus had two out of three chances of responding to any one of the nine stimuli. This figure should be compared with Gesteland's results (*7*) with single olfactory receptors of the frog: under his experimental conditions a given receptor had two out of five chances of responding to any one of 26 stimuli.

Such different selectivity might be explained by stronger stimulation in our experiments than in Gesteland's. In fact we have found that glomerular selectivity increases slightly when the stimulus concentration is decreased near the EOG threshold. Our data appear to support the hypothesis that the 100-million-point olfactory pattern in the mucosa is converted to a homologous pattern of only 2000 points at the glomerular level, which is the real base for central integration.

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11 April 1966

Radioautographic and Electron-Microscopic Evidence of Rapid Uptake of Antigen by Lymphocytes

Abstract. *Iodine-125–labeled ferritin molecules were detected by radioautography in the sinuses of the rat popliteal lymph node shortly after injection into the foot pad; they appeared to be taken up by macrophages and phagocytic reticular cells. Electron microscopic examination of the same tissue also revealed ferritin molecules within small lymphocytes as early as 5 minutes after injection. The antigen appeared to be taken up by the process of pinocytosis and was distributed throughout the cytoplasm and nucleus. While the number of ferritin molecules observed in the lymphocyte was much less than that taken into the macrophage, the observation is significant in understanding the role lymphocytes play during the early phase of antibody response.*

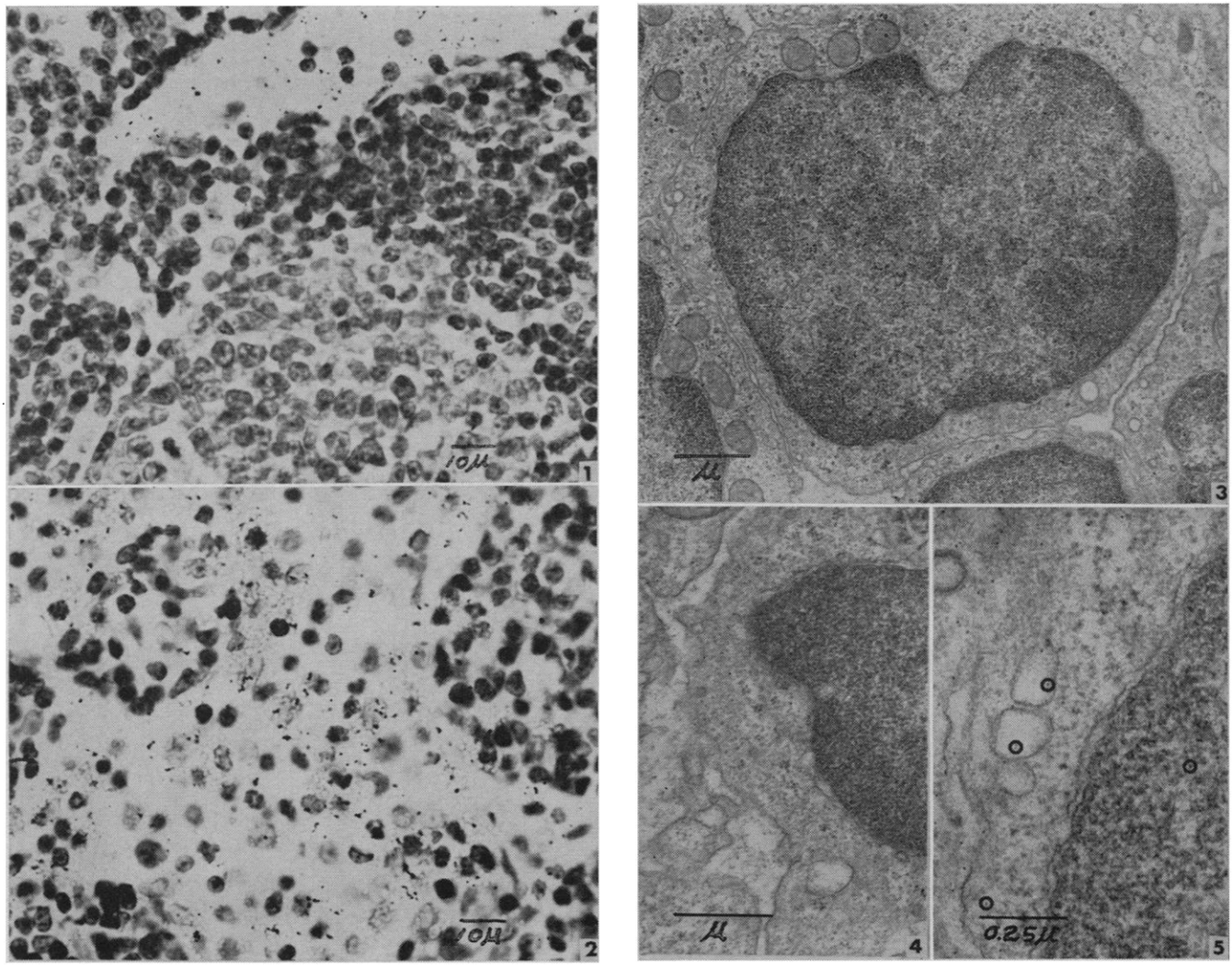
The target cell for antigen in initiation of the process leading to antibody formation is unknown. Various studies have implicated the macrophage (*1*) and the lymphocyte (*2, 3*). The role of the macrophage has been suggested as that of phagocytizing the antigen, processing it, and perhaps transferring it to other cells as a prerequisite to antibody synthesis. On the other hand, depletion of thoracic duct lymphocytes, by chronic drainage, negates the capacity of rats to respond with antibody after the primary injection of antigen (*2*). In our efforts to elucidate the role of the lymphocyte in initiating antibody synthesis, evidence was obtained from autoradiographic and electron-microscopic observations for the presence of antigen in lymphocytes of the popliteal lymph nodes of the rat within minutes after injection of I^{125}-labeled ferritin.

Cadmium-free horse ferritin (*4*) was labeled with I^{125} by a procedure similar to that used for labeling bovine γ-globulin (*5*). The I^{125}-ferritin complex (20 mg/0.2 ml of saline) was injected into the foot pad of Sprague-Dawley rats. Popliteal lymph nodes were dissected and fixed in 2 percent glutaraldehyde in phosphate buffer at 5, 15, and 30 minutes; 1 hour and 5 hours; and 1, 3, 5, 6, 7, 8, 10, 13, 16, 19, and 22 days after the injection. Tissues were embedded in paraffin, sec-

tioned at 6 μ, coated with Kodak NTB-3, and kept in a tightly sealed slide box containing Drierite at 4°C for 2 to 6 weeks. The slides were then developed and stained with hematoxylin and eosin. For electron microscopy small pieces of tissues fixed in glutaraldehyde were later fixed in 2 percent buffered OsO_4 and embedded in Epon. Ultrathin sections were cut on a LKB microtome, stained with saturated uranyl nitrate and 0.1 percent phosphotungstic acid, and observed in a Hitachi HU-11 electron microscope. Serums from these animals were collected, and the antibody titers were determined by passive hemagglutination.

At 5 minutes after injection the radioautographic grains were mainly localized within subcapsular, perinodular, and medullary sinuses of the popliteal node. The grains were primarily located in the spaces of these sinuses, and only a small number of them were superimposed on the reticular cells and macrophages (Fig. 1). One hour after injection, the sinuses were cleared of most of the free granules, and there was an increased concentration of granules within the cytoplasm of macrophages (Fig. 2). The number of macrophages containing antigen decreased with time, but antigen persisted in some throughout the 22-day experiment period. Occasionally only a few grains were localized in the lymphoid nodules and medullary cords. Thus, the results from the radioautographic observations seemed to support the contention that the primary cells concerned with antigen uptake are the macrophages and phagocytic reticular cells of sinusoids and that little antigen reaches the lymphocytic population.

Contrary to the results of autoradiographic observations, those of electron-microscopic studies show that 5 minutes after injection a relatively large number of ferritin granules are located in the cytoplasm of small lymphocytes throughout the medullary cord and cortical nodules. They are contained in small peripheral vacuoles and vesicles (Figs. 3 to 5) which are generally recognized as being responsible for pinocytosis. Some of the ferritin molecules are located in the nucleus as well



Figs. 1 and 2 (left). Radioautographs of a portion of a rat popliteal lymph node 5 minutes (Fig. 1) and 5 hours (Fig. 2) after injection of I^{125}-labeled ferritin. Five minutes after injection silver grains are primarily localized in the sinus and few are superimposed on lymphoid cells of the nodule, whereas after 5 hours (Fig. 2) most grains are found in relation to macrophages. Figs. 3 to 5 (right). Electron micrographs of lymphocytes from a rat popliteal lymph node 5 minutes after injection of I^{125}-ferritin. The low magnification picture (Fig. 3) identifies the small lymphocyte which contains a number of peripheral vesicles in which the electron-opaque ferritin molecules can barely be seen. At higher magnifications (Figs. 4 and 5) the marker molecules are seen both in the pinocytic vesicles as well as in the ground cytoplasm and nucleus (circles, Fig. 5).

as throughout the cytoplasm (Fig. 5). The ferritin molecules are engulfed by macrophages as evidenced by a large number of vesicles containing numerous granules along the surface of the cells. While the relative number of ferritin molecules taken up by the macrophages is far greater than that observed in the pinocytic vesicles of the lymphocytes, a single lymphocyte contains as many as several dozen molecules at a given plane of section, estimated to be 500 Å in thickness.

The use of ferritin molecules in tracing the fate of antigen by electron microscopy must be interpreted with caution since many, if not all, of the macrophages contain a large number of ferritin molecules within their cytoplasm. However, under the conditions of this study no such molecules were observed in lymphocytes from normal rats. This finding supports the contention that the molecules observed in the lymphocyte minutes after injection were antigenic ferritin. The meaning of this observation in terms of the role of the lymphocyte in antibody synthesis remains to be elucidated. Nevertheless, it is significant that antigenic ferritin molecules are taken up within a very short time after injection by small lymphocytes which are to be mobilized shortly afterwards (*6*). These small lymphocytes can be transformed into large blast cells by antigen (*7*). Whether or not the uptake of ferritin by small lymphocytes represents specific recognition of ferritin molecules as antigen awaits a comparative study of the fates of isologous and heterologous ferritin molecules.

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RNA Composition and Base Pairing

Abstract. *If RNA may contain a small proportion of adenine-guanine base pairs, these could interrupt the continuity of helical structure in a polynucleotide, in keeping with current theories of RNA structure, and could also account for the experimentally observed tendency for 6-amino bases to equal 6-keto bases and for purines to exceed pyrimidines.*

The striking regularities in the nucleotide composition of DNA (*1*) stem directly from the structure of the molecule, in which A (*2*) is paired with T and G with C (*3*). The regularities—A = T, G = C, A + G = C + T (Pu = Py), and A + C = G + T (6 Am = 6 K)—are imposed by this structural feature.

It has been pointed out that the nucleotide composition of the total RNA from various sources, reflecting mainly the composition of the ribosomal RNA, tends to show one of these regularities, 6 Am = 6 K, but not the others (*4*). Tentative explanations were proposed in terms of structure (*4*) and, later, of information theory (*5*). Since then evidence has accumulated which indicates that both the ribosomal and transfer RNA's possess a relatively high, though incomplete, degree of base pairing of the DNA type, with U replacing T (*6, 7*). Furthermore, x-ray studies of two viral RNA's (*8, 9*) and of fragments of ribosomal RNA (*10, 11*) have shown

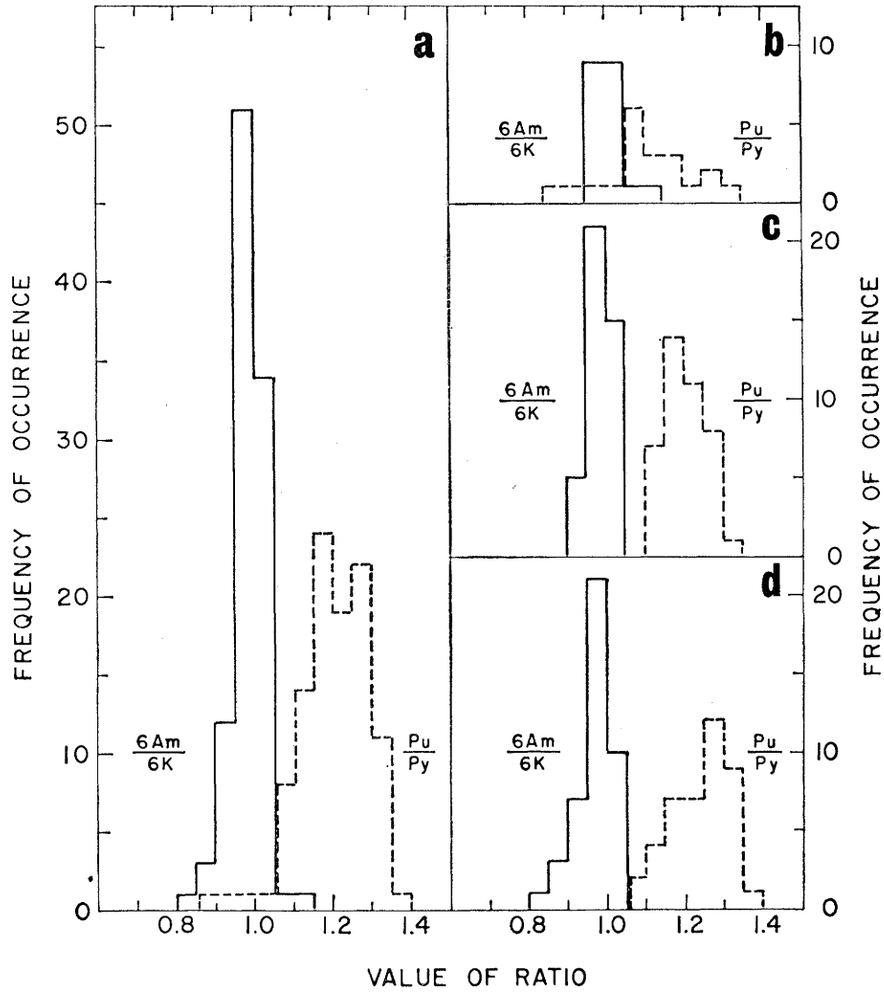

Fig. 1. Frequency distributions of the compositional ratios 6 Am/6 K and Pu/Py of total RNA from 103 different species. The data were taken from references *4* and *12*; where more than one value was listed for a single species, the average value is plotted. *a*, The total data (103 species), which were then divided into three groups as follows: *b*, animals (20 species); *c*, algae and higher plants (41 species); *d*, bacteria and fungi (42 species). Mean values of 6 Am/6 K for groups *a, b, c,* and *d* are 0.98, 1.00, 0.98, and 0.96, respectively. Mean values of Pu/Py for groups *a, b, c,* and *d* are 1.19, 1.11, 1.19, and 1.23 respectively.