tionship between virus and lymphocyte existing at the time of examination. The association of rubella virus with the lymphocytes of infants with the congenital rubella syndrome has not been demonstrated (*15*), but the problem is worthy of continued investigation. The difficulty encountered in isolating the virus from lymphocytes may result from the inability to remove cell-associated neutralizing antibody or from the relationship of the virus and cell (*16*).

A possible basis for the abnormal lymphocyte responsiveness produced by rubella virus and NDV might be an alteration of receptor function at the lymphoid cell surface. Gesner and Ginsberg (*17*) showed that glycosidases at the cell surface contribute to the recognition functions controlling traffic of lymphoid cells within the lymphoid system. Borberg *et al.* (*18*) interfered with the agglutinating effect of PHA on red cells by administering *N*-acetylgalactosamine before treatment with PHA. Furthermore, treatment of lymphocytes with certain enzymes that act on cell surfaces abolishes responsiveness to PHA (*19*). The effect of PHA on lymphocytes is to produce rapid changes in nucleic acid metabolism (*20*); this indicates that the surface action is quickly translated to an influence on cytoplasmic and possibly nuclear metabolism. Our results show that rubella virus and NDV can exert their inhibiting influence even when virus is added to the lymphocyte cultures as late as 24 hours after addition of PHA, which suggests that this inhibition occurs at a site other than the cell surface.

Another explanation for the abnormal lymphocyte responsiveness is the alteration of metabolic pathways by virus thus preventing the mitogenic effect of PHA. Nonlymphoid cells chronically infected with rubella virus have a reduced growth rate (*9, 21*). Although it has been reported that synthesis of protein and ribonucleic acid of cells infected with rubella was unimpaired (*22*), the virus may still produce sufficient alterations to inhibit stimulation of synthesis. In fact infection of human lymphocytes with rubella virus in vitro interferes with protein and ribonucleic acid synthesis induced by PHA (*23*). The finding that virus irradiated with ultraviolet light does not inhibit the PHA responsiveness of lymphocytes supports this hypothesis and suggests that the intact virus genome is required.

The inhibition by these viruses of the response of lymphocyte to PHA suggests that a similar alteration of cellular function may contribute to the persistent shedding of virus in the congenital rubella syndrome. This alteration of lymphocyte responsiveness might also prove a valuable factor in the detection of viruses that cannot be identified by presently available methods. Mella and Lang (*24*) have reported that the number of cells undergoing mitosis was reduced in preparations of leukocytes from patients with infectious hepatitis and, furthermore, that this effect can be produced by the addition of serum from patients with hepatitis to normal leukocytes. Mycoplasma organisms also inhibit mitosis (*25*). The spectrum of agents which might be revealed by this abnormality of lymphocyte responsiveness awaits analysis.

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Gene Activation without Histone Acetylation in Drosophila melanogaster

Abstract. *Chromosomal puffing, generally believed to represent gene activation in* Drosophila, *was induced in the presence of radioactively labeled acetate. There is no preferential uptake of these labeled molecules in regions of gene activation. It is concluded that the acetylation of histones does not play a general role in the regulation of RNA synthesis in* Drosophila melanogaster.

The view has recently been offered that histone acetylation brings about a change in the structure of the chromatin and is a necessary prerequisite for the synthesis of RNA's at previously repressed gene loci (*1*). This viewpoint derives, in part, from studies on the human lymphocyte. For example, when RNA synthesis in suspended lymphocytes is increased following addition of phytohemagglutinin (a kidney bean protein fraction), the increase in the uptake of uridine-2-C^{14} into RNA is preceded by an impressive augmentation in the uptake of sodium acetate-2-C^{14} into histones (*1*).

Although these findings support the view that histone acetylation serves to change the fine structure of the chromatin and thus to allow DNA to serve as a template for RNA synthesis, there is little evidence to show that histone acetylation is spatially correlated with the genetic loci which are activated. The present investigation attempts to determine whether histone acetylation occurs at the site of gene activation in a system, the Dipteran polytene chromosome, in which gene activation is morphologically manifested by chromosomal puffing.

In these experiments, two adjacent puffs (86F and 87A-B of the standard

salivary chromosome map), described by Ritossa (*2*), are of special interest, because they can easily be induced by subjecting the third instar larva of *Drosophila melanogaster*, or its excised salivary gland, to a temperature of 37°C. Although these puffs are normally not seen during this stage of larval development, they are similar to other puffs with respect to their ability to incorporate uridine-H^3. These same puffs, as well as similar heat-induced puffs in *D. busckii* and *D. hydei*, can also be induced by agents which inhibit respiration (*2, 3*), suggesting that the heat treatment induces puffing through some intermediary cellular system and not by acting directly on the chromatin. Such a system of indirect induction is ideal for this investigation in that it allows the cell an opportunity to respond via its own regulating mechanisms, among which acetylation might be included.

Autoradiography was used to determine whether chromosomal acetylation could be demonstrated in regions 86F and 87A-B at any time in the course of puff induction in these regions. Sodium acetate-H^3 (New England Nuclear; specific activity 142 mc/mmole) was used as a source of radioactively labeled acetyl groups in all experiments and was made available to the excised gland in a physiological saline solution (*4*) layered over with mineral oil.

Since it has been suggested that histone acetylation is prerequisite to gene activation, and since puffing begins in regions 86F and 87A-B immediately upon exposure of the gland to 37°C, it follows that the salivary gland nucleus must contain an adequate pool of sodium acetate-H^3 prior to its exposure to 37°C. To insure such a pool the excised gland was first incubated in physiological saline, which was maintained at 25°C and contained sodium acetate-H^3 (50 μc/ml), for 30 minutes. This period was sufficient to render the nuclei and cytoplasm, as observed in sectioned glands, equally heavily labeled The glands were then transferred to another solution, also containing sodium acetate-H^3 (50 μc/ml), which was maintained at 37°C. Upon transfer of the glands to this temperature, puffing begins immediately and reaches a plateau, with respect to size, after 30 to 45 minutes of continuous maintenance at 37°C. Since these puffs, as well as similar puffs in *D. busckii* and *D. hydei*, can be induced by several methods which are known to depress the level of adenosine triphosphate synthesis in cells (*2, 3*), the fact that regions 86F and 87A-B fail to puff during the 30-minute pre-incubation period at 25°C indicates that the glands are under relatively aerobic conditions. Thus it appears likely that there is adequate energy available for the activation of acetate into acetyl CoA. Furthermore, some preparations were extracted with 5 percent trichloroacetic acid (for 5 minutes at 5°C) to show that the acetate was actually bound into molecules of high molecular weight. When the acid-extracted preparations are compared with non-extracted preparations, there appears to be little difference in the amount of labeling.

If activation of a gene involves an acetylated state of that gene, then that state might either persist as long as the locus is active, or else might occur only at the time of initiation of activity. In view of these two possibilities, salivary chromosome preparations were made of the initial stages (1, 2, 3, and 5 minutes after exposure of the gland to 37°C) as well as maximal stages (30 to 45 minutes, 37°C) of puffing. All glands were fixed in acetic alcohol, stained in lacto-aceto-orcein, and squashed in 45 percent acetic acid. Cover slips were removed by the dry-ice technique and slides were coated with Kodak NTB-2 nuclear emulsion and exposed for 60 to 90 days.

The autoradiographs in all cases (chromosome preparations from 20 pairs of glands) demonstrate that there is little uptake of labeled acetate into the chromosomes in general and that there is definitely no preferential uptake of label in regions 86F and 87A-B during the initiation or development of puffing in these regions (Fig. 1). Moreover, no preferential uptake of label into any other regions, puffed or unpuffed, has been observed.

Since there is a definite absence of chromosomal acetylation, it is necessary to demonstrate that the treatment itself is not toxic to the excised gland or its chromosomes. Thus the ability of the experimentally treated gland to incorporate uridine-H^3 was examined by subjecting it to an additional 15-minute

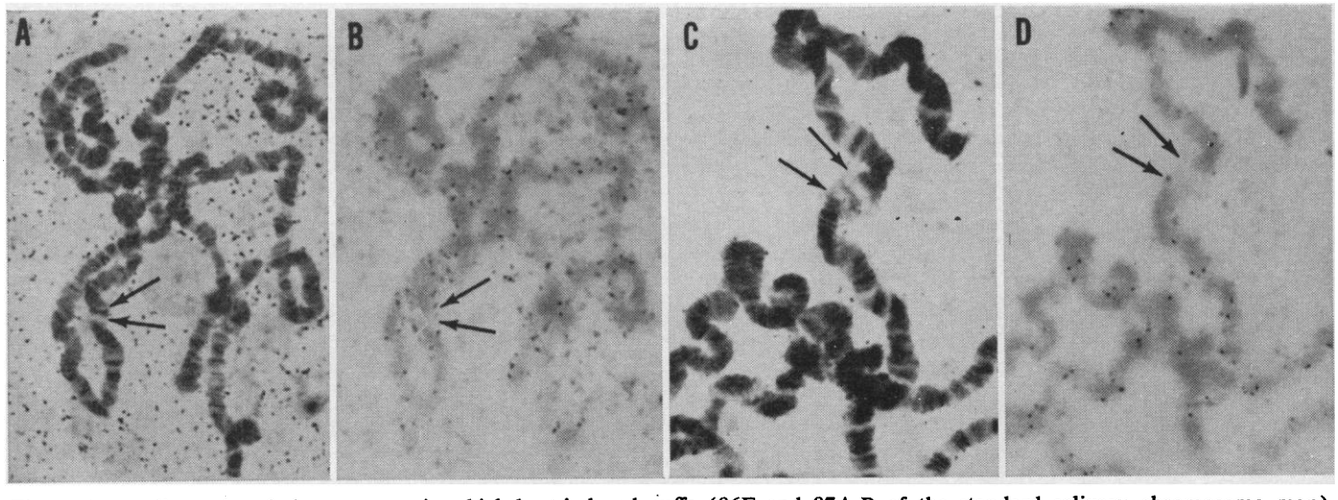

Fig. 1. Autoradiographs of chromosomes in which heat-induced puffs (86F and 87A-B of the standard salivary chromosome map), at arrows, have reached maximum size (from glands incubated at 37°C for 45 minutes, following a 30-minute pre-incubation at 25°C, sodium acetate-H^3 being present during both incubations). Note that in B and D, which focus on grains lying directly above chromosomes in A and C, respectively, there is little label over the puffs or over the chromosomes in general. In some squash preparations, as in A and B, chromosomal label can be compared to label in the neighboring mixture of nucleoplasm and cytoplasm, but preparations rendered free of nucleoplasm and cytoplasm by the squash technique, as in C and D, give better resolution of chromosomal label. Exposed 70 days (about × 2000).

post-treatment in physiological saline (37°C) containing uridine-H^3 (25 μc/ml) as well as sodium acetate-H^3 (25 μc/ml). In all cases (chromosome preparations from three pairs of glands) there was extensive chromosomal labeling and heavy labeling in the puffed regions, suggesting that the glands were still able to synthesize RNA after the experimental treatment.

As a result of these observations, it is concluded that gene activation, as displayed by the heat-induced puffing of regions 86F and 87A-B, does not require the acetylation of histones in these same regions. Although the heat-induced puffs might represent a special class of puffs which might not require histone acetylation, the absence of preferential or extensive uptake of sodium acetate-H^3 in any other chromosomal region, puffed or unpuffed, suggests that acetylation of histones does not play a general role in the regulation of RNA synthesis in *D. melanogaster*. It must be added, however, that although extensive acetylation of chromosomes was not detected in these observations, it might serve more specifically to regulate the activity of a very few genetic loci, as appears to be the case for the hormone ecdysone (*5*). It is also entirely possible that gene activation by means of histone acetylation might require the transfer of very few acetyl groups to the histones, which could go undetected by this method. Even if this were the case, the polytene nature of the salivary chromosomes would seem to offer more sites for acetylation, which would thus enhance the possibility of detecting this phenomenon. Finally, there is also the possibility that acetyl groups are lost from the histones under the conditions employed to prepare the chromosome squashes. However, this seems highly unlikely in view of the fact that acetyl groups remain intact during the several extraction and separation procedures, involving the use of various acid solutions, employed by Phillips (*6*) and are only subsequently removed by partial hydrolysis of the histones in 1*N* or 6*N* H_2SO_4 at 100°C for 5 hours.

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Intracellular Olfactory Response of Hippocampal Neurons in Awake, Sitting Squirrel Monkeys

Abstract. *Intracellular recordings of archicortical neurons in a primate have been made and the responses of these neurons in awake, sitting animals have been observed. Electrical stimulation of the olfactory bulbs elicits excitatory postsynaptic potentials in these neurons, which unlike those evoked by septal stimulation, are subliminal for generating spikes. The olfactory and septal pathways may be considered representative of inputs from exteroceptive and interoceptive systems. The findings are discussed in relation to a paradigm for archicortical conditioning.*

The archicortex of the hippocampus represents the simplest type of cerebral cortex. Its predominant nerve cell is pyramidal in shape and is characterized by a long apical dendrite and several basal dendrites. The axon departs from the base of the cell. We are interested in the mode of action of various inputs to the dendrites and cell bodies of these neurons because such knowledge may contribute to an understanding of mechanisms by which cortical cells are conditioned and participate in memory and learning.

On the basis of extracellular recordings of unit and slow potentials in anesthetized monkeys, Gergen and MacLean (*1*) concluded that impulses from the septum excite hippocampal pyramids more effectively than do those conducted by olfactory pathways. They suggested that the septal input is more effective because it exerts its major excitatory action closer to the trigger zone of the cell. The septal pathway has been shown to be activated by stimulation of the hypothalamus and other structures (*2*) which play an important role in aversive, appetitive, visceral, and humoral reactions. It is probable that olfactory impulses are relayed in part by the perforant pathway from the hippocampal gyrus, which terminates on the apical dendrites (*3*).

Extracellular recording with microelectrodes is useful for detecting the discharge of single nerve cells, but it is inadequate for revealing excitatory or inhibitory postsynaptic potentials developing in an individual cell. To obtain more information about the differential effects of septal and olfactory inputs on pyramidal cells, we have undertaken experiments involving intracellular recordings in awake, sitting squirrel monkeys. Such experiments have been made possible for the first time by adaptation of a technique for intracerebral exploration with microelectrodes (*4*). Heretofore, intracellular recording has commonly involved open operations on the brain, paralysis of the animal, pneumothorax, and other measures to prevent cerebral movement caused by vascular pulsation and respiration. Our technique provides an essentially closed system, and experiments can be conducted without the use of open surgery and the depressing effects of a general anesthetic.

Ten squirrel monkeys were fitted with a special stereotaxic device according to the method described (*4*). Paired Teflon-coated stainless steel stimulating electrodes were permanently implanted in the olfactory bulbs, septum, and fornix. Testing stimuli were one to three shocks (negative constant current 0.5 msec pulses) with an intensity of 0.3 to 1 ma. The shocks to the olfactory bulb were of sufficient intensity to cause sneezing if applied at a frequency of 30 per second. The electrodes in the fornix enabled identification of hippocampal pyramids by means of the antidromic spike. Intracellular and extracellular recordings were obtained with glass micropipettes filled with 2*M* potassium citrate or 3*M* KCl.

Fifty impaled units in the hippocampus responded to one or more of the inputs used. They were tested on the average for a period of 6 minutes. Twelve of 50 units responded to stimulation of the bulb with three shocks with EPSP's (excitatory postsynaptic potentials), the latencies of which