chain leading from water to ferredoxin is located within the membrane. That this may also eventually be true for mitochondrial cristae is indicated by the data of Chance which show that the cytochrome chain is not contained within the elementary particles, but is within the mitochondrial membrane (*24*).

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2 March 1964

Synthesis of RNA in vitro Stimulated in Dipteran Salivary Glands by 1,1,3-Tricyano-2-Amino-1-Propene

Abstract. *1,1,3-Tricyano-2-amino-1-propene stimulates by more than one order of magnitude the incorporation in vitro of uridine into the RNA of dipteran salivary glands. The inhibition by actinomycin D is blocked. Higher doses cause a complete inhibition from which a proportion of the glands recover rapidly upon withdrawal of the substance.*

1,1,3-Tricyano-2-amino-1-propene (tricyamp) stimulates net RNA synthesis in rabbit nerve cells in vivo (*1*). Our observations in vitro suggest that there is also a dramatic stimulation in salivary glands. This small molecule, presumably not encountered in normal metabolism, may prove useful for work on other cells as well.

Larval salivary glands of the chironomid *Smittia parthenogenetica* were incubated for 1 or 2 hours at 25°C in standard medium (*2*) containing tricyamp (*3*) at different concentrations. Then they were incubated in the same medium for either 30 minutes with tritiated uridine (40 or 60 μc/ml, 3.0 c/mmole; Radiochemical Centre) or for 45 minutes with tritiated guanosine (50 μc/ml, 0.4 c/mmole). Controls were treated similarly but tricyamp was omitted from the medium. The glands were fixed in trichloroacetic acid, squashed in acetic acid, and then fixed in trichloroacetic acid (*2*). Autoradiographs showed that incorporation of uridine was markedly enhanced in the whole cell after treatment for 1 hour with 100 μg (Figs. 1 and 2). The stimulation is more obvious in chromosomes and specially nucleoli than in cytoplasm, owing to the normally greater incorporation in the nuclear structures. Treatment with 50 μg of tricyamp had no appreciable effect, while treatment with 175 μg inhibited incorporation almost completely, and treatment with 250 μg inhibited incorporation completely. Treatment for 2 hours with 50 μg caused a similar stimulation (see Fig. 2).

Incorporation of guanosine was also markedly enhanced by treatment for 1 hour with 100 μg of tricyamp, though probably less than that of uridine. All label was sensitive to ribonuclease. Pending further characterization, this suggests that the stimulated incorporation is into RNA.

Recovery from the inhibition caused by 250 μg of tricyamp was indicated in about one-quarter of the glands upon withdrawal of the substance and further incubation with tritiated uridine alone. Controls were incubated with tricyamp present or absent throughout the incubations. Incubation for 30 minutes with uridine, immediately after withdrawal of tricyamp or after an intervening 1.5 hours of incubation in standard medium, showed a stimulation of incorporation as in Fig. 2. After 3 hours in standard medium, the incorporation was of the order of controls. Sodium thiosulphate has a detoxicating action on animals poisoned by tricyamp (*4*), but in our experiments the presence of 250 μg of sodium thiosulphate after withdrawal of tricyamp had no effect. These observations are consistent with an initial rapid decrease of the intracellular concentration of tricycamp

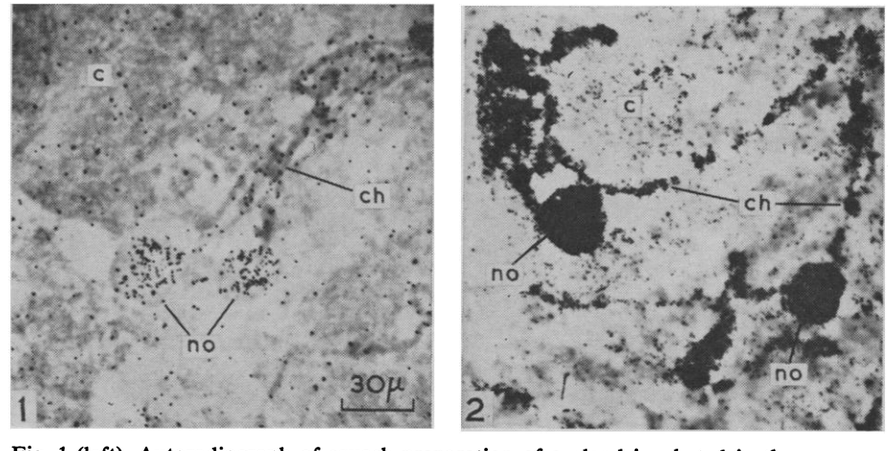

Fig. 1 (left). Autoradiograph of squash preparation of a gland incubated in the presence of ^{3}H-uridine; fixed in trichloroacetic acid and stained with methyl green-pyronin; *no*, nucleolus; *ch*, chromosomes; *c*, cytoplasm. Fig. 2 (right). Same as Fig. 1, except that 100 μg of 1,1,3,-tricyano-2-amino-1-propene (per milliliter) was present before and during incubation.

in those glands that recovered from inhibition.

In other experiments tricyamp (50 μg) and actinomycin D (0.5 μg) (*5*) were offered simultaneously for 2 hours, and the glands were then incubated with tritiated uridine in the presence of these substances. Controls were incubated with actinomycin alone or with neither substance. As reported earlier (*6*), actinomycin by itself almost totally suppressed uridine incorporation into nucleolar RNA and greatly inhibited the chromosomal incorporation (the effect on cytoplasm could not be studied since it is not appreciably labeled in the present incubations, Fig. 1). In the presence of tricyamp the nuclear incorporation was restored to the level of the controls. In parallel experiments tricyamp also relieved the rather specific inhibition of chromosomal incorporation of uridine or guanosine caused by dichlororibofuranosylbenzimidazole (*7*). The observations point in part to the action of tricyamp as probably stimulating DNA-dependent RNA synthesis, but whether this applies to the nucleolus as well as chromosomes is unknown.

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Antibody Production by Nonimmune Spleen Cells Incubated with RNA from Immunized Mice

Abstract. *RNA isolated from the spleens of B_6AF_1 mice immunized to sheep red blood cells was incubated with the spleen cells of nonimmunized mice. The number of antibody-forming cells detected by the Jerne plaque technique was significantly higher among the cells incubated with the RNA from immune animals than among cells incubated without RNA, or with RNA from nonimmune animals.*

There have been several reports concerning "transformation" or induction of mammalian cells by the addition of specific informational RNA depleted of protein. The production of mature viral protein after the addition of polio virus RNA to cultures of HeLa cells may be considered a transformation, although the infectious RNA is assumed to have unique properties of stimulating self-replication (*1*). Niu *et al.* have reported a series of experiments in which RNA obtained from an organ with highly specific function is capable of providing information for the synthesis of specific protein by cells less well differentiated (*2*).

There have been at least two reports which suggest the transformation of lymphoid cells with RNA obtained from cells with a prior exposure to antigen. Fishman (*3*) produced antibody to bacteriophage and Mannick and Egdahl (*4*) produced reactivity to transplantation antigens. In our work, RNA isolated from the spleen of mice immunized with sheep red blood cells converted a small percentage of nonimmune isologous mouse spleen cells into antibody-forming cells. Antibody production by the converted cells was detected by the plaque-assay method (*5*).

We used B_6AF_1 male mice (Jackson Laboratories, Bar Harbor, Maine) 8 to 10 weeks old. The donor mice were immunized by intraperitoneal injections of 50 percent washed sheep red blood cells in 0.1 ml phosphate buffered saline (*p*H 7.4) over a period of 6 to 16 days. Two to three days after the last injection, the mice were killed by cervical dislocation and their spleens were removed for RNA extraction by a modification of the method of Scherrer and Darnell (*6*). The spleens were homogenized in equal parts of 90 percent phenol (containing 0.1 percent 8-hydroxyquinoline) and a mixture of 0.01*M* acetate buffer (*p*H 5.0); 0.5 percent sodium dodecyl sulfate, and 10 μg of bentonite per milliliter. After homogenization, the solution was heated to 60°C for 4 minutes, and was cooled rapidly to 10°C in a bath composed of alcohol and dry ice. After centrifugation at low speed to separate the phases, the aqueous layer containing the RNA was removed. The phenol layer was again extracted with buffer as before, and the aqueous phases were combined and treated three times with the 90 percent phenol mixture, heating and cooling each time. Finally, 2.5*M* sodium acetate was added to the aqueous phase to a final concentration of 0.3*M*, and the RNA was precipitated with two volumes of absolute ethanol. The white precipitate was washed three times with 75 percent ethanol and after the final wash, the RNA preparation was dissolved in Eagle's basal medium (*7*) (without added serum or antibiotics) at *p*H 7.4. The dissolved RNA was added to a cell suspension made from the spleens of nonimmunized B_6AF_1 mice.

The RNA extracted from spleens of 20 donors was added to a pooled cell suspension obtained from spleens of five recipients in 1.5 ml of Eagle's basal medium. Donor RNA was added to the recipient cells and the suspensions were incubated at 37°C for 30 minutes in a water bath equipped with a shaking device. At the end of this period, 0.2 ml of the cell suspension was added to each of a series of tubes containing 0.15 ml of 25 percent washed sheep red blood cells in 2 ml of 0.7 percent agar in Earle's basic salt solution with glucose at 38° to 40°C. Immediately after the addition of the cell suspension to the melted agar, the warm mixtures were overlaid on a solidified base of 1.4 percent agar in Earle's basic salts contained in 100-mm petri dishes.

After a few minutes at room temperature to allow the overlay to solidify, the dishes were incubated at 37°C for 3 hours. After incubation, fresh frozen guinea pig serum was diluted 1:1 with Eagle's basal medium, and 1.5 ml was added to each petri dish. The plates were incubated for an additional 30 minutes at 37°C and were stored at 4°C overnight. Each plate was examined grossly and microscopically for specific zones of hemolysis (Fig. 1) by two observers who were not aware of the group to which