It may be possible to form other G-6-PD hybrids. Rat and type B human enzyme were chosen for hybridization in these studies because rat enzyme appeared to have the most rapid mobility among those of species readily available (mouse, rat, cow, sheep, rabbit, coyote). Thus, there was a well-marked difference in the mobility of the two enzymes. Cow enzyme had the least electrophoretic mobility, but attempts to hybridize this enzyme with the rapidly moving human type A enzyme have failed.

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10. Investigation supported, in part, by NIH grant HE-07449.

23 August 1965

Submaxillary Gland of Mouse: Effects of a Fraction on Tissues of Mesodermal Origin in vitro

Abstract. *A macromolecular fraction from mouse salivary glands markedly enhances growth of mesenchymal tissues in vitro. The same fraction causes dedifferentiation of tissues of mesodermal origin, such as muscle and cartilage. Dedifferentiated tissues do not divide but survive in good condition, while controls in the same cultural media undergo regressive changes.*

Mouse salivary gland contains several factors endowed with unique and remarkable biologic properties. The most striking factor is NGF, a protein that specifically stimulates growth of sympathetic nerve cells in vitro and in vivo; we have extensively studied its chemical and biologic properties (*1*).

Subsequently another growth-promoting factor of a protein nature, EGF, specific for epidermal cells, has been isolated from extract of mouse salivary gland and purified (*2*). Another fraction of homogenate of the same gland is under investigation regarding its ability to induce intense leukocytosis when minute amounts are injected into various animals.

We now report certain effects of still another macromolecular fraction of salivary gland on cultures of embryonic tissues of mesodermal origin; this fraction stimulates the growth of mesenchymal cells and causes loss of differentiative marks in muscle and cartilage.

The active fraction was partially purified by precipitation with streptomycin sulfate and ammonium sulfate, gel filtration, and column chromatography; most of the biologic activity was precipitated by 40 to 70 percent ammonium sulfate. The precipitate was dissolved in a little distilled water, dialyzed, and run through a Sephadex G-100 column. At least three protein peaks were separated, most of the biologic activity being associated with the third peak. The active fractions were pooled, concentrated, and refractionated through a DEAE Sephadex A-50 column at *p*H 7.2; parabolic-gradient salt elution was used. Most of the biologic activity was localized in the fraction eluted with 0.1 to 0.2*M* sodium chloride (Fig. 1). When assayed with casein as substrate, the fraction revealed proteolytic activity. Biologic activity was tested in vitro on tissues dissected from chick embryos. Tissue fragments of about 1 mm^3 were explanted in Eagle basal medium in small falcon dishes and placed in an incubator conditioned with CO_2 at 38°C; they were then fixed and examined histologically.

Growth stimulation by the salivary-gland fraction was clearly evident in explants of lung from 8- to 9-day chick embryos. At this stage the lung primordia consist of loose mesenchyme in which bronchial tubules grow by a process of budding and branching; control cultures examined after 24 hours in vitro show fairly good maintenance of the original structure. Addition of the salivary-gland fraction at concentrations ranging between 5 and 50 μg per milliliter of culture medium caused marked changes: the mesenchyme underwent very active and vigorous growth, without appreciable change in the epithelium lining the bronchial tubules.

The growth-stimulating effect of the extract was further investigated by separating the two lung components: mesenchyme and bronchi. The bronchi were stripped from the surrounding mesenchyme, and mesenchymal and epithelial components were cultured in the presence of the active fraction. Mesenchymal tissue in control media underwent little change during 24 to 48 hours in vitro; there were some necrotic areas in the central part of the explants but the peripheral parts closely resembled the tissue of origin in cell density and in the loose texture of the intercellular matrix. Fragments in experimental cultures differed strikingly from the tissue of origin and from the cultured controls; a dense cell population replaced the loose—even scattered—fibroblasts of the original tissue, and the cells were arranged in tightly packed ribbons of spindle-shaped fibroblasts which filled all available space in the explants. As a result, there was a dense and vigorously growing cell population with no intercellular matrix (Fig. 2, *a* and *b*). Autoradiographic experiments with H^3-thymidine yielded evidence of many mitotic figures in the experimental cultures; such figures were practically absent in controls (Fig. 3, *a* and *b*).

Explants consisting of one or two bronchi stripped from the surrounding mesenchyme underwent little change when cultured for 24 to 48 hours in Eagle medium alone. In the presence of the salivary-gland fraction the few mesenchymal cells that still adhered to

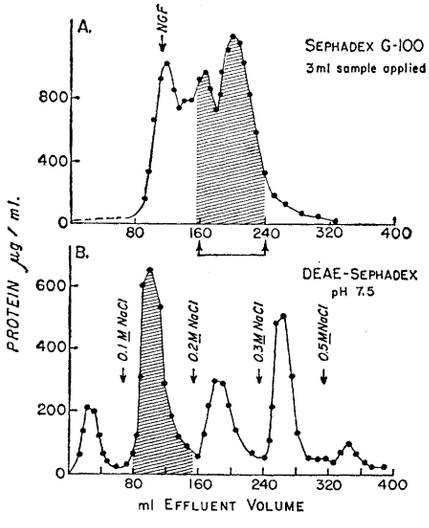
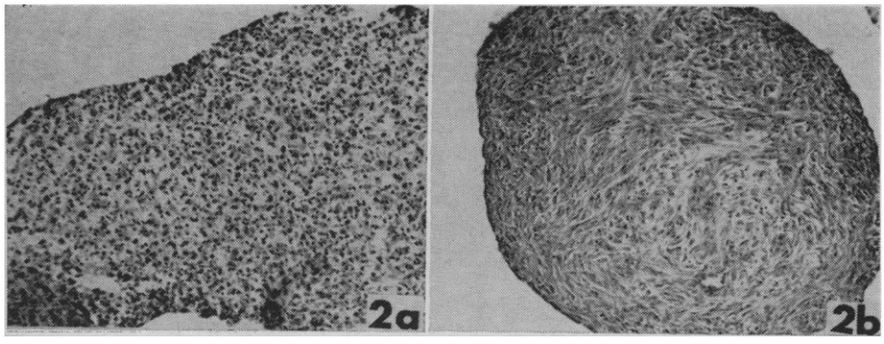
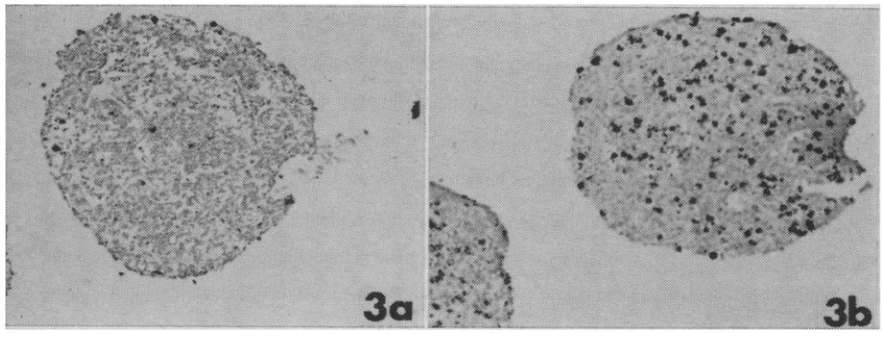
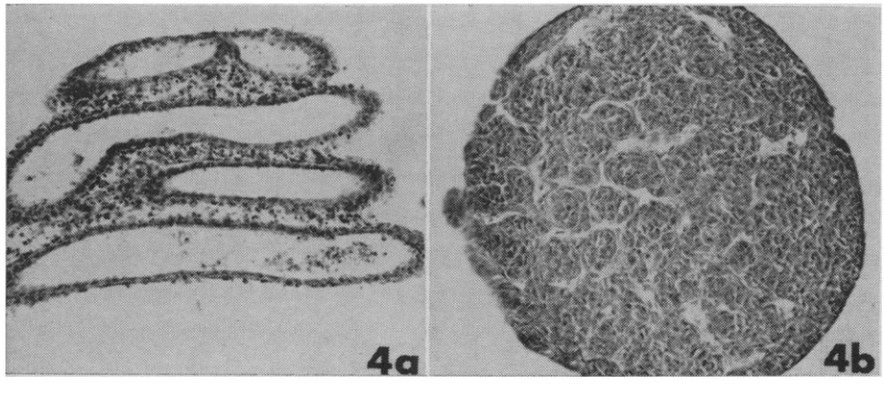
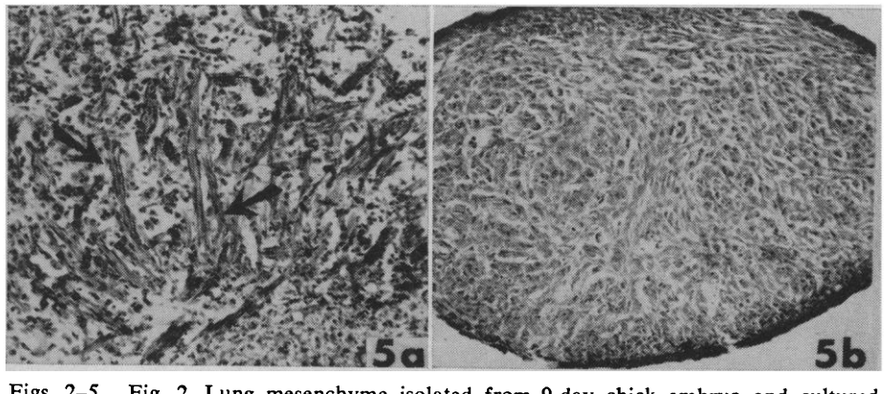


Fig. 1. (*A*) Fractionation of salivary-gland extract through Sephadex G-100 column after ammonium sulfate precipitation: activity of nerve growth factor localized in the first peak; biologic activity in the hatched area. (*B*) Further chromatography of the active fractions from Sephadex G-100 through a DEAE Sephadex column: stepwise elution with increasing concentration of sodium chloride; biologic activity in the hatched area.









Figs. 2–5. Fig. 2. Lung mesenchyme isolated from 9-day chick embryo and cultured in synthetic liquid medium for 24 hours without (*a*) and with (*b*) the active fraction of salivary-gland extract. Fig. 3. Autoradiography of lung mesenchyme, from 9-day chick embryo, exposed to H^3-thymidine (0.5 μc per milliliter of culture medium) for 3 hours without (*a*) and with (*b*) the active fraction of salivary-gland extract. Fig. 4. Bronchial tubules isolated from 9-day chick embryo and cultured in synthetic liquid media for 24 hours without (*a*) and with (*b*) the active fraction of salivary-gland extract. Fig. 5. Skeletal muscle, explanted from the neck region of an 11-day chick embryo, cultured in synthetic liquid medium for 24 hours without (*a*) and with (*b*) the active fraction of salivary-gland extract. Arrows in *a* point to muscle fibers.

the epithelial tubules underwent such active growth as to efface the tubules, which had been the main component of the small explants (Fig. 4, *a* and *b*).

The same salivary-gland fraction was then tested on tissues of mesodermal origin that had already undergone differentiation into muscle or cartilage. Control and experimental cultures of skeletal muscles from 10- to 12-day chick embryos differed strikingly when inspected under the dissecting microscope after the first day of incubation. Control fragments were irregular in shape and had a tenuous texture, while experimental fragments appeared as small spherical or ovoidal masses of tissue of dense and homogeneous consistency. Differences between experimental and control cultures were obvious at the histological level (Fig. 5, *a* and *b*). Well-preserved striated muscle fibers were scattered in control cultures among the loose mesenchyme. In media supplemented with the salivary-gland fraction all muscle fibers had undergone a process of dissociation; after the first day of culture the explants consisted of individual myoblasts and polynucleated cells that were to undergo further dissociation into single units during subsequent culture. Almost complete loss of myosin was indicated by the loss of eosinophilia in myoblasts of experimental cultures. Decisive evidence of the loss of myosin was found by Holtzer (*3*), using fluorescence-labeled antimyosin antibodies to compare the myosin content of our experimental and control cultures; he estimated the content of treated fragments to be about 2 percent of that of controls.

Autoradiographic studies clearly showed that the myoblasts, individualized from the muscle fibers and almost completely deprived of myosin, did not incorporate H^3-thymidine. Mitotic activity also was absent in tissues kept for as long as 10 days in Eagle medium supplemented with the active fraction. After this period, controls in Eagle medium alone had undergone extensive regressive changes, while myoblasts in media supplemented with the salivary-gland fraction were remarkably well preserved; the absence of mitotic figures in this cell population was surprising in view of the lack of differentiating marks and of the healthy appearance of the cells.

Preliminary experiments indicate that actinomycin D at 0.15 μg per milliliter of culture medium inhibits the effect of the salivary-gland fraction on skeletal muscles; this suggests that this effect

may be mediated by synthesis of some RNA species. We performed a few experiments with smooth muscles dissected from stomach and blood vessels of 8- to 10-day chick embryos. In the presence of the active salivary-gland fraction the myoblasts lost their characteristic spindle shape and became round; sharp decrease in their eosinophilia was most likely caused by loss of myosin—as with the striated muscles.

The effect of the salivary-gland fraction on cartilage tissue was studied by culturing vertebrae of 10-day chick embryos for 48 to 72 hours, after which time cartilage fragments in control cultures remained well preserved. Similar fragments in the experimental culture showed sharp decrease in susceptibility to metachromatic staining of the intercellular matrix and changes in the morphology of the cells, which progressively reverted to more undifferentiated types.

Our observations on muscle and cartilaginous tissues indicate a process of dedifferentiation of these tissues as an effect of the salivary-gland fraction. It was therefore of interest to study possible effects of the same fraction on precursors of the same tissues. Rows of somites from chick embryos at stages 14 to 17 (*4*) were dissected together with fragments of neural tube, or without nerve tissue if explanted from older embryos at stages 27 to 28. The explants consisting of 5 or 6 somites were cultured in plasma clot or in Puck liquid media with 15 percent calf serum for 5 to 6 days; control and experimental cultures were then fixed, stained, and examined. Nodules of cartilage and striated muscle fibers were numerous in control cultures, among mesenchymal cells—which constituted the bulk of the explants. Experimental cultures consisted of a homogeneous cell population, with no differentiative marks evident in the many explants examined. It is significant that neither the stimulatory effect on mesenchymal tissue nor the dedifferentiative effect on mesodermal derivatives was obtained with extracts of organs such as thymus, liver, kidney, and pancreas.

The question now is how this salivary-gland fraction produces the marked morphological changes described. These effects may be due to the proteolytic activity that appears to be consistently associated with the active fraction throughout all purification steps. If so, the biologic effects may result from liberation of active peptides from a larger protein molecule, as well as from direct action of the enzyme on the cell surface.

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5. Assisted by grants from the National Institutes of Health and the National Science Foundation.

18 September 1965

Mescaline, 3,4-Dimethoxyphenylethylamine, and Adrenaline: Sites of Electroencephalographic Arousal

Abstract. *Transections of the brain of rabbit reveal that electroencephalographic arousal produced by injections of adrenaline takes place at the midbrain level, while mescaline and 3,4-dimethoxyphenylethylamine induce such arousal lower in the brainstem, at the medullary level.*

It has been shown (*1, 2*) that psychotomimetic indole amines such as psilocin, bufotenin, and lysergic acid diethylamide have a site of electroencephalographic (EEG) arousal below the midbrain and above the first cervical segment of the spinal cord, while their nonpsychotomimetic congeners evoke EEG alerting at the midbrain level. Moreover the chemical constituents of the psychotomimetic congeners have an *N*-dimethyl or *N*-diethyl configuration, while their nonpsychotomimetic congeners lack such configurations.

Other workers (*3*) have found 3,4-dimethoxyphenylethylamine (DMPEA) in the urine of schizophrenic patients. Friedhoff and Van Winkle (*4*) have implicated this amine as an endogenous psychotomimetic substance because homogenates of liver obtained by biopsy from schizophrenic patients were capable of *O*-methylating both *O*-hydroxy groups of dopamine, whereas liver samples obtained from normal subjects induced no such reaction. DMPEA exhibited a catatonic effect similar to that produced by mescaline in cats (*5*); it is closely allied in chemical structure to mescaline, both being *O*-methylated catecholamines.

Eighty-six adult New Zealand albino rabbits weighing 2.5 to 3.0 kg were tracheotomized under ether and local anesthesia, curarized, and artificially respired. For monopolar recordings of an electroencephalogram, coaxial electrodes were placed superficially on motor and on limbic cortical areas, and also deeply in the head of the caudate nucleus, hippocampus, amygdala, and reticular formation, according to the map of Sawyer *et al.* (*6*). Blood pressure was monitored by means of a mercury manometer connected to a femoral artery. Intravenous and intracarotid injections of drugs were made through a polyethylene cannula inserted in a femoral vein and a T-shaped cannula connected to a common carotid artery. Transections of brain were performed (i) at the precollicular, prepontine plane, just above the midbrain; (ii) at the postcollicular, postpontine plane, just below the midbrain; and (iii) through the first segment of the cervical spinal cord (*1*).

In nine intact animals, 20 to 30 seconds after intracarotid injection of 50 to 150 mg of DMPEA, the EEG resting pattern changed from high voltage and slow waves to one consisting of decreased voltage and fast waves in the motor and limbic cortical areas, caudate nucleus, amygdala, and reticular formation, and of waves of predominantly large amplitude, 4 to 6 cy/sec, in the hippocampus. Arousal was also induced by intravenous injection of mescaline (10 to 40 mg/kg) into six intact animals and by intracarotid injection of 2 to 10 μg of adrenaline in five intact animals. Duration of the arousal produced by these drugs depended on the doses. Larger doses of DMPEA and mescaline produced protracted arousal lasting for more than 30 minutes. Arousal obtained by adequate doses of DMPEA and mescaline persisted even after first