of donor chromosomes be transferred to recipient cells? How many of the host cells develop donor characteristics? Both questions are fundamental to any hypothesis of genetic transformation of the host cells.

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7. Factors: 220 kv; half-value layer, 0.89-mm copper; 15 ma; distance, 60 cm; 1.0-mm aluminum plus 0.25-mm copper filtration.
8. Ralston-Purina brand of oxytetracycline; 25 mg/ml.
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27 December 1965

Glucose-6-Phosphatase in Tubular Endoplasmic Reticulum of Hepatocytes

Abstract. *The histochemical localization of glucose-6-phosphatase activity in neonatal mouse liver was studied under electron microscopy. The activity was demonstrated in the tubular endoplasmic reticulum, which pervades the glycogen areas of the cell during glycogenolysis. Activity was also demonstrated in the nuclear envelope and ergastoplasm.*

A number of electron microscopical studies have demonstrated a tubular form of endoplasmic reticulum in hepatocytes of mammals and other vertebrates (*1–5*). This tubular endoplasmic reticulum (TER) has been described as a branching or anastomosing latticework of agranular tubules in continuity with the ergastoplasm and, under physiological conditions, confined to cytoplasmic areas in which glycogen deposits are found. Peters, Kelly, and Dembitzer (*4*) have reported on the sequence of morphological events associated with the differentiation of TER during hepatic development in the mouse. TER is absent throughout the late gestational period when massive stores of glycogen accumulate. It becomes evident for the first time shortly after birth and proliferates extensively while glycogen rapidly declines on the first postnatal day. From these observations it was concluded that TER is related to glycogenolysis but not also to glycogen synthesis and storage as previous authors (*2*) had suggested. In view of biochemical information (*6*) that a rapid increase in glucose-6-phosphatase activity coincides with the early neonatal decline in glycogen, it was further postulated that the TER might function in glycogenolysis as a site of glucose-6-phosphatase activity. Histochemical studies on adult rat liver have shown the enzyme to be present in the ergastoplasm, nuclear envelope, and some smooth membrane (*7*). In order to determine whether TER is also a site of glucose-6-phosphatase activity the present histochemical study utilized the developing mouse liver at an age when hepatocytes are known to exhibit large aggregates of TER.

Samples of livers from 23- to 27-hour-old strain C3H mice were fixed for 1 hour in a 4-percent glutaraldehyde solution at *p*H 7.1. This fixative, as well as the subsequent wash, incubation medium, and postfixative, contained 0.05*M* sodium cacodylate as buffer. By the addition of 0.35*M* sucrose in the latter three solutions the osmolality throughout all the procedures was maintained at approximately 550 milliosmoles. The same *p*H was used in all solutions except the incubation medium, which was at *p*H 6.8. Low temperatures were maintained during fixation with an ice-water bath and during the wash period by refrigeration at 1°C; incubation, however, was at room temperature. After removal of excess glutaraldehyde by washing the samples for 2 hours, 35-μ frozen sections were obtained. The incubation media consisted of the cacodylate-sucrose solution and 3 m*M* lead nitrate, either with or without the addition of 4 m*M* substrate. The test substrate was glucose-6-phosphate, and the control substrates were fructose-6-phosphate; ribose-5-phosphate; fructose-1,6-diphosphate; adenosine monophosphate; β-glycerophosphate; and adenosine triphosphate. Incubation time for glucose-6-phosphate was 3 to 15 minutes, and for the control substrates, 15 minutes. After incubation, the sections were postfixed 1 hour in 1-percent osmic acid, dehydrated in graded concentrations of ethanol, and embedded in Maraglas resin prior to thin-sectioning for electron microscopy. Thin sections were studied either unstained or stained with lead citrate and uranyl acetate, used singly and in combination. For light microscopy 10-μ sections were handled similarly through the incubation, after which they were rinsed briefly in the cacodylate-sucrose wash solution and treated with dilute ammonium sulfide. An additional series of 10-μ sections was incubated in acetate buffer at *p*H 5.0 for 15 minutes prior to a 15-minute incubation in glucose-6-phosphate medium. This procedure is known to inhibit glucose-6-phosphatase activity while not affecting acid and alkaline phosphatases (*8*).

Under electron microscopy, the sections incubated in the glucose-6-phosphate medium showed lead reaction product in the ergastoplasm and TER (Fig. 1, top), and nuclear envelope. No reaction product appeared in the pericanalicular bodies, microbodies, the Golgi complex, mitochondria, or plasma membrane. With incubation periods of 10 to 15 minutes the reaction product appeared as large, very dense, closely spaced clumps often filling long stretches of the cisternae (Fig. 1, top). The ergastoplasm and aggregates of TER were easily distinguishable, even

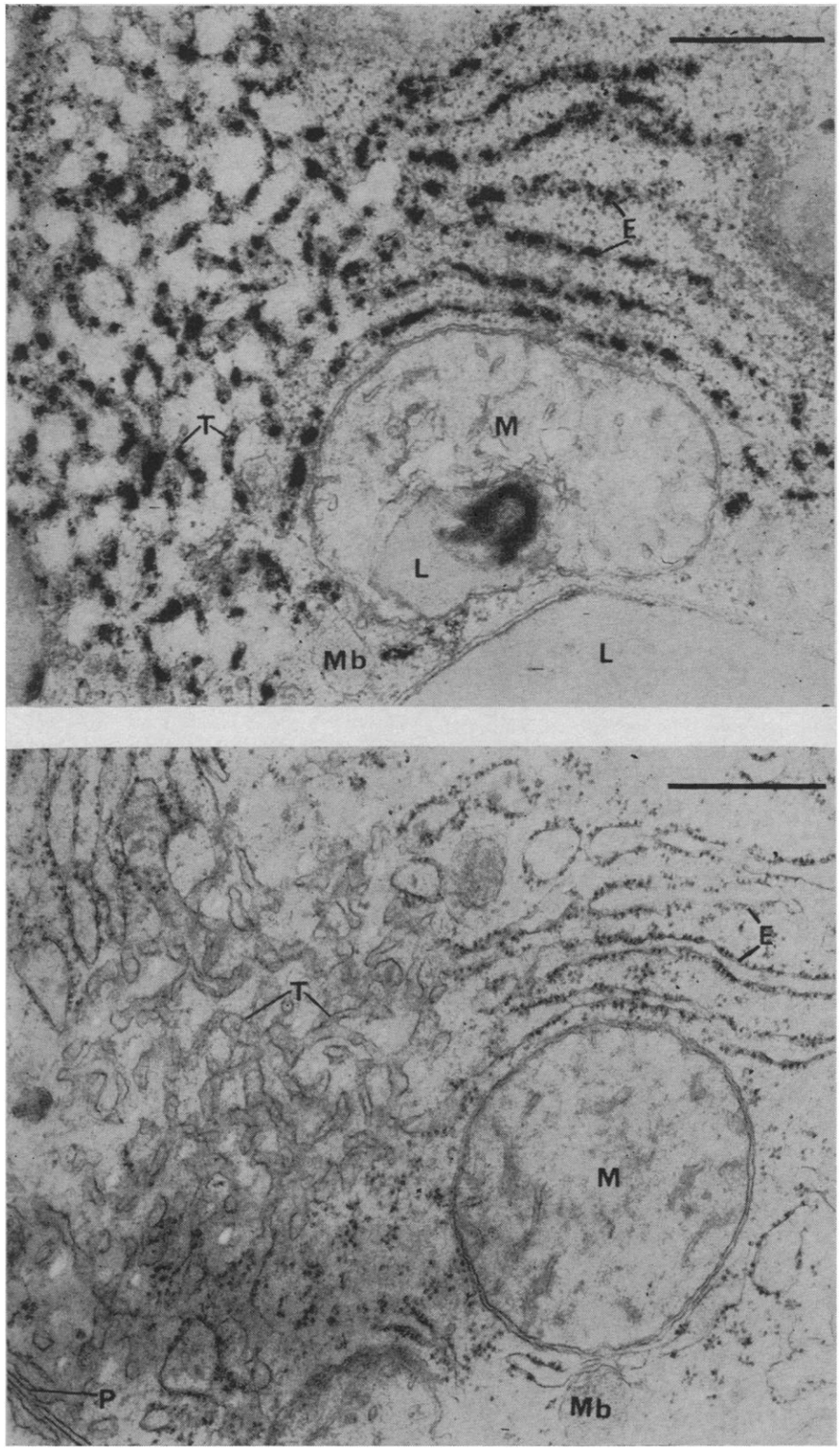

Fig. 1. Electron micrographs showing comparable regions of parenchymal cell cytoplasm from livers of 1-day-old C3H mice. Fixed in glutaraldehyde, incubated in media containing lead ions, and postfixed in osmic acid. (× 22,100). (Top) Incubated in presence of glucose-6-phosphate. Dense lead deposits indicate localized glucose-6-phosphatase activity in cisternae of tubular endoplasmic reticulum (*T*) and ergastoplasm (*E*). Note absence of reaction product in the mitochondrion (*M*), microbody (*Mb*), and lipid droplets (*L*). The myelin figure between the lipid droplet and mitochondrion is common in preparations at this age and may be artifactual. (Bottom) Incubated without substrate. The absence of reaction product affords unobscured view of membranes of the ergastoplasm (*E*) with their associated ribosomes and the membranes of tubular endoplasmic reticulum (*T*) as they typically appear within glycogen areas. Glycogen was demonstrable in these areas in unincubated controls. *M*, mitochondrion; *Mb*, microbody; *P*, plasma membrane. Bars represent 1 μ.

where deposits of reaction product obscured the membranes, because of the characteristic arrangement of these organelles in the 1-day-old mouse hepatocyte. This morphological arrangement was observed more readily in sections incubated in the substrateless medium, where there was no reaction product (Fig. 1, bottom). No localized reaction product was noted after incubation in control substrates or after incubation in acetate buffer at *p*H 5.0 prior to incubation in the glucose-6-phosphate medium.

Tice and Barrnett (*7*) reported glucose-6-phosphatase activity in the ergastoplasm and nuclear envelope of adult rat hepatocytes but noted that the glycogen areas under the conditions of their study were devoid of glucose-6-phosphatase activity as well as cell organelles. They did, however, describe reaction product in a moderate proportion of distorted small vesicles of the smooth-surfaced endoplasmic reticulum and little or no activity in larger, swollen, smooth-surfaced vesicles. Some of the small vesicles containing reaction product were situated at the periphery of the cell, and occasional ones abutted on glycogen areas. It is also known from cytochemical studies on rat livers that some microsomal subfractions of smooth membrane show glucose-6-phosphatase activity, whereas others do not (*7* and *9*). However, as in earlier histochemical studies, the smooth membranes were not identifiable as TER.

The present work, identifying the TER as a site of glucose-6-phosphatase activity, provides additional evidence for the relationship of TER to hepatic glycogenolysis. This is not to suggest that glycogenolysis is the sole function of the TER. It has been demonstrated that the activity of hepatic esterase sensitive to E600 (diethyl-*p*-nitrophenyl ester of phosphoric acid) is localized in the TER as well as the nuclear envelope and ergastoplasm (*10*). Furthermore, the presence of glucose-6-phosphatase may itself indicate multifunctional potential in view of biochemical evidence for the identity of this and two other enzymes, namely, inorganic pyrophosphatase and pyrophosphate phosphotransferase (*11*).

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Density-Current Plumes

Abstract. *Diurnal solar heating produces an unstable warm zone just off the bottom of the inshore regions of a salt lake. The warm water rises in plumes in which brine shrimp become entrapped through apparently negative photokinetic behavior. The plumes of concentrated shrimp resemble those composed of insects in air.*

My observations of brine shrimp and their environment may increase our knowledge in two areas recently discussed in *Science*. The report of Peterson and Damman (*1*) and comments thereon (*2*) present two well-established observations: Visible plume-like formations occur over trees; and flying insects sometimes appear in plume-like formations, often over trees. From thermal considerations it has been inferred that these plumes may at times contain convectively rising warm air.

I have observed similar plumes of arthropods in an aquatic medium. In inshore waters of Mono Lake, California, when the population of brine shrimp (*Artemia salina*) is at maximum during the summer, concentrations of thousands of these plankton may be seen rising in graceful turbulent plumes from stones or calcium carbonate concretions. The plumes, varying in size from a few cubic decimeters to several cubic meters, may contain shrimp at more than 1000/dm^3. A similar phenomenon was observed in Great Salt Lake, Utah, in 1872 (*3*). The plumes appear to be closely associated with thermal and radiative processes of the inshore region, and I offer the following observations to support an explanatory hypothesis that may be relevant to both the convection plumes over trees and the density currents in aqueous media described by Bradley (see *4*).

The plumes of shrimp at Mono Lake appear only in shallow regions, to a depth of about 4 m, when direct sunlight enters the water; they appear most strongly developed soon after noon on calm, clear days; they are not seen when wind actively stirs the water.

The plumes often hover for long periods over a rock and always have their bases on the sunny side—never on the shady side; they slowly disperse when artificially shaded. Plumes may drift along the shore and move gradually upslope. The axis of the plume is frequently inclined away from the sun. Within the water of the plume, temperatures are several tenths of 1°C higher than in the adjacent strata.

Filtered plume water, when compared with filtered water from outside the plume, has a considerably higher energy absorption at several specific ultraviolet wavelengths and in the red region (Fig. 1); this fact suggests that the plume water may contain one or more dissolved organic compounds, possibly including certain breakdown products of plant pigments. Addition of 30 mg of vitamin-free casein to lake water produced an absorption peak at 230 nm comparable to the prominent peak found in natural waters. By wet-oxidation methods, the dissolved organic carbon of pelagic Mono Lake water was determined to be 62 mg/liter (*5*)—a very high value for natural media. The casein added to this water did not affect specific viscosity, which was 1.20 [considerably lower than that reported (*4*) for $NaHCO_3$ of comparable density]. Possibly, however, some of the naturally occurring organic compounds may reduce the viscosity (*6*) and "lubricate" the stream-tubes comprising the rising density currents. Garman (*3*) noted that breezes produced no ripples on water overlying swarms of shrimp; this fact suggests that plume water has a lowered surface tension—comparable to "slicks" at sea.

Dye-marker experiments and temperature observations indicate that just above the bottom in shallow regions there is a layer of water about 1 cm thick; it is warmer than the overlying water and presumably enriched with organic compounds from the mud (Fig. 2). This thermal layer causes a marked density instability, since the lake water expands considerably more per 1°C than pure water (*7*). The instability is apparently relieved by upward flows along warmed transisothermal surfaces (such as rocks) or in local up-funneling "thermals" that may travel along or across bottom contours, draining the warm water upward as they move. Grids of fluorescein dye laid by

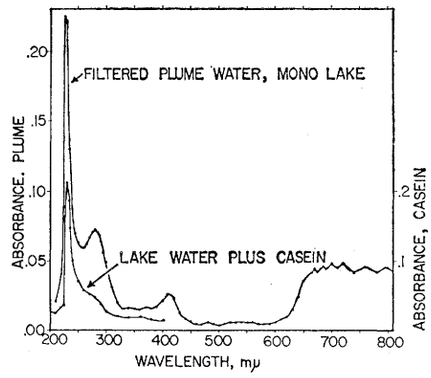

Fig. 1. Absorbance spectra of Mono Lake water from within a plume of shrimp (23 July 1964) and of pelagic water to which 30 mg of vitamin-free casein had been added (7 November 1964). The first measurement was made against water collected adjacent to the plane; the second was made against a normal pelagic-water blank; 1-cm quartz cells were used in a Beckman-DU spectrophotometer. All water had been filtered through a Whatman GF/C pad.

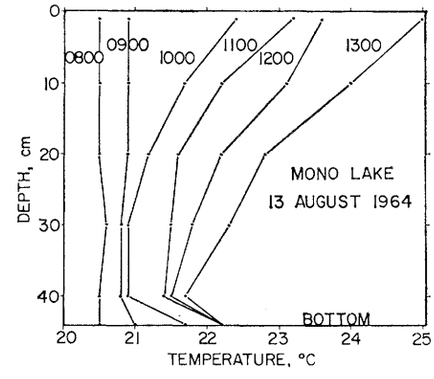

Fig. 2. A series of temperature profiles with depth in a shallow, inshore location in Mono Lake. Observations began at 0800 hours P.S.T. before significant heating from the sun, which was then about 30 deg above horizon. The thermistor-bridge circuit used, recently calibrated, was accurate within 0.01°C.