crystal chemistry (*8*). This problem no longer exists since the wolframite-type molybdates have been synthesized. The reason for the requirement of pressure to produce wolframite-type molybdates is part of the common and largely unexplained problem of polymorphism. It is evident from this and other investigations that prophecies concerning possible high pressure polymorphs are justified. However, to explain why pressure is required for their production is not generally possible with present theories of crystal chemistry (*9*).

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## Morphogenesis of Syncytiotrophoblast in vivo: An Autoradiographic Demonstration

Abstract. *In an in vivo autoradiographic study of immature primate trophoblast, tritiated thymidine was first found only in nuclei of cytotrophoblast but after 22 hours, labeled nuclei appeared in syncytiotrophoblast as well. Hence syncytiotrophoblast of the monkey appears to originate by differentiation of cytotrophoblast.*

The origin and function of syncytiotrophoblast has been a controversial subject (*1, 2*). Electron microscopic and immunohistochemical studies of benign and malignant trophoblast have strongly supported the concept that syncytiotrophoblast develops from cytotrophoblast as the differentiated form of trophoblast and has as a major function the synthesis of chorionic gonadotropin (*3, 4*). These conclusions would be proved if it could be shown that placental cytotrophoblast differentiated in vivo into syncytiotrophoblast.

Therefore an attempt was made to selectively label and follow the fate of cytotrophoblastic cells. Rhesus monkeys were selected because they have a chorio-allantoic type of placenta resembling closely that in man. Tritiated ($H^3$) thymidine, which is incorporated into newly synthesized deoxyribonucleic acid (DNA), was chosen for the label since, of the two trophoblastic cell types, mitosis has been demonstrated only in cytotrophoblast.

To ensure the use of immature developing trophoblast, early pregnancies were detected by assaying for chorionic gonadotropin, a hormone detectable in the serum of rhesus monkeys only between the 14th and 30th days of a 5- to 6-month gestation (*5*). The assay for chorionic gonadotropin was based on the appearance of ovarian hyperemia in weanling rats 3 to 18 hours after intraperitoneal injection of 1 ml of monkey serum. Pregnant monkeys were injected intravenously with 0.5 $\mu$c of $H^3$-thymidine per gram of body weight, and at intervals thereafter (Table 1) they were anesthetized with pentobarbital and either a hysterotomy with placental biopsy or hysterectomy was performed. All tissues were fixed in Bouin's fluid, embedded in paraffin, sectioned at 4 $\mu$, coated with Kodak AR 10 stripping film, and exposed at 4°C in plastic boxes containing Drierite. After sufficient exposure the slides were developed, stained with Harris' hematoxylin, differentiated in 0.2 percent HCl, and blued in 0.5 percent sodium acetate.

Immature chorionic villi were obtained from four monkeys at seven intervals and, as indicated in Table 1, in the first 12 hours all labeled nuclei appeared in cytotrophoblast; no labeled nuclei were seen in syncytiotrophoblast (Fig. 1). At 22 hours there were a few labeled nuclei in cells apparently intermediate in position between cytotrophoblast and syncytiotrophoblast, and a few more in outright syncytiotrophoblast. At 48 and 72 hours, labeled nuclei were found in both cytotrophoblast and syncytiotrophoblast in approximately equal numbers (Fig. 2). Since there was no evidence of independent synthesis of DNA in syncytiotrophoblast, which is in accord with the in vitro studies of Richart (*2*), the hypothesis that the syncytium increases by amitosis would appear to be untenable. Thus we concluded that syncytiotrophoblast is derived by differentiation of cytotrophoblast.

To substantiate this conclusion we studied immature villi and trophoblastic columns from these monkeys with an electron microscope. Our results (*6*) show that syncytiotrophoblast contains many dilated cysternae of endoplasmic reticulum, a feature of functioning differentiated cells (*7*), while cytotrophoblast contains many free ribosomes with few profiles of endoplasmic reticulum, a feature of rapidly dividing, undifferentiated cells (*8*). Cells midway in position between cytotrophoblast and syncytiotrophoblast (intermediate cells) possess features of both cell types. This suggests a developmental relationship between the undifferentiated and differentiated cells such as has been shown for other systems (*8*). Previous ultrastructural studies have shown similar intermediate cells in malignant human trophoblast (*3*).

Syncytiotrophoblast of the monkey seems to originate solely by differentiation from cytotrophoblast and, as the differentiated form of trophoblast, should be expected to perform the specialized functions of trophoblast. In

Table 1. Localization of nuclei in trophoblast labeled with $H^3$-thymidine. Time is given in hours after administration of $H^3$-thymidine.

| Time (hr) | Trophoblast | | |
|---|---|---|---|
| | Source | Cyto- | Syncytio- |
| 1 | Biopsy | + | − |
| 2½ | Biopsy | + | − |
| 4 | Hysterectomy | + | − |
| 12 | Hysterectomy | + | − |
| 22 | Hysterectomy | + | + |
| 48 | Biopsy | + | + |
| 72 | Hysterectomy | + | + |

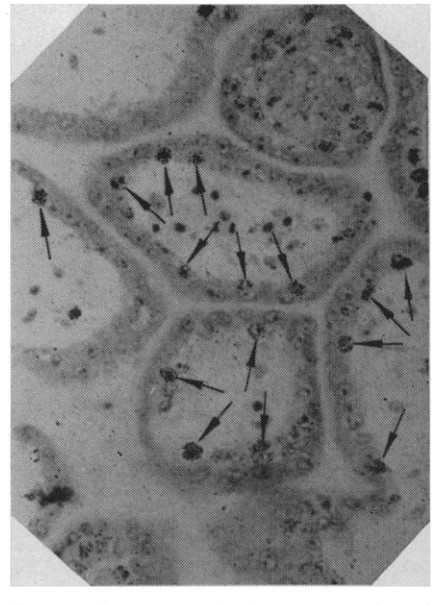

Fig. 1. Placental villi from a monkey 1 hour after it had received $H^3$-thymidine. Only cytotrophoblastic nuclei are labeled (see arrows). There are no labeled nuclei in syncytiotrophoblast.

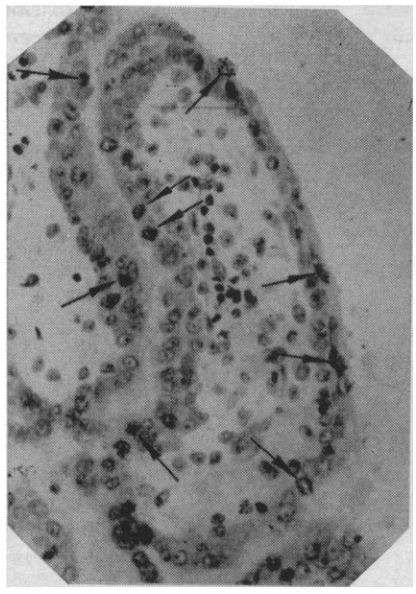

Fig. 2. Placental villi from a monkey 48 hours after it had received $H^3$-thymidine. A large proportion of labeled nuclei are now in syncytiotrophoblast (arrows).

this context our earlier immunohistochemical demonstration of human chorionic gonadotropin in the cytoplasm of human syncytiotrophoblast must indicate that syncytiotrophoblast synthesizes this hormone. Cytotrophoblast on the other hand contains no chorionic gonadotropin (*4*), is an embryonic form of trophoblast which divides rapidly, is ultrastructurally undifferentiated, and lacks the organelles for specialized function. Similar evidence demonstrating that human placental cytotrophoblast in vitro can differentiate into syncytiotrophoblast has been found (*9; 10*).

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## Pleistocene Marine Microfauna in the Bootlegger Cove Clay, Anchorage, Alaska

Abstract. *Ostracods and Foraminifera, associated with molluscs, indicate a marine depositional environment for part of the Bootlegger Cove Clay. The definite Arctic and North Atlantic affinities of the microfauna suggest a possible migration through the Bering-Chukchi seaway during the late Pleistocene.*

Anchorage, Alaska, situated at the head of Cook Inlet, is built on a dissected gravel plain. Underlying these gravels and younger deposits, and exposed in bluffs along Knik Arm and Cook Inlet, is the Bootlegger Cove Clay. The depositional environment under which the clay was deposited has been the subject of debate. It may be lacustrine or freshwater, estuarine to marine, or, as some workers conclude, partly marine and partly lacustrine. Miller and Dobrovolny (*1*) state that its environment is unknown but favor a lacustrine environment. Karlstrom (*2*) and Trainer (*3*) recognize a vertically restricted middle marine zone in the Bootlegger Cove Clay. Karlstrom considers that this marine zone is underlain and overlain by lacustrine clay and silt. Discovery of a marine microfauna in the Bootlegger Cove Clay supports the view that, in part, the clay was deposited in a marine, or estuarine, environment.

The Bootlegger Cove Clay is of Pleistocene age and occurs between tills of Knik and Naptowne age. Its stratigraphic position and lithology were described in detail by Miller and Dobrovolny. They consider the Knik Glaciation to be of pre-Wisconsin age. Karlstrom believes that the Knik Glaciation was pre-Wisconsin but post-Illinoian (*2*). Peat beds appearing to occur at the same stratigraphic horizon as the Bootlegger Cove Clay have been dated as "older than 38,000 radiocarbon years" (*1*, p. 15). Shells within the Bootlegger Cove Clay were dated by ionium-uranium methods as 46,000 to 31,000 B.C. (*2*, p. 330).

A major point in the discussion about the origin of the formation has been whether fossil molluscan shells collected along the bluff have been washed up from the inlet, or whether they were in material that was "unquestionably undisturbed." The marine molluscs collected along the bluff in sections 22 and 23, T13N, R4W, S.M. by Miller and Cooley have been identified by F. S. MacNeil as *Buccinum* cf. *B. physematum* Dall, *Odostomia (Evalea)* sp., *Nuculana fossa* Baird, *Cardium ciliatum* Fabricius, *Macoma* cf. *M. sabulosa* Gmelin, *Saxicava pholadis* Linné, and *Mya truncata* Linné (*1*, p. 45).

Up to now, no reference has been made to any evidence of a fossil microfauna by any previous investigators. In July 1962 J. R. Williams of the U.S. Geological Survey and I revisited the exposures of the clay along Knik Arm. In a freshly exposed bluff in NW¼-SW¼ sec. 22, T13N, R4W, S.M., 5.7 km (3½ miles) southwest of Anchorage, a shell bed was clearly exposed in an undisturbed section of the bluff. The bluff, the top of which was approximately 15.3 m (50 ft) above water level at high tide, was channel-

Table 1. Sampled section along bluff of Knik Arm in NW¼SW¼ sec. 22, T13N, R4W, S.M.

| Sample No. (from top to bottom) | Description | Thickness of bed * (m) |
|---|---|---|
| | *Top stratum* | |
| 8 | Clean gray sand and soil. No fossils. | 0.14 |
| | *Bootlegger Cove Clay* | |
| 7 | Silty clay, dark greenish gray, lighter gray laminations. No fossils. | 1.68 |
| 6 | Silty clay, dark greenish gray. No fossils. | 2.54 |
| 5 | Silty clay, greenish gray. Uppermost pelecypod fragment in outcrop. Foraminifera and Ostracoda rare. | 1.37 |
| 4 | Silty clay, greenish gray, with sandy partings. Foraminifera and Ostracoda present. | 1.02 |
| 3 | Silty clay, greenish gray, with sandy partings; contains small pebbles and occasional coal fragments. Molluscs abundant upper 1.3 m (50 inches), lacking lower 0.25 m (10 inches). Foraminifera and Ostracoda abundant. | 1.42 |
| 2 | Silty clay, dark greenish gray, containing angular pebbles and cobbles. Foraminifera and Ostracoda rare. | 1.37 |
| | *Bootlegger Cove Clay but offset 3.6 m to east* | |
| 1 | Silty clay, dark greenish gray, angular and rounded pebbles and cobbles. Foraminifera rare, no Ostracoda. | 2.74 |
| 0 | Remainder of bluff covered by slump to high tide level. | 2.67 |

* English equivalents are as follows: sample 8, 0 ft, 5.5 in.; sample 7, 5 ft, 6 in.; sample 6, 8 ft, 4 in.; sample 5, 4 ft, 6 in.; sample 4, 3 ft, 4 in.; sample 3, 4 ft, 8 in.; sample 2, 4 ft, 6 in.; sample 1, 9 ft, 0 in.; and sample 0, 8 ft, 9 in.