Initial attachment and proliferation of rat keratinocytes on selected membranes and plastic culture vessels have been compared and reported (25). The extent of attachment and growth of cells on opaque membranes were determined after fixing the cells and substratum in 10% phosphate-buffered formalin or Carnoy's solution (90% butanol, 10% acetic acid). The fixed culture was stained with hematoxylin, dehydrated, cleared with xylene, and mounted on microscopic slides with Permount and coverslips. Quantification of initial attachment and subsequent proliferation was accomplished by counting stained nuclei per unit area of the substratum. This was performed electronically with the Bioquant Image Analysis system on the Apple IIE microcomputer and accessories. Cross sections for microscopic examination were obtained by first fixing selected cultures in 10% buffered formalin, embedding in paraffin, sectioning with a rotary microtome, and mounting sections on microscopic slides and, finally, staining with hematoxylin and eosin (H&E).

Epidermal cultures grown on synthetic membranes were also examined at the ultrastructural level. Cultures were fixed in Karnovsky's fixative, washed, and prestained with 2% osmium tetroxide, stained enblock with uranyl acetate, dehydrated, and embedded in resin. The embedded specimens were polymerized, sectioned, poststained with uranyl acetate and lead citrate, and observed with an AEI Corinth 275 transmission electron microscope (TEM).

Figure 3 illustrates the initial attachment (see Fig. 3A) and ensuing proliferation (see Fig. 3B) of rat keratinocytes on synthetic membranes, compared with growth on a plastic substratum. The data in this figure indicate that cultivation on some of the selected membranes was superior to that on plastic culture vessels used as controls. Observation of cultures after a 1-day incubation showed that aggregates of keratinocytes had attached and spread out, forming growth centers. The large variation in cell number per unit area seen in Figure 3A reflects the different size and unequal distribution of growth centers in early cultures. After 10 days of incubation, monolayers had formed with varying degrees of compactness, depending on the substratum (see Fig. 3B). Membranes specially prepared for tissue culture procedures (i.e., TCM200, TCM-450, HA-TF, and RA-TF) supported attachment and proliferation equal to or better than the plastic substratum. The increased attachment and proliferation of keratinocytes on Puropor nylon membranes (P200 and P450) over controls was statistically significant and proliferation on P200 was superior to the others examined. Therefore, this membrane was selected for further study in producing stratified, differentiated keratinocyte cultures in vitro (25).