^{3}H-uridine. The cell pellet was divided into 2 portions and RNA was extracted by both methods. Purified RNA was annealed with adenovirus DNA on nitro-cellulose filters (Table II). The RNA sample purified by the hot phenol-SDS method showed about 9% homology with adenovirus DNA and RNA purified by DEPC-SDS method also showed over 7% hybridization. In both cases very low background binding to blank filters was found. This result is in agreement with previously reported hybridization analysis of adenovirus RNA prepared with penol[15]. Therefore, RNA prepared with DEPC method was suitable for DNA-RNA hybridization experiments.

Polyacrylamide gel electrophoresis of RNA. The integrity of cytoplasmic RNA prepared by phenol-SDS or DEPC-SDS from various cell lines was studied by polyacrylamide gel electrophoresis. Figure 1 shows the electrophoresis profiles of ^{14}C-uridine labeled RNA prepared by the phenol-SDS procedure from KB cell cytoplasm and ^{3}H-uridine labeled RNA prepared by the DEPC-SDS method from the same cells. The profiles of RNA extracted by both methods are exactly the same. The ratio of 28 S RNA to 18 S RNA in both samples was also very similar. RNA samples prepared by these 2 methods, from cynomolgus monkey kidney cells (YCB-1) showed the same result (Figure 2). Similar results with mouse cells were also obtained. Thus, it is obvious that the DEPC-SDS method is satisfactory for gel electrophoresis studies to determine the molecular weight of RNA and its size distribution.

Discussion. RNA preparations purified by both DEPC and phenol extraction methods showed the same profile with intact 28 S and 18 S ribosomal RNA. Thus, the DEPC method can provide undegraded RNA molecules for size analysis by polyacrylamide gel electrophoresis.

RNA samples, prepared by both methods, from adenovirus 2 infected KB cells annealed with homologous viral DNA with similar efficiency, indicating the suitability of the method for the extraction of viral RNA from infected cells. This results suggests the possibility of applying the DEPC-SDS method for RNA purification with viral or cellular DNA-RNA hybridization experiments.

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A New Substrate for Cultures of Dissociated Primary Rat Brain

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Summary. Different substrates were used to coat plastic petri dishes for the cultivation of dissociated fetal rat brain cells. Only on surfaces which were coated with a mixture of serum and non-reconstituted collagen, did the majority of the inoculated cells attach singly or as aggregates within 24 h. The attachment of the cells was followed by the outgrowth of cellular processes either from single cells or from aggregates in the same time period. This did not occur on collagen or serum treated or on regular plastic dishes. Under the latter conditions a similar outgrowth was observed only after 3–5 days.

A number of different substrates have been used for the cultivation of dissociated brain cells in culture. The most widely used substrate is collagen isolated from rat tendons, which is reconstituted either by ammonia vapors[3] or by riboflavin[4]. Non-reconstituted collagen has also been described as a substrate for nerve cell cultures[5]. Alternatively, polylysine coated surfaces have been recommended to circumvent possible selectivity during the attachment and cultivation of dissociated brain cells[6]. Other workers have resorted to untreated Falcon plastic dishes for the cultivation of dissociated brain cells from different species[7–9].

Here we report the short-term effect of a two-component substrate on attachment and outgrowth of cellular processes from dissociated fetal rat brain cells.

Material and methods. Cell cultures were prepared by a slightly modified Shapiro and Schrier method[7]. Timed Fisher strain rats (17–19 days pregnant, Iffa Credo, Lyon, France) were anesthetized by exposure to CO_2 for 1 min, the fetuses removed and their brain tissue dissociated mechnically by forcing it through a 210 µm nylon cloth glued to a 10 ml plastic syringe. A single cell suspension was obtained by gravity flow through 130 µm nylon cloth. Coated (see below) or uncoated Falcon tissue culture dishes (35 × 10 mm) were used and inoculated with 1.5 mg/cm^2 wet brain tissue (1 ml of a 15 mg/ml cell suspension).

The culture medium consisted of Dulbecco's modified Eagle medium (Gibco), supplemented with 4.5 g/l glucose, 20% (v/v) fetal calf serum (Gibco), 10 units/ml Na-Penicillin G and 10 µg/ml streptomycin sulfate. Cultures were incubated at 37 °C (95% humidity) in 10% CO_2, 90% air.

Petri dishes were coated in the following way. We added either 2 drops of diluted or undiluted serum or 2 drops of a 1 mg/ml collagen solution (calf skin, A grade, Calbiochem) in 0.017 *N* acetic acid or 2 drops of a mixture of fetal calf serum and collagen (1 mg/ml non-reconstituted) in a 1:3 ratio. Serum dilutions were routinely made with H_2O. The liquid was evenly dispersed on the surface of the dish and dried completely at room temperature in a sterile hood under air current. Coated dishes were equilibrated in the CO_2 incubator with 1 ml of serum supplemented medium before being inoculated with the cell suspension.

Results and discussion. When fetal calf serum (5 to 100%) or reconstituted or non-reconstituted collagen were used separately as substrates, dissociated brain cells did not attach to the coated surface within 24 h (Figures A and B). Most of the cells were floating singly or as cell aggregates of variable size. When serum and collagen (1.0 mg/ml) were mixed (1:3, v/v) and used as

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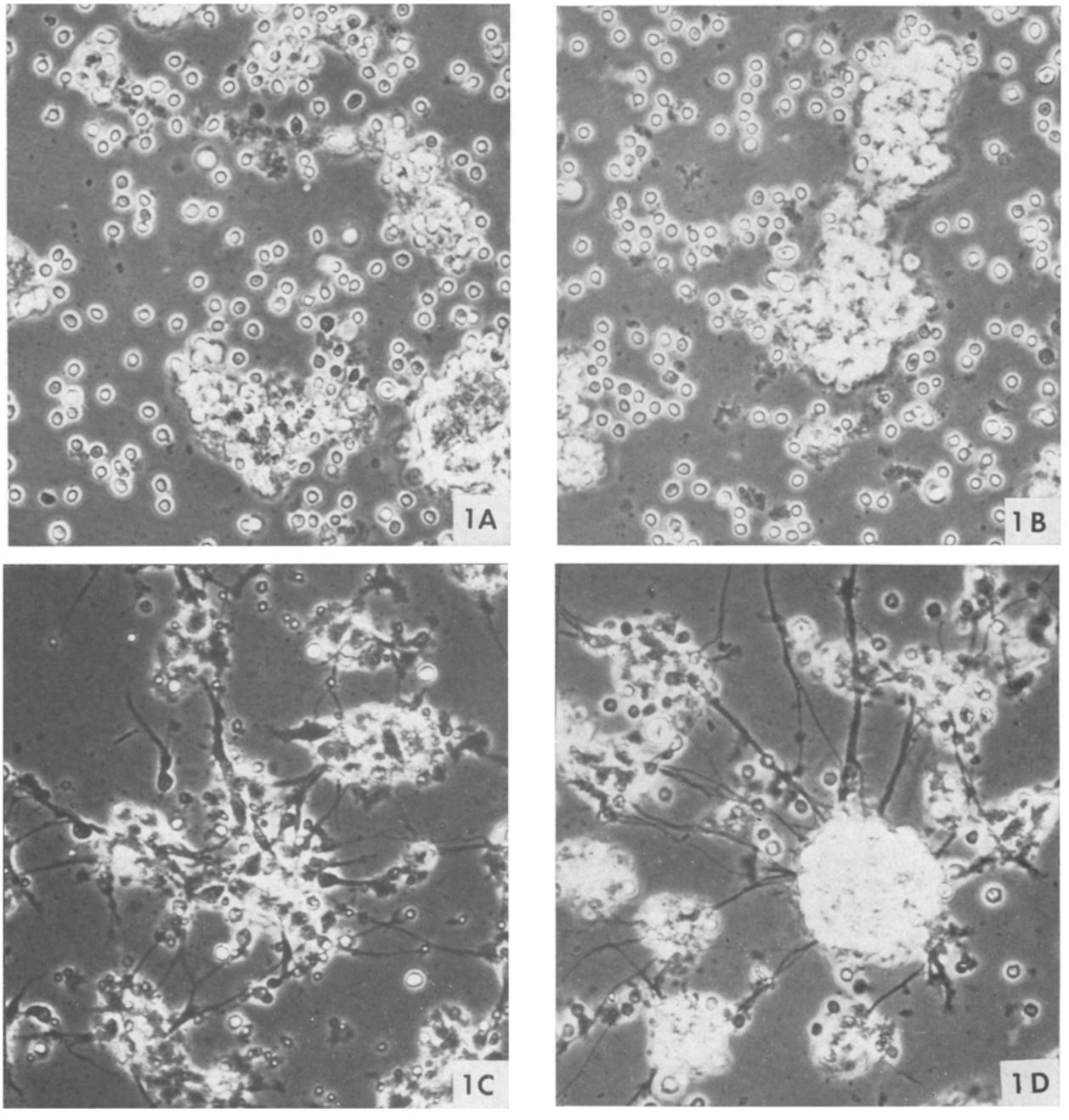

Mechanically dissociated cells from fetal rat brain were inoculated on different substrates and observed after 20–24 h in culture. The plastic dishes were coated either with fetal bovine serum (A) or collagen (B). The mixed substrate, consisting of fetal bovine serum and collagen (1:3 v/v), was used to coat dishes C and D. Phase contrast. ×150.

substrate, the cells adhered to the surface after 20–24 h of incubation. In addition to the rapid attachment of the cells on the serum-collagen coated dishes, a network of cellular processes could be observed. Processes originated from single cells and appeared also in a solar radiation arrangement from cell clumps (Figure C and D). Serum concentrations of $\leq 5\%$ mixed with a constant amount of collagen (0.5 mg/ml) did not permit the attachment and outgrowth of cells. Untreated or heat-inactivated fetal calf, horse or bovine serum mixed with 0.5 or 1.0 mg/ml non-reconstituted collagen (1:3), all produced similar positive results.

The attachment and behaviour of dissociated cells on poly-L-lysine treated surfaces was compared to cultures on serum-collagen coated plastic dishes. Although cellular attachment appears to be much faster on polylysine than on serum-collagen, the outgrowth of cellular processes and the formation of a dendritic network seems improved in the latter.

Whether the effect of the dual substrate is due to growth promoting factor(s)[10–12], charged protein(s) in the serum[13] or a combination of the two has yet to be elucidated.

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