perturbations when switching. The rate of extension is adjustable from 7 μm/min to 3500 μm/min and calibration is effected by means of a built in dial-gauge (G) with a range of 5 mm and a resolution of 1 μm (Tenso, Huggenberger, Zürich). The construction of the system is sufficiently accurate to ensure smooth movement at the micron level, and a compressed automobile valve-spring (F) (c. 5 kg/cm, BMC, Coventry, U.K.) is used to eliminate backlash in the screw system.

The force transducer (4.5 kg max., type UF2, Pye-Ether, Stevenage, U.K.) is rigidly mounted on a brass plate attached perpendicularly to the sliding saddle. The mounting holes in this plate are slotted so that accurate alignment of the system is possible. The compliance of the transducer is such as to produce an error of only 0.04 mm at maximum loading.

Two types of specimen grips are used, depending upon the nature of the specimen. For horizontal operation with very fragile specimens, the specimen ends are cemented to 2 flattened rods which screw into the force transducer and sliding frame respectively. For routine measurements on less fragile samples, Instron pneumatic grips (K) (type 2710-002, Instron Ltd., High Wycombe, U.K.) are used, and the device is operated in the vertical mode. The upper grip is suspended on gimbals.

Each measuring system, for force and displacement, utilizes a coherent amplifier designed and described by GERARD[2]. The transducers are activated by stable audio-frequency oscillators, which also supply the reference signals for the coherent amplifiers. The output from each transducer is fed into a coherent amplifier and a D.C. voltage is obtained which is accurately proportional to the property being measured. These rectified outputs drive the appropriate channels of an X-Y recorder. In this way, accurate measurements of force versus extension are recorded continuously over wide ranges of sensitivity and with a linearity of better than 1%[3].

Zusammenfassung. Es wird eine einfache und ökonomische Vorrichtung zur kontinuierlichen Aufzeichnung von Druckbelastungs- und Spannungs-Kurven biologischer Baustoffe beschrieben.

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A Microtube Oscillation Method for Cellular Reaggregation

Cell aggregation has been used to study both histotypic rearrangement (MOSCONA[1]) and the strength of cellular adhesion (EDWARDS[2]). A comparison is made in the Table, between various methods, contrasting numbers of cells needed, the interference of a solid surface, and the facility for observing the formation of individual aggregates. (CURTIS and GREAVES[3], CUNNINGHAM and HIRST[4], JONES and KEMP[5], OPPENHEIMER and OLDENKRANTZ[6], HENKART and HUMPHREYS[7], ROUX[8] and HERBST[9]).

This paper describes the use of an acoustic pump for studying aggregation of very small numbers of cells in suspension, under direct observation.

Materials and methods. HeLa cells, CBM17 cells, (FRANKS and HENZELL[10]) or primary mouse embryo cells were suspended, at a density of between 10^4 and 10^5 per ml in culture medium with Hepes buffer and calf serum.

Aggregation chambers were made from the fine ends of disposable Pasteur pipettes. These were bent through 90° about 1 cm from the end, and again in a plane perpendicular to the first bend a further 3 cm along the tube. They were cut 3 cm beyond that and were flame polished. Each tube was fitted with a minute, centrally bored, silicone rubber bung, and was sterilized by dry heat.

The bungs fitted into a short plastic tube connected to the ear mould connection of a Madresco OL 575 hearing aid earpiece. The latter served as a pressure transducer, driven by an oscillator operating at low audio frequencies (20 to 200 Hz).

The open end of the tube was submerged in liquid paraffin in a Petri dish; the entire apparatus being held with Plasticene upon an aluminium plate with a central hole. A second tube, not connected to a transducer was

Method	Direct observation of aggregation	Cell numbers needed	Third phase attachment	Principle	References
Shakerflask	None	High	None	Cell collision	1
Turbidimetric	None	High	None	Cell collision	3–5
Miniature shaker	None	Moderate	None	Cell collision	6, 7
Settling	Satisfactory	Low	Dominant	Cell rearrangement	8, 9
Microtube oscillation	Satisfactory	Low	None	Cell collision and clustering	

placed with the submerged end parallel to the aggregation tube (Figure 1).

The oscillator was switched on, and using a fine glass micropipette, cells in suspension were introduced into each tube. A volume of about 0.025 ml gave a cylinder of medium contained in a glass tube, closed at each end by a paraffin/medium meniscus. The cylinder connected to the earpiece oscillated along the axis of the cylinder.

The tube contents were observed and photographed with an inverted microscope; a small substage condenser aperture being used to give optimum contrast. The oscillator was turned off during exposures, with the exception of Figure 2.

Results. Cells oscillated over a distance of 100 µm at the tube axis and 10 µm at the tube wall. They collected in zones, dependant upon oscillator frequency (Figure 4A), and formed small aggregates which moved to one or other meniscus (Figure 4B). Individual cells and aggregates could be watched whilst moving, appearing as small rods (Figure 2). Aggregates, built up over a period

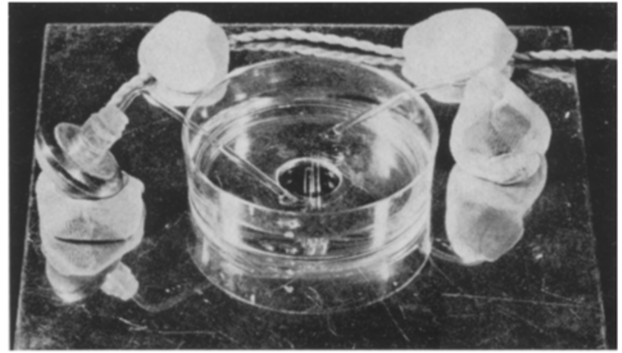

Fig. 1. Microaggregation apparatus assembled beside a 5 cm Petri dish.

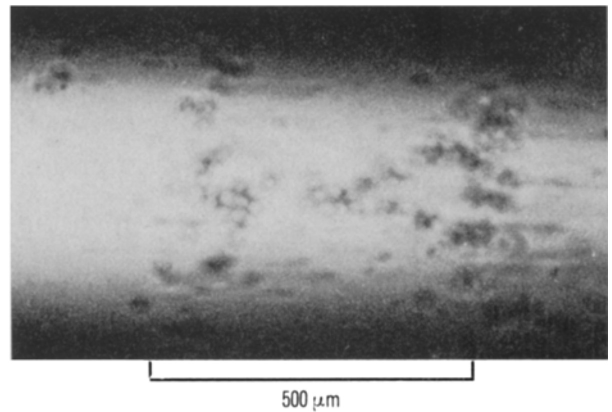

Fig. 2. Microscopic appearance with oscillator on.

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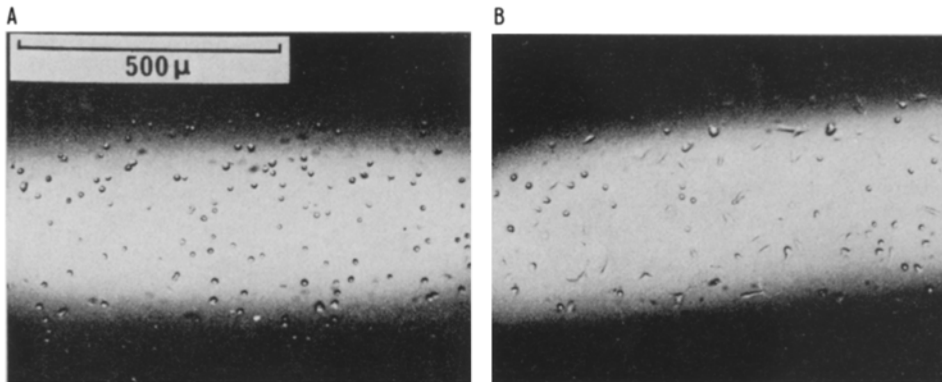

Fig. 3. CBM17 cells in control culture showing the flattening of well distributed single cells. A) appearance at 10 min; B) appearance at 5 h.

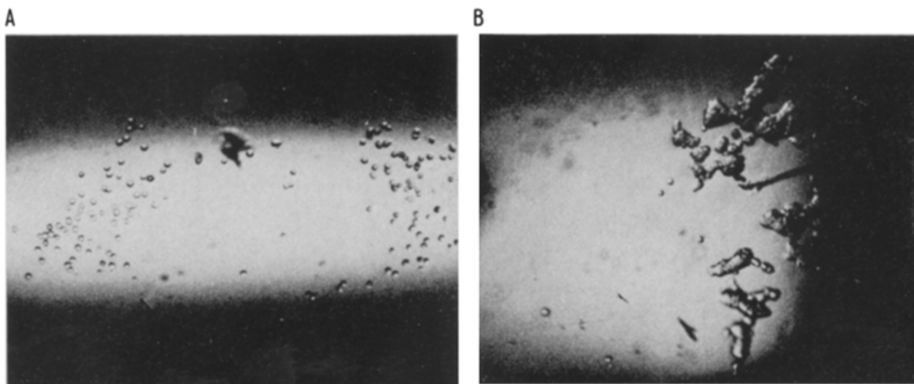

Fig. 4. Cells in oscillated culture showing clustering and aggregate formation. A) at 10 min with clustering in zones; B) at 5 h with clustering in zones.

of several hours, flattened on the tube wall if allowed to settle and retain contact. In the 'still' tube, cells flattened and grew as a monolayer (Figures 3A and B). If the liquid paraffin was replaced by tissue culture medium, the oscillations caused a rapid and controllable jet of medium distal to the orifice (Stoker[11]).

Discussion. Cells in oscillating columns of culture medium could not settle. Cells at the centre of such columns moved more rapidly than those at the periphery, hence collisions occurred. Stable clustering at sites determined by oscillator frequency indicates an harmonic effect, and this with the gathering at the menisci show that factors other than collision and shearing apart were involved in these experiments. Control and test cultures were viable and of greatest importance, could be observed during formation without the interference of a static surface.

The method reduces the number of cells and medium volume considerably, compared with suspension techniques previously described, and allows observation of form and size throughout experiments. It has been suggested that numerical assessment of aggregation is feasible.

Résumé. Description d'un appareil pour l'étude de la réagrégation des cellules. Observation de la formation d'agrégats individuels ne comprenant que de très petits nombres de cellules. Les cellules individuelles et les agrégats sont maintenus en suspension dans une colonne liquide oscillante.

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Freies Thyroxin im Serum durch Ultrafiltration

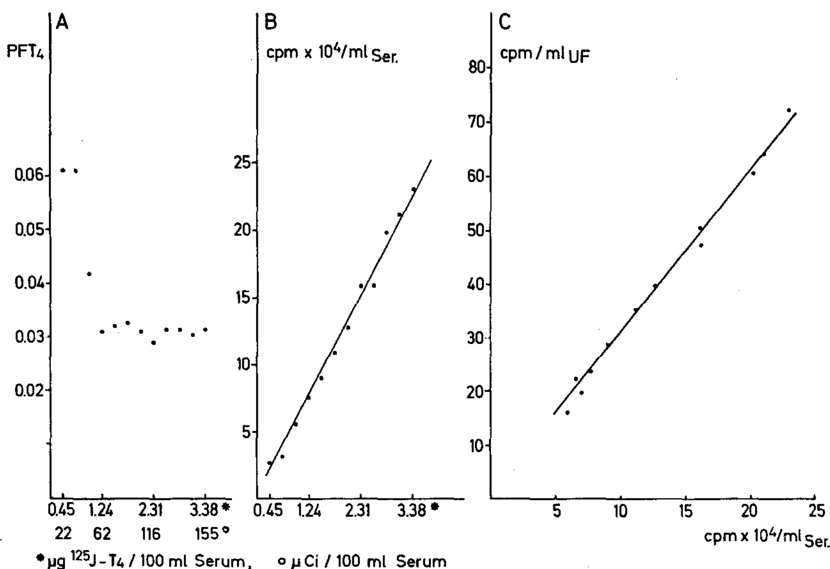
Während die quantitative Erfassung der Schilddrüsenhormone durch Radioimmunoassay (Trijodthyronin, T_3) und Proteinbindungsanalyse (Thyroxin, T_4) routinemässig durchgeführt werden kann, fehlen bisher rasch durchführbare Nachweismethoden für den nicht an Trägerproteine gebundenen Anteil (FT_4). Dieser tritt in Beziehung zu Rezeptoren der Zellmembran und vermittelt die biologische Wirkung des Hormons. Da die T_4-Affinität der Proteine variabel sein kann, ist die FT_4-Bestimmung für die Interpretation abnormaler T_4-Werte diagnostisch wertvoll.

Methode. Blutentnahme (nüchtern) aus ungestauter Vorarmvene in 10-ml Vacutainer-Röhre (Schuco-International, Hamburg), Zentrifugieren und Übertragung von ca. 4.5 ml Serum in eine gleiche vorbeschriftete Röhre (pH-Protektion); nach Zusatz von 1–2 µCi/ml ^{125}J-Thyroxin (Amersham-Buchler, Braunschweig) kurz schütteln und 30 min stehen lassen (Raumtemperatur). Anschliessend Ultrafiltration während 2 h unter 3 atü N_2 in handelsüblichen Druckkammern (Millipore, Neu-Isenburg) über Cellulosenitrat-Membran (Ausschlussgrenze MG 10000, Sartorius, Göttingen) nach dem von PUTMAN[1] angegebenen Verfahren, das sich für Serienbestimmungen eignet. Bei Einsatz von 4 ml Serum beträgt die durchschnittliche Ausbeute an Ultrafiltrat 1.4–1.7 ml, die Mehrfachbestimmungen zu je 0.4 ml gestattet. Präzipitation und Waschen erfolgen nach STERLING und BRENNER[2] mit $MgCl_2$-Lösung (10%) in *Tris*-NaCl, pH 9.3 unter gleichzeitigem Zusatz von Kaliumphosphat-Puffer (0.15 *M*, pH 7.4) und Thyroxin (1 mg L-Thyroxin/ml 0.033 *n* NaOH). Berechnung:

$$PFT_4 = \frac{\text{cpm/ml Ultrafiltrat}}{\text{cpm/ml Serum}} \times 100 .$$

Die in Tabelle I angegebenen Werte für «absolut freies Thyroxin» (AFT_4) errechnen sich aus dem zeitgleich bestimmten T_4 (modifiziert nach MURPHY und JACHAN[3]).

Die Assoziationsbindung zwischen Hormon und Trägerprotein ist stark pH-abhängig. Mit der beschriebenen Technik ist der CO_2-Verlust gering und die Aktivitätsausbeute im Ultrafiltrat proportional der dem Serum zugesetzten Menge (Figur C). Die Reproduzierbarkeit für PFT_4 ist entsprechend konstant (Figur A), wenn nicht



Freies Thyroxin (PFT_4) Qualitätsprüfung. A) Variationskoeffizient in der Serie 3.95 (gleiche Probe, $n = 9$), Mindestzugabe 62µCi/100ml. B) Kontrolle des tracerbeschickten Serums. C) lineare Wiederfindung im Ultrafiltrat ($r = 0.997$).