maximal von 6 Kulturen je zwei Doppelansätze gleichzeitig mit Colcemid versehen, bzw. 12 Einzelansätze verarbeitet werden. Es ist aber durchaus möglich, ein grösseres Gerät zu bauen. In Figur 2 wird das Gerät schematisch dargestellt. Es besteht aus einem Zugmagneten (1) 220 V, 50 Hz, 3,8 kp Dauerbelastung, 42 mm Hub, einer Grundschale (2) aus Sustamid zur Aufnahme der Kulturflaschen (6), einer Druckfeder (3), einer Einhängescheibe (4), für die Einwegspritzen (7) und einer Druckscheibe (5), beide Scheiben ebenfalls aus Sustamidmaterial.

Der Zugmagnet steht in Verbindung mit einer elektrischen Zeitschaltuhr (8), die ausserhalb des Brutschrankes (9) steht und die Vorwahl des gewünschten Zeitpunktes der Einspritzung des Mitostatikums gewährleistet.

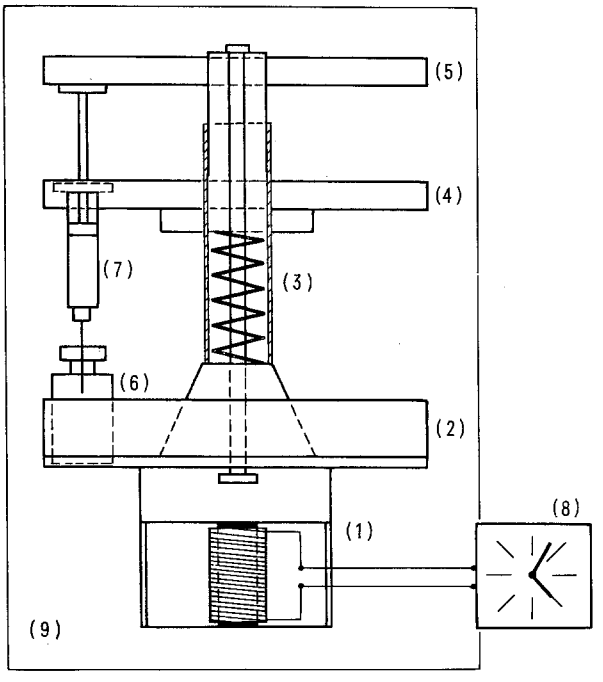

Fig. 2. Schematische Darstellung des automatischen Pipettiergerätes.

Die Zeitschaltuhr kann 24 h vorher eingestellt werden und schaltet das Pipettiergerät in einem 15 min Intervall ein und aus. Während der 15 min Schaltzeit fibriert der Zugmagnet und gewährleistet somit eine Durchmengung des Mitostatikums mit der Medienmenge.

Einstellung des Gerätes. Eine den Kulturflaschen entsprechende Anzahl an 1 ml Einwegspritzen wird mit der nötigen Menge Colcemid (zumeist 0.3 ml einer 10 µg/ml Colcemidlösung) versehen, die Nadel mit der Spritze in die Kulturflasche (besitzt als Verschluss einen Gummistopfen) eingestochen, die Nadel bis zum Anschlag vorgeschoben, die Spritze in die Einhängescheibe eingehängt und der Spritzenkolben bis zur Druckscheibe zum Anschlag aufgezogen. Damit entsteht ein Luftpolster zwischen Kanülenansatz und Mitostatikaflüssigkeit, wodurch ein Austropfen verhindert wird.

Zum eingestellten Zeitpunkt schaltet die Zeitschaltuhr den Zugmagnet ein, dieser zieht die Druckscheibe an und drückt somit die aufgesogene Menge Colcemid mit Druck in die Kulturflasche. Der Druck reicht für die leichte Aufwirbelung der Zelloberfläche aus, womit die Zellen in innigen Kontakt mit dem Mitostatikum gelangen. Druck und Hubweg können eingestellt werden. In Serienversuchen konnte die einwandfreie Wirksamkeit des Colcemids nach einer Verweildauer von 24 h im Pipettiergerät ermittelt werden.

Summary. An instrument for the automated and time-variable supply of mitostatica to leucocyte cultures for chromosome preparations is described. This instrument allows a routine laboratory to prepare cultures with a better time schedule. The action of colcemid was successfully tested after a 24-h period in the incubator before adding to the culture.

G. STRANZINGER und A. KUCHLER[1]

Institut für Tierzucht der Technischen Universität München, D-805 Freising-Weihenstephan (Bundesrepublik Deutschland, BRD), 18. September 1974.

[1] Der Hersteller des Gerätes ist: Elektromeister Alfred Kuchler, D-8399 Prienbach 153, Bundesrepublik Deutschland.

Measurement of Fibrinolysis in Rat System by One-Dimensional Diffusion Method

The most commonly used method of estimating fibrinolytic activity is based on the external lysis of preformed fibrin plate[1]. Most recently a one-dimensional diffusion method using small fibrin tubes was developed by YASUKOUCHI and WATANABE[2], and in the human system it has been proved to be more advantageous than the conventional fibrin plate technique. Methods measuring fibrinolytic activity in human system cannot be applied to rat, a species convenient for studying fibrinolysis, without modification[3]. In the present experiments the applicability of the above-mentioned method to rat system was established and the optimal conditions were determined.

Materials and methods. Blood was obtained from the inferior vena cava of Wistar male rats by a plastic syringe in deep pentobarbital anaesthesia. Euglobulin (Eug) fraction was prepared from the pooled plasma of 12 animals according to GALLIMORE et al.[3]. The precipitated Eug was redissolved in 1/10 volume of 0.12 *M* pH 7.4 sodium acetate solution (10 × Eug) and then diluted as indicated in the Table and the Figures.

[1] T. ASTRUP and S. MÜLLERTZ, Arch. Biochem. Biophys. *40*, 346 (1952).
[2] T. YASUKOUCHI and T. WATANABE, Thromb. Diath. haemorrh. *28*, 329 (1972).
[3] M. J. GALLIMORE, H. M. TYLER and J. T. B. SHAW, Thromb. Diath. haemorrh. *26*, 297 (1971).

To prepare fibrin tubes 0.5 ml thrombin (2 U/ml, Hoffman-La Roche) was mixed to 4.5 ml fibrinogen (Sigma Chemical Co.) solution (0.1% clottable protein in 0.1 *M* pH 7.4 *Tris* HCl buffer) in a petri dish. The mixture was pipetted into small calibrated glass tubes (7 cm long with a uniform diameter of 3.5 mm) exactly to the height of the calibration (50 mm from the bottom). After the coagulation had taken place, 50 µl, if not otherwise indicated, of Eug solution was added onto the top of the fibrin column. The length of the fibrin column dissolved after 18 h incubation at 37 °C was taken as a measure of the fibrinolytic activity. Each of the data was calculated as an average of triplicate determinations. The fibrin plate assay was carried out according to GALLIMORE et al.[3]. Occasionally tubes or plates were preheated at 70 °C for 60 min in order to inactivate their plasminogen content[4].

Urokinase (850 Ploug units/mg, Calbiochem) and EACA were dissolved in the diluted Eug solutions.

Results and discussion. Under the experimental conditions, the plasminogen content of both substrated fibrinogen and test Eug was activated (see differences between heated and nonheated fibrin tubes in Figure 1).

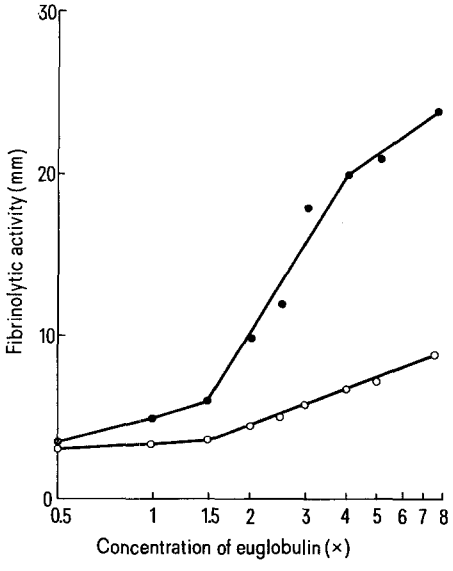

Fig. 1. Relation between fibrinolytic activity and Eug concentration. ○, heated; ●, nonheated.

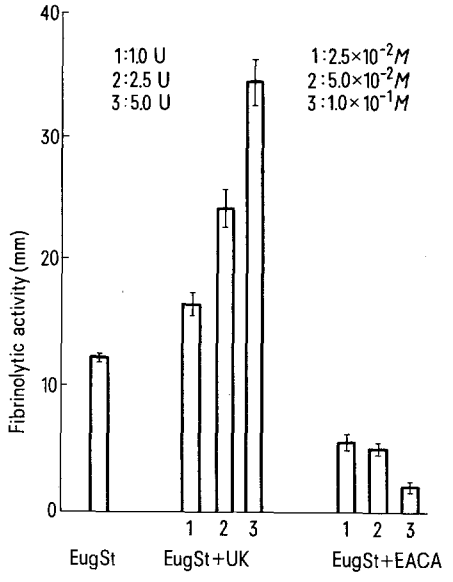

Fig. 2. The sensitivity of the fibrin tube assay to the activation (urokinase) and inhibition (EACA) of fibrinolysis. EugSt: 50 µl of 2.5 × Eug.

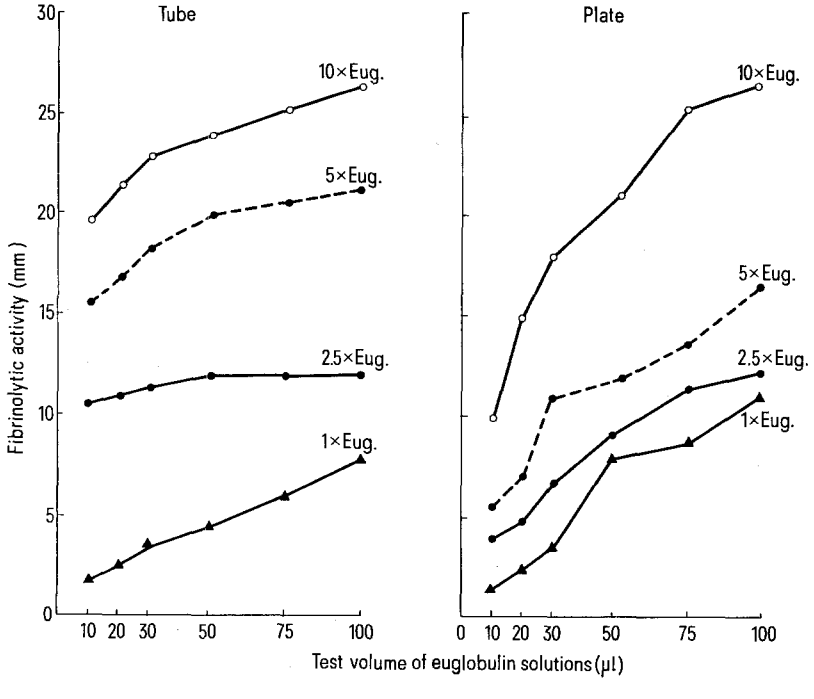

Fig. 3. Relation between fibrinolytic activity and volume of testmaterial using various concentration of Eug.

When the logarithm of Eug concentrations was plotted against their fibrinolytic activity, a straight line was obtained over a broad range, i.e. the results agreed well with the law of one-dimensional diffusion. On the basis of Figure 1, 2.5 × concentrated Eug seems to be optimal for routine investigations. In this range changes (increase as well as decrease) of fibrinolytic activity are well evaluable (Figure 2).

Compared to the plate technique, the lysis of fibrin tubes was less influenced by the volume of the samples and 2.5 × concentrated Eug is an optimal one from this point of view, too (Figure 3). Its volume dependence can be neglected above 50 µl. Other advantages described by YASUKOUCHI and WATANABE[2] for human system, i.e. a clear line of demarcation between lyzed and remaining fibrin, small amount of fibrinogen required, stable enzyme activity of tested materials, were found in rats, too. Results shown in the Table indicate that, estimating at optimal conditions, the reproducibility of tube assay is also superior to that of the plate technique.

To summarize, we suggest the following conditions for the estimation of fibrinolytic activity in rat system by fibrin tube assay: 0.1% fibrin tube as substrate, 50 µl of 2.5 × concentrated Eug as test sample, 18 h incubation at 37 °C. It can be used in each case when the ASTRUP technique is indicated, but the tube one is much more advantageous.

Reproducibility and accuracy of fibrin plate and fibrin tube assays

Method	Fibrinolytic activity[a]							Mean ± SD
Plate[b]	6.5	7.4	9.0	9.6	10.0	5.8	7.2	7.9 ± 1.54
	6.0	7.9	9.5					
Tube[b]	11.2	12.3	12.1	11.8	11.5	11.9		11.9 ± 0.34
	12.1	12.0	11.5	11.9				

[a] Diameter or length of digested fibrin (mm). [b] Test sample: 50 µl of 2.5 × concentrated Eug.

Zusammenfassung. Feststellung, dass die eindimensionale Diffusions-Methode eine sensitive, einfache und reproduzierbare Technik zur Bestimmung der Veränderungen der fibrinolytischen Aktivität bei Ratten ist.

L. FÉSÜS and EVA ÖLVETI

Department of Pathophysiology, University Medical School of Debrecen, 4012 Debrecen, Pf. 23 (Hungary), 22 April 1974.

[4] M. LASSEN, Acta physiol. scand. *27*, 371 (1953).

^{125}I in Electron Microscope Autoradiography

^{125}I has been used fairly widely in light and electron microscope autoradiography, particularly in studies on iodine metabolism[1–3]. Theoretical considerations[4] suggest that the isotope is likely to possess a high potential for electron microscope autoradiography and this is borne out by experimental studies[5]. A drawback to the wider use of iodine is that its introduction can alter the biological and physicochemical properties of proteins[6]. This report describes studies employing the isotope in a biological situation where this difficulty has been overcome and where the high resolution attainable with ^{125}I can more readily be demonstrated.

In these studies electron microscope autoradiography has been used to measure the rate of growth of collagen fibrils in vitro. As observed by BENSUSAN and SCANU[7], full iodination of the collagen was found to have a profound effect on growth rates. We were nevertheless able to estimate the growth rate of normal fibrils with a method of iodination utilizing carrier-free radioiodide, thus providing maximum specific activity for minimum iodination. A specific activity sufficient for autoradiography could be achieved for a degree of iodination so small that growth rates were not measurably affected. Such an economical use of ^{125}I is possible largely because of its half life of 60 days. This is long enough to permit convenient handling, but is sufficiently short to utilize a substantial fraction of the iodine atoms during the autoradiographic exposure. In addition each nucleardisintegration yields (on average) 1.7 electrons[4].

Solutions of calf skin collagen were prepared by standard methods[8]. Labelling of the protein with ^{125}I followed essentially the chloramine-T method of GREENWOOD et al.[9] who obtained specific activities up to 300 mCi/mg on human growth hormone without loss of anti-serum affinity. Calf skin collagen has only 11 tyrosines per molecule of 3000 residues, but an activity of 1 mCi/mg (corresponding to iodination of less than 1% of these tyrosines) was found to be adequate for electron microscope autoradiography with only 3 days exposure. The emission of γ-radiation by ^{125}I meant that the activity could conveniently be monitored on a sodium iodide crystal scintillation counter.

After separation from excess radioiodide by dialysis, collagen fibril precipitation was initiated by warming. Aliquots were removed at an early stage when the fibrils were short. These short labelled fibrils were then placed in a large excess of unlabelled collagen solution, allowing further (unlabelled) growth to take place. After known times, drops of precipitate were removed for autoradiography. The specimen was mounted on one side of a 400 A thick carbon-formvar film and a monolayer of Ilford L4 emulsion was applied to the other. Details fo the autoradiographic technique are described separately[10].

[1] H. FUJITA, Virchows. Arch. Abt. B. Zellpath. *2*, 265 (1969).
[2] A. TIXIER-VIDAL, R. PICART, L. RAPPAPORT and J. NUNEZ, J. Ultrastruct. Res. *28*, 78 (1969).
[3] J. J. MARCHALONIS, R. E. CONE and V. SANTER, Biochem. J. *124*, 921 (1971).
[4] W. G. MYERS and J. C. VANDERLEEDEN, J. nucl. Med. *149* (1960).
[5] N. O. KUHN and C. G. HARFORD, Science *141*, 355 (1963).
[6] R. H. RICE and G. E. MEANS, J. biol. Chem. *246*, 831 (1971).
[7] H. B. BENSUSAN and A. W. SCANU, J. Am. chem. Soc. *82*, 4990 (1960).
[8] D. S. JACKSON and E. G. CLEARY, Meth. biochem. Analysis. *15*, 25 (1967).
[9] F. C. GREENWOOD, W. M. HUNTER and J. S. GLOVER, Biochem. J. *89*, 114 (1963).
[10] R. A. HAWORTH and J. A. CHAPMAN, in press.