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.

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^{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].

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Figure 1a is an autoradiograph showing typical growth of unlabelled collagen on 2 labelled fibrils. The polarity of fibrils could be found from the 640 A periodic staining pattern, visible at higher magnification (see Figure 1b). Growth is apparent at both ends, with rather more growth at the N-terminal end than the C. When, however, the experiment was repeated using collagen fully iodinated with I_3^-, precipitation was a factor of 15 to 20 times faster and growth was observed only at the N-terminal end of the fibril[10].

In order to check the effect of the small degree of iodination involved in measuring normal fibril growth rates, the experiment was repeated using short fibrils labelled (by GREENWOOD's[9] method) to different extents. It was found (see Table I) that doubling the degree of iodination (labelling) did not significantly affect the growth rate measured. It may therefore be concluded that the degree of iodination with this method of labelling was sufficiently small for its chemical effect to be neglected.

To obtain some measure of the resolution achieved on the autoradiographs, histograms were compiled showing the number of grains found in 180 A intervals from the fibril axis. Distances were measured from the centre of the fibril (average diameter approximately 800 A) to the centre of the smallest circle to circumscribe a grain. In the histogram shown in Figure 2 every grain was counted up to a distance of 0.8 μm from the fibril axis. Of 300 grains counted, half fell within a distance of about 500 A from the central axis of the fibril. In another histogram, taken from autoradiographs in which the contribution of grains caused by non-precipitated collagen dried down in the background could not be neglected, a similar figure

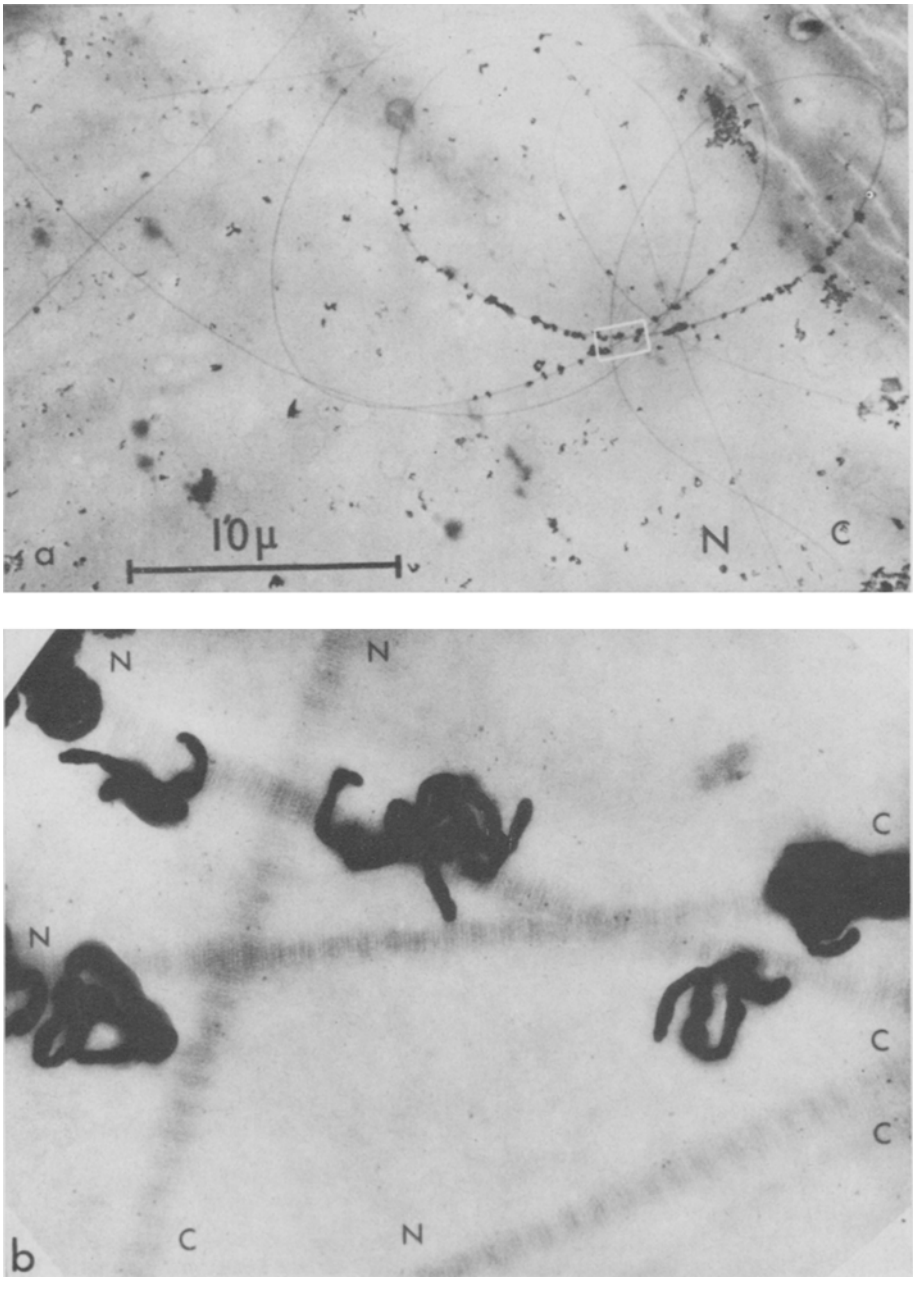

Fig. 1. a) An autoradiograph of two collagen fibrils showing growth of unlabelled collagen at both ends. The developer used was Microdol X. b). An enlargement of the area outlined by the small rectangle in Figure a), showing the 640 A periodic staining pattern and the polarity of the fibrils.

Table I. Axial growth of normal collagen fibrils at 30 °C in phosphate buffer pH 7.0. I = 0.2, C_o = 750 μg/ml.

Specific activity of labelled collagen	N end (molecules/sec)	C end
0.4 mCi/mg	1.73 ± 0.06	1.27 ± 0.08
0.2 mCi/mg	1.89 ± 0.08	1.11 ± 0.06

Errors represent standard deviations on measurements of about 20 fibrils in each case.

Table II. The energies of β^-'s emitted by ^{125}I (taken from reference[4])

Energy (keV):	2.77	3.6	22.5	31.0	34.3
Fraction of β^-'s with this energy	0.291	0.488	0.142	0.067	0.012

The high proportion of electrons with low discrete energies is due to their atomic, rather than nuclear, origin. They are emitted in consequence of the electron capture and γ-emission processes by which the ^{125}I nucleus decays and are a mixture of internal conversion and Auger electrons[14].

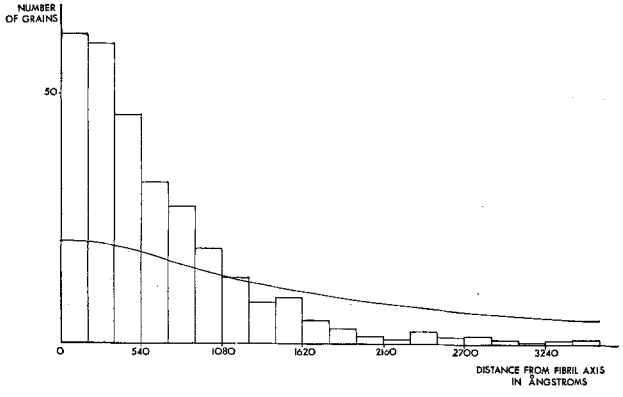

Fig. 2. For explanation see text.

of 540 A was obtained from 600 grains, after a correction for this background had been made.

The theoretical curve in Figure 2 represents the probability of a hit as a function of distance, assuming that electrons pursue a straight line path and are not absorbed. It is derived from the curve of DONIACH and PELC[11] for a point source, by numerical integration over a line source of fibrillar dimensions in the same geometry as the experiment. The large difference between the histogram and the curve (whose areas are the same) indicates that the assumption about electron paths is not valid for ^{125}I. This is likely on theoretical grounds. Since most of the decay electrons of ^{125}I have a very low energy (see Table II), these will have a range in silver bromide of less than a single L4 crystal diameter[12]. Shielding of one crystal by another will therefore be significant, resulting in an enhanced probability of hits directly over the source and an improvement of resolution[13].

Our results confirm the potential of ^{125}I for electron microscope autoradiography and demonstrate the high resolution that can be achieved. They show that problems arizing from the effects of iodination on protein behaviour can be minimized by a suitable method of iodination.

Résumé. Le ^{125}I possède un grand potentiel pour l'autoradiographie en microscope électronique. Il permet d'atteindre la haute résolution. Le nombre des problèmes que posent les effets de l'iodination sur le comportement des protéines peut être considérablement réduit par une méthode appropriée d'iodination.

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Differential Staining of the Satellite Regions of Human Acrocentric Chromosomes

Cytogeneticists have reported that all 10 acrocentric chromosomes of the D and G groups in humans probably bear satellites on their short arms; furthermore, all of these could be expected to function in the organization of nucleoli[1,2]. Certain of these acrocentric chromosomes approach each other with their satellite ends more often than would be randomly expected[3,4]. Cytogeneticists are presently investigating this preferential satellite association as a possible cause of chromosomal non-disjunctions and translocations[5]. However, these investigators are handicapped because these regions do not usually stain and they have to arbitrarily define these associations. OHNO et al.[2] suggested that at metaphase the satellite regions become very short and stain poorly because of the deficiency of DNA. Therefore, there was a need for a technique to differentially stain these important satellite regions.

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