This technique was employed to determine cross-measurements of motor neurons in lumbar spinal cord segments of the albino rat[4]. Upon completion of the study, measurements were repeated on 10% of the cells (r = 0.92). Using an ocular micrometer, another 10% of the cells were remeasured (r = 0.88). Thus, the technique is both reliable (r = 0.92) and valid (r = 0.88)[5–7].

Several advantages exist with this technique: 1. Any quantity of measurements can be made in less time than using an ocular micrometer or polar-planimeter for the same measurements; 2. tracing a projected image is not necessary; and 3. the mean cross-measurement can be used to determine the cross-sectional area of the cell. Disadvantages are minimal in that: 1. a mean diameter is calculated from 4-cross-measurements; and 2. two people are sometimes required depending upon the magnification of the projected image.

Thus, this technique allows the investigator to make crossmeasurements of individual cells quickly and easily with a high degree of accuracy and reliability.

[4] T. GILLIAM, unpublished Ph. D. thesis (1973).
[5] H. ELIAS, A. HENNIG and D. SCHWARTZ, Physiol. Rev. *51*, 158 (1971).
[6] E. R. WEIBEL and ELIAS, *Quantitative Methods in Morphology* (Springer Verlag, Berlin 1965).
[7] E. R. WEIBEL, G. S. KISTLER and W. F. SCHERLE, J. Cell Biol. *30*, 23 (1966).

A Simple Staining Technique to Demonstrate Chromosomal DNA Replication

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Summary. With the BrdU technique here described, Giemsa stained metaphases with either early or late replicational patterns can be obtained within 1 day after cell harvesting, showing a better resolution than ^{3}H-thymidine autoradiography.

The detection of chromosomal DNA synthesis by ^{3}H-thymidine labelling can be replaced by the use of its non-radioactive analogue 5-bromodeoxyuridine (BrdU) which leads to different coiling[1,2] and fluorescence-staining in unifilarly and bifilarly substituted DNA[3–5]. Recently a modification of the latter technique producing permanent preparations was developed[6–10].

Here we describe a simple and less time-consuming technique for the detection of early and late replicational patterns of mammalian chromosomes in Giemsa-stained preparations by combining the BrdU technique of LATT[3] with the staining procedure of KORENBERG and FREEDLENDER[7].

Material and methods. Various normal and abnormal human cell lines and a permanent line of *Microtus agrestis* were grown in Eagle's MEM supplemented with 20% and 10% fetal calf serum, respectively. Blood cultures were set up according to the method of ARAKAKI and SPARKES[11].

In one experimental series the cells were cultivated for most of their S-phase in the presence of 100 μM BrdU (Serva) and 0.4 μM FdU (to block endogenous thymidine synthesis). Similar results were obtained if 200 μM BrdU without FdU was used. Thus, the agent(s) were added 17 h before harvesting; 12 h later the medium was removed, the cultures were washed twice with a balanced salt solution and then reincubated in normal medium containing 0.2 mM TdR.

In another series 100 μM BrdU and 0.4 μM FdU were administered only for the last 5 h. In both protocols, Colcemid (Ciba) was present during the last 2 h. The cells were harvested as usual, treated with 0.075 M KCl for 12 min and fixed in methanol/acetic acid (3:1). The fixed cells were dropped on cold, wet slides and air-dried.

After storage for at least 1 day at room temperature, the slides were incubated at 88 °C for about 20 min in 1 M NaH_2PO_4 which was adjusted with solid NaOH to pH 8. Longer storage of slides usually requires longer incubation times. In case of poor chromosome morphology, older slides should be used. Then the slides were briefly rinsed in distilled water and stained with 2% Giemsa (Merck) diluted in phosphate buffer/aqua dest (1:9) for 7 min.

Results and discussion. With the simple procedure here described, those chromosomal regions which have incorporated BrdU during S-phase become only faintly

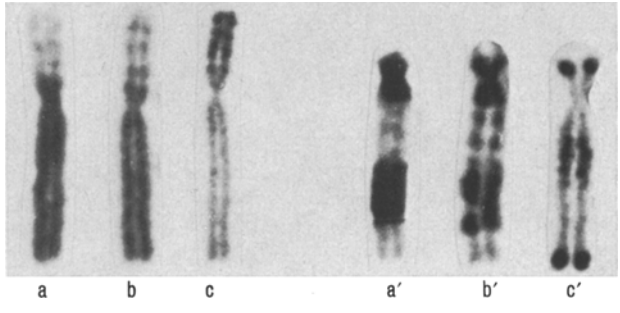

Fig. 1. X-chromosomes (left) and derived Y-chromosomes of an established cell line of *Microtus agrestis* after C-banding (a, a') and after incorporation of BrdU at early (b, b') and late S-phase (c, c').

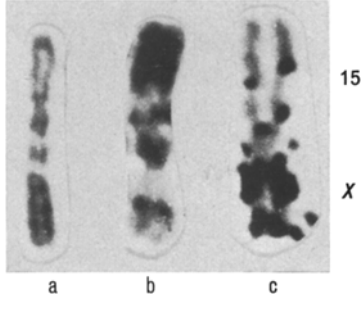

Fig. 2. Human X-autosome translocation: tdic (X; 15) (p21; p12) with regularly late replicating X. After BrdU incorporation at early (a) or late (b) S-phase the dark or the faint bands correspond with the silver grain patterns of the ^{3}H-TdR autoradiography (c).

stained with Giemsa. This can be clearly demonstrated in the European field vole *Microtus agrestis*, where most of the late replicating constitutive heterochromatin is confined to the large sex-chromosomes. If BrdU is present at the beginning (end) of the S-phase, these heterochromatic regions are darkly (faintly) stained (Figure 1).

Similarly, the facultative heterochromatic *X*-chromosome in mammals is distinguishable from its euchromatic homologue which can be especially useful for analysis of the inactivation patterns of abnormal *X*-chromosomes (Figure 2). Generally, a close correlation exists between the banding patterns of the chromosomes after BrdU incorporation at early (late) S-phase and G-banding (R-banding), which has been already described[5,9,10]. Thus, this BrdU technique has some clear advantages over the ^{3}H-TdR autoradiography: it is cheaper, quicker and provides a better resolution for examining the timing of chromosomal DNA replication. As in most cell lines the duration of G_2 phase is rather uniform, the time of BrdU removal or application is fairly constant, irrespectively of any differences in generation times.

Furthermore the technique can be used instead of G-banding, resulting in a higher yield of well banded metaphases, as well as in the additional identification of both *X*-chromosomes in the female.

[1] A. F. Zakharov and N. A. Egolina, Chromosoma *38*, 341 (1972).
[2] A. F. Zakharov, L. I. Baranovskaya, A. I. Ibraimov, V. A. Benjusch, V. S. Demintseva and N. G. Oblapenko, Chromosoma *44*, 343 (1974).
[3] S. A. Latt, Proc. natn. Acad. Sci., USA *70*, 3395 (1973).
[4] S. A. Latt, J. Histochem. Cytochem. *22*, 478 (1974).
[5] B. Dutrillaux, Chromosoma *52*, 261 (1975).
[6] My. A. Kim, Humangenetik *25*, 179 (1974).
[7] J. R. Korenberg and Freedlender, Chromosoma *48*, 355 (1974).
[8] P. Perry and Sh. Wolff, Nature, Lond. *251*, 156 (1974).
[9] K.-H. Grzeschik, My. A. Kim and R. Johannsmann, Humangenetik *29*, 41 (1975).
[10] J. T. Epplen, J.-W. Siebers and W. Vogel, Cytogenetics *15*, 177 (1976).
[11] D. T. Arakaki and R. S. Sparkes, Cytogenetics *2*, 57 (1963).

Affinity Chromatographic Preparation of Arterial Heavy Meromyosin Subfragment-1

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Summary. Heavy meromyosin subfragment-1 (HMM S-1) was prepared by papain digestion of arterial myosin or actomyosin and was purified by agarose-ATP affinity chromatography. Proteolysis of crude arterial myosin suspensions was preceded by solubilization. HMM-S-1 thus obtained consisted mainly of a 90,000 dalton polypeptide and fully retained the K^+- and Ca^{2+}-ATPase of the parent myosin. Its affinity to agarose-ATP was comparable to that of skeletal muscle HMM S-1.

The study of arterial and other smooth muscles is faced with unsolved preparative problems originating from a peculiar solubility behavior of its actomyosin and from difficulties in separation of actin from its myosin preparation. Since agarose-ATP affinity chromatography technique proved useful for the purification of active fragments of myosin from striated muscles[3] we were tempted to try and apply this procedure in order to prepare pure and well defined heavy meromyosin (HMM S-1) from arterial muscle. A preparation of this subfragment has already been reported by Huriaux[4] who overdigested myosin with trypsin instead of papain, which proved to be so specific and useful for the preparation skeletal myosin s_1 (c.f.[5]).

Arterial actomyosin was prepared from pig carotides according to Russel[6]. Crude arterial myosin was obtained by centrifugation of a solution of actomyosin (10 mg/ml) in 0.6 *M* KCl, 0.1 m*M* dithiothrietol (DTT), 50 m*M* *Tris* pH 7.6 and 10 m*M* Mg-ATP for 6–12 h at 100,000 *g*. Sepharose adipic hydrazide-ATP (agarose-ATP) was prepared as previously described[7].

Two approaches to the preparation of arterial HMM S-1 were followed:

1. Papain digestion (10 min, 25 °C) of myosin (5 mg/ml) in suspension at low ionic strength (30 m*M* KCl, 10 m*M* imidazole pH 7 and 0.1 m*M* DTT) and purification of the active fragment by adsorption to Sepharose adipic-hydrazide-ATP after removal of insoluble protein by centrifugation (100,000 *g*, 45 min); 2. Papain digestion of arterial actomyosin solution, separation of actin by centrifugation for 45 min at 100,000 *g*, removal of salt, ATP and insoluble protein by dialysis plus centrifugation and application to the affinity column (for details see legend to the Figure).

In the course of examination of the effect on the yield and activities of HMM S-1 of increasing papain: myosin ratios, it was observed that papain at a low level (papain: myosin 1/8000–1/2000 w/w) solubilized most of the ATPase activity within 10 min at 25 °C. SDS gel electrophoresis of the 100,000 *g* supernatant after this period revealed that most of the myosin was in the form of the intact 200,000 dalton heavy chains of myosin, i.e. that solubilization was not due to proteolysis. This unusual behavior prompted us to apply papain in excess (1/200) to insure complete digestion of the myosin. The same papain: myosin ratio was found adequate also in the digestion of actomyosin.

The Figure describes the affinity chromatography step in the purification of HMM S-1 derived from actomyosin.

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[3] R. Lamed and A. Oplatka, Biochemistry *13*, 337 (1974).
[4] F. Huriaux, FEBS Lett. *10*, 194 (1970).
[5] S. Lowey, H. Slayter, A. Weeds and H. Baker, J. molec. Biol. *42*, 1 (1969).
[6] W. B. Russell, Eur. J. Biochem. *30*, 459 (1973).
[7] R. Lamed, Y. Levin and A. Oplatka, Biochim. biophys. Acta *305*, 163 (1973).