3) Rats which received only 2 days of PB treatment (fig. 1).
4) Rats which received the Gerlans treatment including PB treatment and were killed 48 h after the start of PB treatment (fig. 2).
Examination of the metaphases showed that in the control rats, even after hepatectomy, the silver-staining was relatively low and confined to the NOR regions. On the contrary, in the carcinogen-treated rats, different types of silver-stained metaphases are observed. In some metaphases, silver staining is seen, as expected, only on the NORs (fig. 1a), but in other metaphases, a very intensive silver staining is present on the whole chromosomes (fig. 1b) and sometimes also in the nucleo-(cyto-)plasm (fig. 2).
These observations are relevant for diploïd cells and for polyploïd cells as well (fig. 2). Moreover, the unexpected silver-staining found in the nucleo-(cyto-)plasm cannot possibly be considered as a technical artefact since two neighboring cells may or may not present this nucleoplasmic silver-staining. Quantification of the data presented in relation to the carcinogen treatment given is difficult as long as the biological significance of the observed data is not understood.

Discussion. The present interpretation of silver-staining on chromosomes is that, under standardized conditions, silver nitrate binds acidic highly phosphorylated proteins B_{23} and C_{23}[6-8] which must be closely associated with nascent rRNA and with rDNA. One of the possible candidates might be the RNA polymerase I[11]. However since a strict relationship cannot be established between silver-staining and transcriptional activity, it is suggested that silver-staining may detect the presence of a protein (or set of proteins) responsible for chromatin decondensation[2]. In the light of these suggestions, our data may indicate that carcinogens like phenobarbital and 2-acetylaminofluorene induce a quick decondensation of chromatin. Silver nitrate would in this case stain not only the NOR regions but also all decondensing chromosome regions. These findings need confirmation in other systems with different carcinogens before a causative relationship can be proved between carcinogens and chromosome decondensation.
As far as the nucleo-(cyto-)plasmic silver-staining is concerned, the interpretation is difficult. Of course one might think of free rDNA copies or free 'decondensing proteins' but further molecular studies are needed to understand the findings.

1 Goodpasture, C., and Bloom, S. E., Chromosoma *53* (1975) 37.
2 Hernandez-Verdun, D., Biol. cell. *49* (1983) 191.
3 Hofgartner, F. J., Krone, W., and Jain, K., Human Genet. *47* (1979) 329.
4 Krack, G., Gravier, O., Roberfroid, M., and Mercier, M., Biochim. biophys. Acta *632* (1980) 619.
5 Lans, M., de Gerlache, J., Taper, M., Preat, V., and Roberfroid, M., Carcinogenesis *4* (1983) 141.
6 Lischwe, M. A., Richards, R. L., Busch, R. K., and Busch, H., Exp. Cell Res. *136* (1981) 101.
7 Lischwe, M. A., Smetana, K., Olson, M. O. J., and Busch, H., Life Sci. *25* (1979) 701.
8 Mamrack, M. D., Olson, M. O. J., and Busch, H., Biochemistry *18* (1979) 3381.
9 Paweletz, N., Siels, W., and Lettre, R., Z. Zellforsch. *76* (1967) 577.
10 Solt, D. B., Medline, A., and Farber, E., Am. J. Path. *88* (1977) 595.
11 Williams, M. A., Kleinschmidt, J. A., Krohne, G., and Franke, W. W., Exp. Cell Res. *137* (1982) 341.

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A method for karyotyping mouse blastocyst embryos developing from in vivo and in vitro fertilized eggs

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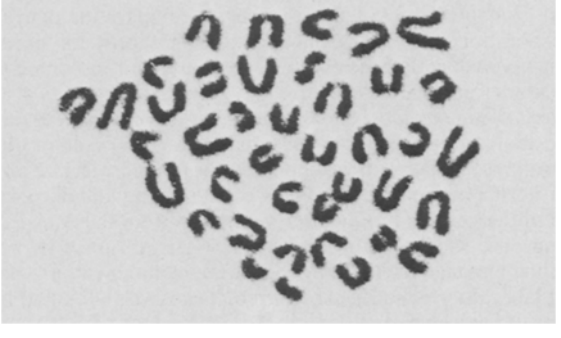
Summary. A method for karyotyping blastocyst-staged mouse embryos is described. The use of this protocol results in the recovery of a high percentage (> 70%) of readable karyotypes and can be completed rapidly.
Key words. Karyotype; mouse blastocyst.

There are two published methods for karyotyping preimplantation staged mouse embryos, one for cleavage-staged embryos[2] and one for blastocyst-staged embryos[3]. Both have disadvantages. We have found that the former method yields a low percentage of readable karyotypes (approximately 25%), and the latter results in the loss of a large number of the embryos because of the extensive length of time required for fixation.
The method we describe here is a modified version of that described by Takagi et al.[3]. With this modified protocol embryos are not lost and a high percentage of readable karyotypes are obtained. The method is fast and reproducible.
Procedure. Blastocyst embryos, developing from eggs fertilized either in vivo or in vitro[4,5] have been karyotyped. Following fertilization, 2-cell embryos, removed either from excised oviducts or from insemination dishes, are placed into 200 µl drops of standard egg culture medium (SECM)[6] under paraffin oil contained in 10 × 35 mm tissue culture dishes (Falcon Plastics). The dishes are placed into a continuous gas flow incubator (5% CO_2 in air) maintained at 37 °C. The embryos are allowed to continue development until they reach the early blastocyst stage[7].
At this stage, the embryos are transferred to 150–200 µl drops of SECM containing 1 µg/ml Colcemid (Sigma Chemical Co.). The drops of supplemented culture medium are under paraffin oil in 10 × 35 mm tissue culture dishes. The dishes are returned to the incubator for 3–4 h. After treatment, the embryos are divided into groups of 10, each of which is transferred to a depression in a spot test plate containing a hypotonic solution (deionized water: SECM; 2:1) which causes the cells to swell. After 5 min at



A representative air-dried, giemsa stained preparation of mouse blastocyst chromosomes. × 2900.

37 °C, each group of embryos is dehydrated and fixed by transferring them to a depression in another spot test plate containing a mixture of cold, 100% methanol and glacial acetic acid (3:1). The embryos are allowed to remain in the solution for 10 min. During this treatment the test plates are kept on ice. Attempting to handle more than 10 embryos through the hypotonic treatment and fixation results in a loss of material.

In order to disassociate the cells of the fixed embryos, each blastocyst is next placed onto an acid cleaned glass slide and one drop of a solution containing EDTA, deionized water and glacial acetic acid is applied directly to the embryo. This solution is made by first dissolving 0.01 g EDTA in 50 ml deionized water. On the day of use, an aliquot of this solution is diluted with deionized water (1:1). Glacial acetic acid is then added to the diluted solution (3:1). As soon as the cells of the blastocyst begin to disassociate from each other, three consecutively applied drops of cold, 100% methanol:deionized water; glacial acetic acid (9:4:3) are placed directly onto the embryo causing the cells to complete disassociation and to lyse. The liquid is allowed to evaporate between applications to the extent that the embryonic cells become visible by microscopic examination. The rapid spreading of the drops of this hypotonic solution is critical for good chromosome spreads. If the drops do not spread out over the slide quickly, the chromosomes remain clumped and are not distinguishable.

Immediately following the partial evaporation of the third drop of the hypotonic solution, three drops of 100% methanol:glacial acetic acid (3:1) are applied, allowing each to evaporate to the extent described in the previous step. The application of this solution dehydrates the material and increases its adhesiveness to the slide. The slide is then placed on a warming plate (37 °C) and allowed to dry. The disassociated and lysed cells are stained with giemsa (Gurr; Hopkin and Williams, Co. Essex, England). Optimum results are obtained if all solutions are prepared immediately prior to use.

Using the described technique we are able to analyze > 70% of the embryos prepared for karyotypic studies. The figure is typical of the karyotypes obtained by this method.

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2 Tarkowski, A. K., Cytogenetics *5* (1966) 394.
3 Takagi, N., Wake, N., and Sasaki, M., Cytogenet. Cell Genet. *20* (1978) 240.
4 McGrath, J., and Hillman, N., Nature *283* (1980a) 479.
5 McGrath, J., and Hillman, N., J. Embryol. exp. Morph. *60* (1980b) 141.
6 Biggers, J. D., Whitten, W. K., and Whittingham, D. G., in: Methods in Mammalian Embryology, p. 86. Ed. J. E. Daniel, Jr. W. H. Freeman and Co., San Francisco 1971.
7 Nadijcka, M., and Hillman, N., J. Embryol. exp. Morph. *32* (1974) 675.

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Incorporation of ^{32}P into renal phospholipids of mice during postnatal growth

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Summary During the first 40 days of life the rate of incorporation of ^{32}P into total phospholipids and into phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, diphosphatidylglycerol, phosphatidic acid and phosphatidylinositol of mouse kidney was by some 25–35% higher than in older animals. Results suggest a different involvement of cellular membranes during of normal and compensatory renal growth.
Key words. Mice; phospholipids; kidney growth.

Cellular membrane synthesis occurs during postnatal growth of the kidney. Rapidly growing neonatal rat kidneys contained high levels of choline kinase activity and the level gradually declined during the first month of life[1]. During the early phase of compensatory renal growth incorporation of ^{32}P into renal phospholipids, particularly phosphatidic acid and phosphatidylinositol, was increased[2]. In this article we describe investigations, using carrier-free ^{32}P, of phospholipid metabolism during postnatal growth of the kidney, in order to compare the data (on incorporation of ^{32}P into phospholipid fractions) for normal kidney growth with those obtained previously during compensatory kidney growth[2].

Materials and methods. C57BL/GoZgb mice, aged 1–120 days, were used in this study. The mice that were 20 days old or older were all males. The 1-day-old puppies were used within 24 h of their normal vaginal birth. The 1-, 5- and 10-day-old mice were used not later than 15 min after separation from the dams. The young ones suckled until 20 days after parturition. The non-suckling animals (over 20 days old) were maintained on a standard laboratory diet (Sljeme, Zagreb, Yugoslavia) and had free access to tap water.

2 h before sacrifice all mice of different ages were injected i.p. with 37 kBq ^{32}P per g body mass as carrier-free ^{32}P-labeled sodium orthophosphate (purchased from Vinča, Beograd, Yugoslavia).

The animals were sacrificed by decapitation. The kidneys of suckling mice were not purposely decapsulated, but capsules were often damaged during dissection. The kidneys of older mice were decapsulated by slitting the capsule at one end and squeezing gently between index finger and thumb. Each kidney was washed with isotonic saline, blotted on filter paper and weighed on a torison balance. The kidneys were pooled from six 1-day-old mice, four 5-day-old mice and three 10-day-old mice. From 20-day-old mice both kidneys were used and in the older mice all estimations were performed on right kidneys only.

Tissue samples were homogenized in 2 ml of ice-cold 0.3 N perchloric acid with a Potter-Elvehjem glass homogenizer as described previously[2]. The phospholipids were extracted by the method of Folch et al.[3] and were analyzed by thin layer chromatography. Aliquots were placed on two silica gel 60 G 254 plates (E. Merck AG, Darmstadt, Federal Republic of Germany), one plate was run in chloroform/methanol/acetic acid/water (65:50:1:4) and the other in chloroform/methanol/saturated ammonia/water (65:35:2:3). Phospholipids were visualized using iodine vapor or UV light. R_f values were compared with those of known standards purchased from Sigma Chemical Co,