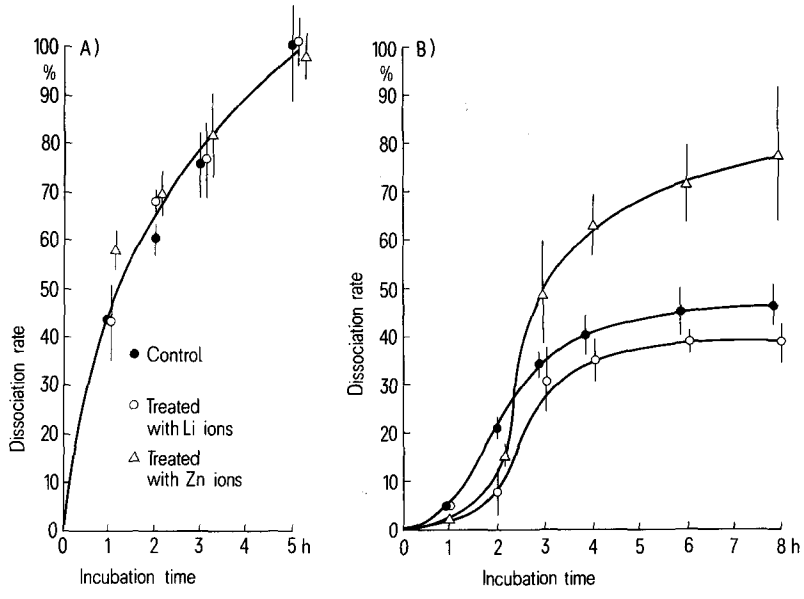

Increase in the dissociation ratio of the blastulae of the sea urchin *Anthocidaris crassipina*. The embryos, cultured for 14 h (A) and 22 h (B) at 20 °C, were suspended in the mixture of 1 M glycine - 2 mM EDTA and Ca, Mg-free sea water (1:1): control (●), Li-treated (○), and Zn-treated (△). Each plot shows the average for triplicate counts. Vertical bar indicates SEM Student's t-test was applied to the difference between each treated group and the control at 8 h of incubation. For the Zn-treated group, $p < 0.01$; for the Li-treated group, $p < 0.02$.

number of these dissociated units increased gradually. Figures A and B show the increase in the dissociation ratio of 3 groups of the blastulae at 14 h and 22 h after insemination, respectively. The rate of increase in the dissociation ratio of the normal blastulae at 22 h after insemination was remarkably lower than that of the blastulae at 14 h after insemination, indicating that cellular adhesion of the blastulae becomes tight as a whole as development proceeds. This tendency was also observed in the vegetalized blastulae. At 22 h after insemination, the rate of increase in the dissociation ratio of the vegetalized blastulae was a little lower than that in the normal blastulae (fig. B). This difference means that the cellular adhesion in the vegetalized blastulae was somewhat tighter than that in the normal blastulae, which could be ascribed to the proportion of the endodermal cells to ectodermal cells being higher in the vegetalized blastulae than in the normal ones. In the animalized blastulae, however, the tendency of strengthening cellular adhesion was not remarkable compared with the normal and vegetalized blastulae, suggesting that Zn ions caused significant inhibition of the strengthening cell adhesion which is a prerequisite for gastrulation, since both the normal and vegetalized blastulae could form archenteron but the animalized ones remained permanently in the blastula stage at the time which gastrulation was expected to occur.

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Contradictory differential staining results with Coomassie Brilliant Blue and silver carbonate on sister chromatids[1]

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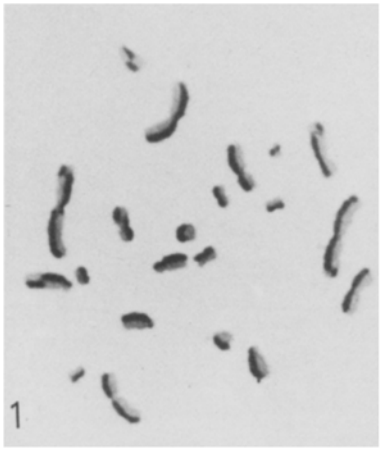
Summary. Coomassie Brilliant Blue, and silver stain, are used in electrophoretic gels to identify polypeptides. The relative staining intensity has been taken as indicating a quantitative difference between proteins. However, when these 2 stains were applied to 2 identically pretreated chromosome preparations, contradictory staining results were obtained.

Whereas Coomassie Brilliant Blue is regularly used by biochemists to identify polypeptides in electrophoretic gels and to determine protein concentration in solutions[2-4], a silver stain has long been used in many histological and cytological studies. Recently, by using silver to stain polypeptides in electrophoretic gels, a 100-fold increase in sensitivity has been achieved over Coomassie Brilliant Blue, and the method has also been used for quantitative evaluation[5-10]. In this communication we report a result showing that Coomassie Brilliant Blue and silver carbonate can give entirely different staining results on the same biological material.

Chinese hamster ovary cells were grown in an incubator at 37 °C 5% CO_2, in McCoy's 5a medium supplemented with 15% fetal calf serum, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 0.03% L-glutamine. 5-Bromodeoxyuridine (BrdUrd, Sigma, final concentration 50 µM) was added to the culture for 24 h (about 2 cycles). Colcemid (final concentration 2×10^{-7} M) was added during the final 3 h of incubation. Chromosomes were prepared by the air-



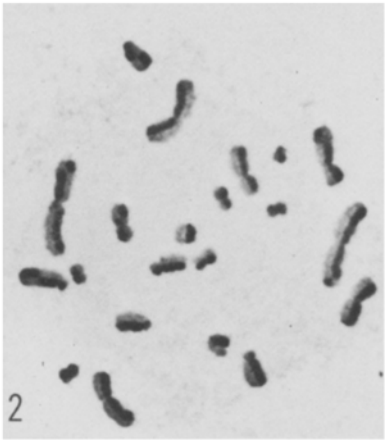


Figure 1. CHO chromosomes replicated 2 cycles in 5-bromodeoxyuridine, pretreated with Hoechst 33258 plus black light at 55 °C, then stained with Coomassie Brilliant Blue R-250.

Figure 2. The same preparation stained with silver carbonate after Coomassie Brilliant Blue R had been removed.

drying technique. Slides between 1 day and 5 months old were subjected to pretreatment as described in the simplified FPG method[11]. Essentially, this pretreatment includes mounting the slides with 2 drops of Hoechst 33258 solution and exposing the slides to black light (peaking at 356 nm) at 55 °C for 10 min. This pretreatment is referred to as HB pretreatment. The HB pretreated slides were either stained in 0.3% of Coomassie Brilliant Blue R-250 (CBBR, Sigma) or Coomassie Brilliant Blue G (CBBG, Sigma) dissolved in MA solution (methanol 50%, acetic acid 10%) with constant stirring for 1–12 h, or they were stained with silver carbonate using the procedure described by Goyanes[12]. To know more about the structural components responsible for the CBBR and silver carbonate staining, after HB pretreatment some slides were subjected to various digestions or extractions before being stained. DNA digestion was carried out by incubation of slides at 37 °C with DNase I (Sigma, type I, 340 Kunitz/ml of 50 mM tris-HCl buffer pH 7.5 containing 3 mM $MgCl_2$), and micrococcal nuclease (Sigma, 10 units/ml of 3 mM sodium phosphate buffer pH 6.8 containing 0.1 mM $CaCl_2$). Protein digestion was carried out at room temperature with pronase (Calbiochem, 500 µg/ml of 0.3 M NaCl and 0.03 M sodium citrate) and trypsin (Gibco, 0.25% in Hanks' balanced salt solution without calcium and magnesium). Histones were extracted with 0.25 N HCl and 0.4 N H_2SO_4. Nonhistones were extracted with 0.14 M NaCl, 0.35 M NaCl, 2 M NaCl and 0.1 N NaOH.

When chromosomes which had replicated during 2 cycles in BrdUrd were subjected to HB pretreatment and then stained with CBBR or CBBG, a sister-chromatid-differential staining (SCD) pattern was obtained, i.e., the BrdUrd bifilarly substituted chromatids (BB chromatids) were darkly stained and the BrdUrd unifilarly substituted chromatids (BT chromatids) were lightly stained[13]. When the identically-pretreated chromosomes were stained with silver carbonate however, the BB chromatids were lightly stained and the BT chromatids were darkly stained. The CBBR stained chromosomes (fig. 1) could be destained in MA solution, air dried and then restained with silver carbonate (fig. 2). In the various digestions and extractions tested, no apparent differences were observed between CBBR-SCD and silver carbonate-SCD in their effect on the formation. Mild digestion or extraction with DNase I, nuclease, pronase, trypsin and 0.1 N NaOH did not abolish the SCD results, however, extensive digestion and extraction did. On the other hand, repeated extraction with 0.25 N HCl, 0.4 N H_2SO_4, 0.14 M NaCl, 0.35 M NaCl or 2 M NaCl did not affect the SCD.

The ultrasensitive silver staining procedure developed for proteins also stains nanogram quantities of RNA and DNA in polyacrylamide gels[14], whereas a preliminary test using commercial DNA, RNA, histones and bovine plasma albumin immobilized on filter papers showed that CBBR and CBBG bound to proteins but did not bind to DNA or RNA. Therefore, CBBR and CBBG probably bind preferentially to chromosomal proteins, whereas the silver carbonate probably binds to other components on chromosomes in addition to proteins. On the other hand, it is also possible that the differences in protein compositions or DNA-protein conformations between the BB and BT chromatids after HB pretreatment may account for the contradictory SCD results. It has been shown that the sensitivity of silver staining is different for individual proteins[15]. Variation of the binding of CBBG to different proteins has also been recognized[4], although the variation for CBBR is smaller than that for silver staining[16]. Ockey[17] has shown that when BrdUrd-containing chromosomes are subjected to UV-treatment, a differential loss of BrdUrd-substituted DNA occurs, and this is the essential mechanism of the FPG staining method. However, this result is disputed by Webber et al.[18]. The present results suggest that some nonhistone proteins or the conformations organized by DNA and some nonhistone proteins may be changed in the HB pretreatment and these also play important roles in the production of SCD.

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