regions of the positive Giemsa bands are involved and that the exchange may be more complex than has been suggested. At present we have no better explanation for the interrupted positive G bands in the exchange regions.

Of particular interest with the trypsin procedure to induce SCD is that frequently one observes interchromatid connections at the point of SCE (Figure 6). Such connections have never been seen in 33258 fluorescence preparations or Giemsa preparations using procedures described previously by other investigators. It is possible that trypsin treatment distorts the distribution of nucleoproteins along the chromatids and the connecting stained material represents an artefact.

Since SCE may be a sensitive and accurate method for measuring genetic instability of mammalian and human cells, improvements in methodology (rapidity, simplicity as well as combination of techniques) should prove to be useful in future investigations. The procedures described in the present paper not only can reveal both SCD and G banding but are also rapid. The trypsin method requires a total of approximately 5 min.

Summary. We report a procedure for combining sister chromatid differential staining and G banding in the same metaphase plate. Mammalian cells in culture are grown in medium containing 5-bromodeoxyuridine for two cell cycles, and conventional air-dried preparations are made. The slides are treated with a trypsin or a urea solution the same way as for regular G banding. This method is simple and fast and provides additional information for cytogeneticists[8].

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Limulus Chromatophorotropin: Action on Isolated *Uca* Legs and in Various Crustaceans

The CNS of the horseshoe crab, *Limulus polyphemus*, contains a chromatophorotropin that disperses melanophore pigment in *Uca* species[1,2]. During our continuing study of this substance[3,4], we wondered if it acted directly or indirectly in *Uca* melanophores. We also wondered if this *Limulus-Uca*-chromatophorotropin, which for convenience we refer to as LUC, was capable of influencing pigment migration in chromatophores of other crustaceans. To obtain answers to these questions, we have tested the effects of LUC extracts on isolated legs of *Uca* species and on eyestalkless representatives of several crustacean families.

All animals (except *Gecarcinus lateralis*[5]) were obtained commercially and held in suitable artificial sea water (Instant Ocean) environments. Eyestalks were removed at least 1 day prior to experiments. Isolated legs, having all stage one[6] melanophores, were obtained from eyestalkless *Uca pugilator* and the recently described *U. panacea*[7]. Legs were removed proximal to the autotomy plane on the basiischium and placed in Syracuse glasses containing physiological saline[8]. The 2nd and 3rd walking legs were used; the right legs as controls and the left legs as experimentals. Extracts were prepared by thoroughly grinding lyophilized *Limulus* CNS in all-glass homogenizers in either saline (legs) or artificial sea water (crabs). The resultant mixture was then centrifuged, boiled for 10 min, recentrifuged, and the supernatant used for injection. Control legs were injected with 10 µl saline, and experimental legs with 10 µl of a 0.1 mg dry CNS/ml saline extract. Preliminary studies showed that melanophores of eyestalkless *Uca* exhibited pronounced responses to injections of 10 µl of one dry CNS/5 ml sea water LUC extract. Therefore, eyestalkless crabs were injected with a volume of that extract equal to the ratio eyestalkless crab weight/*Uca* weight. Control crabs were injected with an equal volume (10–150 µl) of sea water. All injections were made with a Hamilton 100 µl syringe equipped with a 30 gauge needle. Legs were injected through the autotomy plane into the merus, and crabs were injected at the base of the walking legs. All assays were performed in a darkened room. Isolated leg chromatophores were staged[6] before injection and at 15, 30, 60, 90, and 120 min after injections. Eyestalkless crab chromatophores were observed and staged[6] at similar intervals, or until the absence of response was evident. No attempt was made to quantify the response of eyestalkless crab chromatophores to LUC extracts. Instead, we observed the chromatophore response to extracts and to sea water for a period sufficient to demonstrate pigment dispersion or contraction, or no response, in those animals injected with extract. In positive experiments, LUC extracts produced obvious dispersion or concentration of pigment (different by two or more chromatophore stages from the control value) within 15–30 min after injection. Experiments on eyestalkless crabs were conducted three or more times for each species.

To test for a possible direct action of LUC, we first injected isolated legs with 10 µl of LUC extract or 10 µl of saline. The results of these experiments at 15, 30, 60, 90, and 120 min, are shown in Table I. Although the chromatophores of the control legs showed a response to saline injections, the response of legs from both species to LUC was significantly higher ($p < 0.005$, Student's t-test and Mann-Whitney U-test) at all intervals examined. Since these experiments demonstrated that LUC extracts could cause pigment dispersion in the melanophores of

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Table I. Response of isolated *Uca* legs to LUC and theophylline

Treatment	Time after injection (min)				
	15	30	60	90	120
Controls (20)	1.2 ± 0.4	1.6 ± 0.7	2.1 ± 1.1	2.4 ± 0.7	2.6 ± 0.6
LUC (20)	1.8 ± 0.5	2.4 ± 0.7	2.9 ± 0.6	3.0 ± 0.6	3.4 ± 0.5
Theophylline (10)	1.3 ± 0.7	1.7 ± 0.7	2.3 ± 0.7	2.6 ± 0.5	2.8 ± 0.6
Theophylline + LUC (15)	2.3 ± 0.5	2.6 ± 0.5	3.3 ± 0.5	3.5 ± 0.5	3.9 ± 0.4

( ) = the number of legs tested; data presented as the mean chromatophore stage ± S.D.

isolated legs, we infer that the effect of LUC in eyestalkless *Uca* is direct and not mediated by the *Uca* CNS or some other tissue or organ.

Since a direct effect of LUC could be demonstrated, and since chromatophorotropins in other animals may act via the adenylate cyclase-cAMP system[9,10], we wondered if we could potentiate the LUC effect by the addition of the phosphodiesterase inhibitor theophylline. We therefore tested the effects of 10 µl of a 0.1 m*M* solution of theophylline (Sigma) and 10 µl of a LUC extract containing 0.1 m*M* theophylline. The results of these experiments are also in Table I. Theophylline alone caused a response that was not significantly different from that of the controls ($p > 0.2$, Student's *t*-test and Mann-Whitney U-test). LUC in the presence of theophylline caused a response that was significantly higher ($p < 0.05$, Student's *t*-test and Mann-Whitney U-test), at 15,90, and 120 min, than the response to LUC alone. These experiments indicate that LUC may act to stimulate the adenylate cyclase-cAMP system of the *Uca* melanophores. The results are, however, also compatible with other conclusions.

Our experiments on the effects of LUC on the chromatophores of crustaceans other than *Uca* are summarized in Table II. LUC extracts clearly caused black pigment dispersion and white pigment concentration in the chromatophores of *Carcinus maenas*, *Hemigrapsus oregonensis*, and *Pachygrapsus crassipes*, but were without effect on the erythrophores of these species. By contrast, the black and white chromatophores of *Gecarcinus lateralis* and the white chromatophores of *Pagurus pollicarus* were not affected by the extracts, but red pigment dispersed in the erythrophores of both species. We observed no effect of our extracts on *Paneopus herbstii* chromatophores, and we never observed effects on the yellow chromatophores of any species examined.

Table II. Effects of LUC extracts on chromatophores of various decapod crustaceans

Genus	Chromatophore type		
	Black	White	Red
Carcinus (16)	D	C	O
Hemigrapsus (11)	D	C	O
Pachygrapsus (16)	D	C	O
Pagurus (9)	–	O	D
Gecarcinus (9)	O	O	D
Panopeus (9)	O	O	–

() = total number of control and experimental animals. O, no effect; D, dispersion; C, concentration; –, not present.

It is clear from earlier experiments[1-4] that LUC is active as a chromatophorotropin in *U. pugnax*, *U. pugilator*, and *U. panacea* of the family Ocypodidae. The above experiments show that LUC extracts are also active on the chromatophores of at least 4 additional brachyuran families, i.e., Portunidae (*Carcinus*), Grapsidae (*Hemigrapsus* and *Pachygrapsus*), Gecarcinidae (*Gecarcinus*), and Paguridae (*Pagurus*). We have also shown for the first time that LUC extracts stimulate anomuran (*Pagurus*) chromatophores. We therefor conclude that LUC extracts can influence chromatophores in representatives of several crustacean families. This conclusion is reinforced by out casual observation, not repeated owing to lack of animals, that LUC extracts stimulated black pigment dispersion in the chromatophores of the portunid *Callinectes sapidus* and the cancerid, *Cancer antennaris*.

Our extracts seem to work on eyestalkless crabs in one of two ways. In some species (*Carcinus*, both grapsids, and *Uca*) they stimulate black pigment dispersion and white pigment contraction, but have no effect on red chromatophores. In others (*Pagurus*, *Gecarcinus*) they stimulate red pigment dispersion but have no effect on white or black chromatophores. Unfortunately, we do not know if these differences are due to more than one active substance, but the data suggest that more than one chromatophorotropin may be present in the *Limulus* CNS. Our results, and those of others[1,11], demonstrate that some crustaceans have chromatophores that do not respond to LUC extracts[12,13].

Summary. *Limulus* CNS chromatophorotropin causes melanin dispersion in isolated legs of *Uca*. Theophylline potentiation of extract activity suggests that the material may act through the adenylate cyclase-cAMP system. The extracts are also active on the chromatophores of 7 decapod species from 6 families.

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