Induction by hydrocortisone-21-sodium succinate of the 70K heat-shock polypeptide in isolated salivary glands of *Drosophila melanogaster* larvae[1]

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Summary. The vertebrate steroid hormone hydrocortisone-21-sodium succinate induces in isolated salivary glands of *Drosophila melanogaster* 3rd instar larvae a protein identified as the 70K heat-shock polypeptide by 1- and 2-dimensional gel electrophoresis analysis. This response is accompanied by significant induction of the puffs 87A and 87C.

In *Drosophila*, heat-shock induces specific puffs in polytene chromosomes[2]. This response is accompanied by synthesis of a specific set of polypeptides (hsp's)[3]. Recent studies have shown that the heat shock response may be universal, occurring in species ranging from *E. coli* to mammals and under a variety of different stress conditions[4]. It has also been found that synthesis of hsp23 and other low molecular weight hsp's are stimulated by treatment of Schneider's line 3 *Drosophila* cells with the steroid insect hormone ecdysone[5]; moreover, at stages of *Drosophila* development characterized by high ecdysone titers polyadenylated RNA contains substantial amounts of sequences complementary to hsp22 and hsp26 genes[6]. On the other hand, claims of specific induction of puffs by high doses of mammalian steroid hormones[7] have not been confirmed[8] and only traces of hsp22 were induced in primary cultures of *Drosophila* embryonic cells by 10^{-3} M cortisone[9].

We report here induction of a protein by hydrocortisone-21-sodium succinate in isolated salivary glands from 3rd instar *Drosophila melanogaster* larvae, and evidence identifying this protein as the 70K heat-shock polypeptide.

Materials and methods. Salivary glands from late 3rd instar *Drosophila melanogaster* larvae of a wild type (Canton S) strain were used.

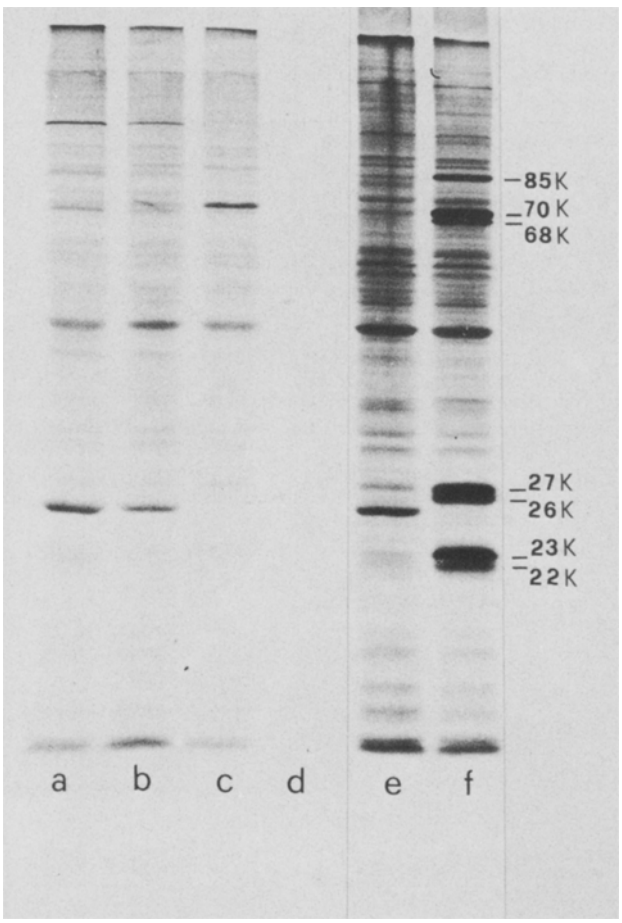

Figure 1. Autoradiogram of 11% SDS-polyacrylamide gel of ^{35}S-methionine-labeled proteins of 5 pairs of salivary glands, prepared as described under 'Materials and methods'. *a* Glands incubated in Poel's medium (control); *b* incubation in medium containing 3 mM hydrocortisone-21-sodium succinate (HCS); *c* 7 mM HCS; *e* glands dissected from non heat-shocked larvae and immediately labeled in Poel's medium (heat-shock control); *f* glands from larvae shocked for 30 min at 36.5 °C, treated as in (e). Exposure time was 7 days at −80 °C.

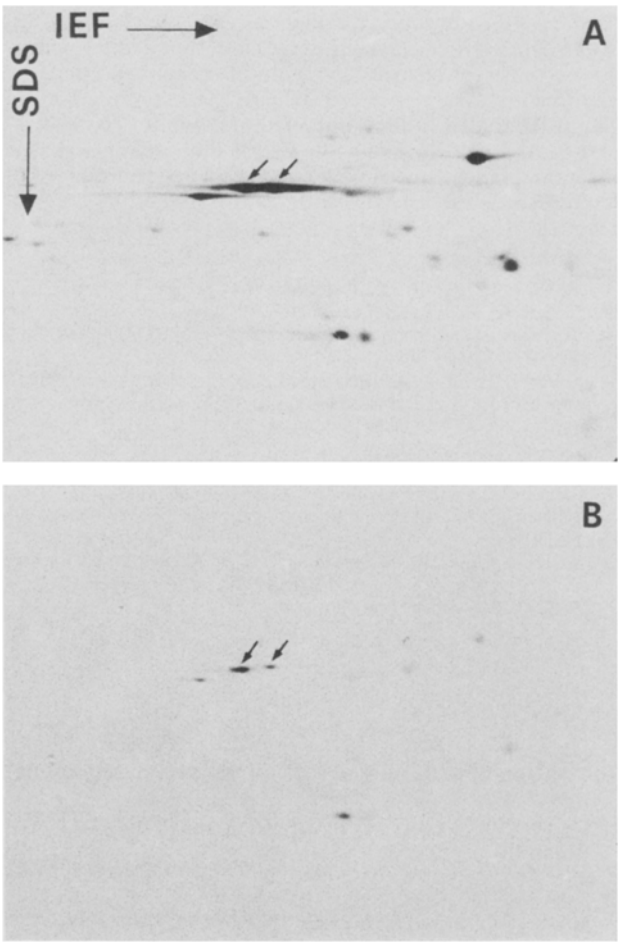

Figure 2. Two-dimensional electrophoretic pattern of ^{35}S-methionine-labeled polypeptides synthesized by 5 pairs of salivary glands from larvae heat-shocked for 30 min at 36.5 °C (A) and synthesized by 5 pairs of isolated salivary glands incubated for 90 min in presence of 7 mM hydrocortisone-21-sodium succinate (B). Two-dimensional gel electrophoresis was performed according to O'Farrell[12]; 2nd dimension was 11% SDS-polyacrylamide. Exposure time was 7 days at −80 °C.

Analysis of polypeptides induced by hydrocortisone-21-sodium succinate (HCS). Five pairs of salivary glands were dissected in insect Ringer and incubated for 90 min at 24 °C in 50 µl of Poel's medium[10] containing appropriate concentrations of HCS or no HCS (controls); HCS was used because it is soluble in the incubation medium at higher concentrations than hydrocortisone or most other hydrocortisone salts. In some experiments 5 µg/ml α-amanitin was added to the HCS containing incubation medium. Glands were labeled for 45 min at 24 °C in fresh medium containing 400 µCi/ml ^{35}S-methionine (sp. act. 600–1300 Ci/mM, Amersham), then transferred to buffer containing 50 µl 0.0625 M Tris-HCl pH 6.8,2% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol, 0.001% bromophenol blue, 10% glycerol and were heated in boiling water for 10 min. After centrifugation the supernatant was run on 11% SDS-polyacrylamide gels[11]. Gels were stained with Coomassie blue and dried; autoradiography was performed using Kodak No-Screen X-ray Film.

Proteins from similarly treated glands were also analyzed on two/dimensional gels according to O'Farrell[12].

Analysis of heat-shock induced polypeptides. Larvae were shocked at 36.5 °C for 30 min; the salivary glands were dissected in insect Ringer, and proteins were immediately labeled with ^{35}S-methionine and analyzed as described above.

Results and discussion. Isolated salivary glands from 3rd instar *Drosophila melanogaster* larvae responded to incubation in presence of hydrocortisone-21-sodium succinate by synthesizing a protein significantly less represented in glands incubated in the same medium without HCS (compare lane a with lanes b–c in fig. 1); this protein was also absent in glands treated with comparable concentrations of sodium succinate, and its synthesis is inhibited by 5 µg/ml α-amanitin (data not shown). Maximum induction occurred at about 7 mM HCS; at higher concentrations, total protein synthesis rapidly diminished and was almost absent at 13 mM HCS (fig. 1, d). In 11% SDS-polyacrylamide gels, the protein induced by HCS migrated to the position corresponding to the 70K; the heat-shock response, however, is clearly much stronger (fig. 1, f). Proteins at the positions of hsp26, hsp68 and hsp83 were also present in small amounts in HCS-treated glands; it should be pointed out that traces of these proteins were also often present in our controls.

To confirm the identification of the major HCS-induced protein as the 70K hsp, ^{35}S-methionine labeled proteins from HCS-treated glands were run on 2-dimensional gels and compared with autoradiograms of similarly labeled proteins from heat-shocked glands (fig. 2, A). In the HCS treated sample, the major newly synthesized protein also migrated to the position of the 70K hsp (fig. 2, B). Moreover, we observed cytologically in HCS treated glands significant induction of the puffs at 87A and 87C, the loci of hsp70 synthesis[13,14].

It is difficult to assess the biological significance, if any, of induction of hsp70 (and possibly other hsp's) by a mammalian steroid hormone such as hydrocortisone. We cannot exclude a specific hormone-mediated response, but, because of the high concentration necessary for induction (compare with 10 µM ecdysone sufficient for hsp stimulation in *Drosophila* cells lines[5]), and of the fact that still higher doses abolished total protein synthesis, we favor the hypothesis that incubation in HCS at the doses employed represents per se a stress situation. If this is the case, HCS is to be added to the list of agents capable of eliciting the 'heatshock' response, at least in isolated salivary glands of *Drosophila melanogaster.*

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Cultivation of arterial endothelial cells from human umbilical cord

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Summary. We have developed a simple method for the isolation of endothelial cells from human umbilical artery. The method provides a sufficient number of cells to be of experimental value. The presence of factor VIII antigen specific for endothelium has been demonstrated by immunofluorescence as well as by the peroxidase-antiperoxidase immune complex method.

Valuable information on the physiological functions of endothelium has been obtained using the method of Maruyama[1] and Jaffe et al.[2] developed for the cultivation of endothelial cells from the human umbilical vein. This method has also been utilized by several investigators for the cultivation of umbilical arterial endothelium, but isolation procedures have not been reported in detail[3-5]. From our experience arterial endothelial cells in large numbers