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Protein Synthesis in Salivary Glands of *Drosophila hydei* after Experimental Gene Induction

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Several treatments, namely incubation at 37°C, in the presence of arsenite, 2,4-dinitrophenol or vitamin B-6, or release from anaerobiosis induce the same set of puffs in the polythene chromosomes of salivary glands of *Drosophila hydei*. Analysis of changes in protein-synthetic patterns (as determined by radioautography of sodium dodecyl sulphate-gel electrophoretograms of extracts from [^{35}S]methionine-labelled salivary glands) showed that concomitant with puff induction by these various treatments the same six strongly labelled polypeptide bands appeared. The amount of radioactive label in these peptides accounted for 25% of the total incorporation of [^{35}S]methionine, except during incubation at 37°C when it accounted for about 50%. The rate of synthesis of these peptides was maximal 1 h after the start of the puff-inducing treatment. The rate of decay of the rate of synthesis showed first-order kinetics both after removal of the puff-inducing stimulus or in the presence of actinomycin, with a half-life of approx. 4 h.

Interference with the respiratory metabolism of salivary glands of *Drosophila hydei* (or *Drosophila melanogaster*) results in the appearance of a specific set of puffs, the 'heat-shock' puffs (Ritossa, 1962, 1964; Berendes *et al.*, 1965; Ashburner, 1970; Leenders & Berendes, 1972; Leenders *et al.*, 1973, 1974*a*, *b*). About 10 to 20 min after these puffs have reached their maximum size in *D. hydei*, the activities of several mitochondrial enzymes start to increase (Leenders & Beckers, 1972; Leenders *et al.*, 1974 *a*, *b*; Koninkx *et al.*, 1975; Koninkx, 1975; Sin & Leenders, 1975). These increases are dependent on transcription and translation *de novo*. Moreover, the increase in activity of mitochondrial NADH dehydrogenase (EC 1.6.99.3) could be correlated with the presence of a puff at locus 4-81B, whereas the increase in mitochondrial tyrosine transaminase activity (EC 2.6.1.5) appeared to be related to the presence of a puff at locus 2-48BC (Leenders & Beckers, 1972; Leenders *et al.*, 1973). It was therefore suggested that the RNA product of these puffs might code for at least part of these enzymes.

On the other hand, Tissières *et al.* (1974) have shown that puff induction by a temperature shock in *D. melanogaster* is followed by a change in the pattern of protein synthesis: six new strongly labelled bands appear. These findings were extended by Lewis *et al.* (1975) who found that a similar set of bands could be induced by a temperature shock in salivary glands of *D. hydei* or *D. similans*. If the appearance of these bands is indeed causally related to the presence of the 'heat-shock' puffs, as has been suggested, then all treatments which induce these puffs should also lead to the appearance of the 'heat-shock' bands. However, Lewis *et al.* (1975) found that puff induction by release from anaerobiosis induced the 'heat-shock' bands only in salivary glands from *D. melanogaster*, but not in those from *D. hydei*, where a partial induction was found, whereas after treatment of *D. melanogaster* glands with 2,4-dinitrophenol, which also induces the 'heat-shock' puffs, only one of the 'heat-shock' bands could be detected.

I have therefore re-investigated the induction of the 'heat-shock' bands under various conditions. Further, preliminary to a study of a possible correlation between the induction of one or more of the 'heat-shock' bands and the observed increases in enzyme activity, an effort has been made to study the kinetics of induction and de-induction of these bands quantitatively.

Experimental

Materials

NNN'N'-Tetramethylethylenediamine, riboflavin, acrylamide, bisacrylamide and sodium arsenite were from BDH Chemicals, Poole, Dorset, U.K.; sodium dodecyl sulphate and cycloheximide were from Serva, Heidelberg, Germany; 2,4-dinitrophenol and oligomycin were from Sigma Chemical Co., St. Louis, MO, U.S.A.; vitamin B-6 was from Eastman Kodak Co., Rochester, NY, U.S.A., actinomycin D was from Calbiochem, Los Angeles, CA, U.S.A., and chloramphenicol from Boehringer G.m.b.H., Mannheim, Germany; Permablend III and Soluene-100 were from Packard Instrument Co., Downers Grove, IL,

U.S.A.; [^{35}S]methionine (specific radioactivity 330Ci/mmol) and [^{3}H]uridine (specific radioactivity 53.5Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were of analytical grade from E. Merck A.G., Darmstadt, Germany.

Incubation and labelling of salivary glands with [^{35}S]methionine

Salivary glands were hand-isolated from late-third-instar larvae of *Drosophila hydei*, reared as a mass culture under standard conditions (Mitchell & Mitchell, 1964). Five pairs of glands, freed of the adhering fat-body as much as possible, were incubated in 50μl of incomplete Poels' (1972) medium (modified to remove exogenous methionine) at 25°C (except for 'heat-shock' experiments) under the various conditions and for the length of time necessary (as detailed in the legends to the Figures) to induce the puffs. In all experiments, the effectiveness of puff induction was checked by examining a squash preparation of one of the glands. Glands were labelled at 25°C for 15min in 5μl of incomplete Poels' (1972) medium containing 10μCi [^{35}S]methionine, except after treatment with 2,4-dinitrophenol or vitamin B-6. In those cases, the labelling period was 30min and 50μCi [^{35}S]methionine was used. Samples were prepared for electrophoresis as described by Tissières *et al.* (1974), except that the dried glands were dissolved by boiling them for 2min in 35μl of sample buffer (for composition see below). A sample (2μl) was then counted directly in 10ml of scintillant containing 20ml of methoxyethanol, 5.5g of Permablend III and 30ml of Soluene-100/litre of toluene. Liquid-scintillation counting was performed with a Packard Tri-Carb liquid-scintillation spectrometer, model 3004. A sample of the remainder was applied to a slab gel such that all slots contained an equal amount of radioactivity (this usually varied between 150000 and 1850000c.p.m.).

Gel electrophoresis and radioautography

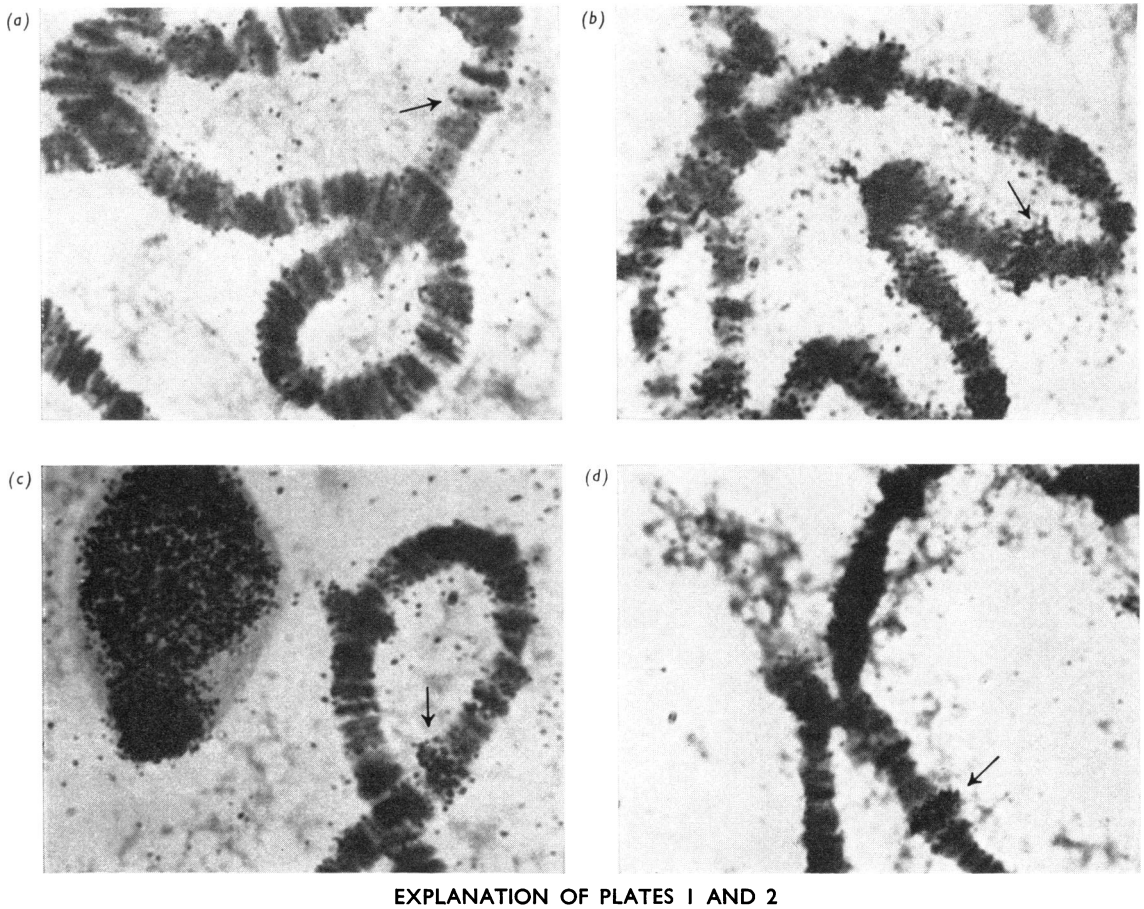
The protein samples were run for 3h at 16mA on 10% (w/v) polyacrylamide-slab gels, 1.5mm thick in a discontinuous sodium dodecyl sulphate system at room temperature (23°C). The system used was slightly modified from that described by Tissières *et al.* (1974). The small-pore gel buffer was composed of 375mM-Tris/HCl, pH8.9, 4M-urea and 0.1% sodium dodecyl sulphate. The large-pore buffer was composed of 62.5mM-Tris/HCl, pH6.7, 4M-urea and 0.1% sodium dodecyl sulphate. The sample buffer contained 0.01M-sodium phosphate, pH7.6, 1% sodium dodecyl sulphate, 4M-urea, 1% 2-mercaptoethanol and 0.001% Bromophenol Blue. The reservoir buffer was composed of 3.0g of Tris, 14.4g of glycine, 0.1% of sodium dodecyl sulphate and water added to 1 litre, pH8.3. After drying, the slab gels were exposed to Kodak RP/R 14 X-ray film for 4–72h. The radioautographs were quantified by scanning with a Vitatron densitometer. From this densitogram, the areas under the various peaks were then determined.

Results

Correlation between puffing patterns induced by interference with the cellular respiratory metabolism and changes in protein-synthetic patterns

As mentioned above, several treatments that interfere with the respiratory metabolism all induce the same set of puffs: namely 2-32A, 2-36A, 2-48BC and 4-81B in *D. hydei* (Leenders & Berendes, 1972; Leenders *et al.*, 1974*a*, *b*). The size of the puff induced is virtually the same irrespective of the puff-inducing stimulus, except for the puff at locus 2-48BC, which becomes very much larger during treatment with vitamin B-6 (Leenders *et al.*, 1973). Not only are puff sizes the same, the amount of uridine incorporated in the puff area, as assayed radioautographically, is also very similar (Plates 1 and 2). In order to determine whether the induction of similar puffs is accompanied by similar changes in protein-synthetic patterns, salivary glands were pulse-labelled with [^{35}S]-methionine at various times after the start of the puff-inducing treatment. The gland extracts were then electrophoresed on polyacrylamide gels in the presence of sodium dodecyl sulphate and the pattern of labelled protein was determined by radioautography. Plate 3 shows such an experiment with temperature as the puff-inducing stimulus. In agreement with Lewis *et al.* (1975), new bands appear during the temperature treatment, whereas some of the bands present in the untreated control sample disappear. The same set of bands appear during recovery from anaerobiosis, although the bands of lower molecular weight are less prominent than after a temperature shock (Plate 4*a*). In contrast with the pattern obtained after temperature treatment, however, the bands also present in control glands continue to be synthesized. The pattern obtained during incubation of the glands in arsenite (Plate 4*b*) is very similar to that obtained after release of the glands from anaerobiosis: the extra bands are added to the normal pattern of protein synthesis and do not replace it.

Glands incubated in medium containing 2,4-dinitrophenol or vitamin B-6 did not incorporate enough [^{35}S]methionine to allow a radioautographic analysis of the protein-synthetic pattern. This failure is presumably due to the very low cellular concentration of ATP under these conditions. Therefore glands were pre-incubated with either 2,4-dinitrophenol or vitamin B-6 to induce the puffs, then rinsed with and further incubated in normal medium and pulse-labelled with [^{35}S]methionine.



EXPLANATION OF PLATES 1 AND 2

Radioautographs of salivary-gland chromosomes displaying region 4-81*B*

After incubation of the glands in Poel's (1972) medium under various conditions to induce the puffs (see below), the glands were labelled for 5min with [^{3}H]uridine (1mCi/ml) and prepared for radioautography as described by Berendes (1966). Exposure time was 3 days. (Plate 1*a*) Control (untreated glands); (Plate 1*b*) 2h incubation at 37°C; (Plate 1*c*) 2h N_2 anaerobiosis followed by 45min recovery in air; (Plate 1*d*) incubation for 2h in the presence of 0.1mM-sodium arsenite; (Plate 2*a*) incubation for 2h in the presence of 1mM-2,4-dinitrophenol; (Plate 2*b*) incubation for 2h in the presence of 50mM-vitamin B-6 (Plate 2*c*) the banding pattern of the salivary-gland chromosomes after aceto-orcein staining (incubated as in Plate 2*a*).

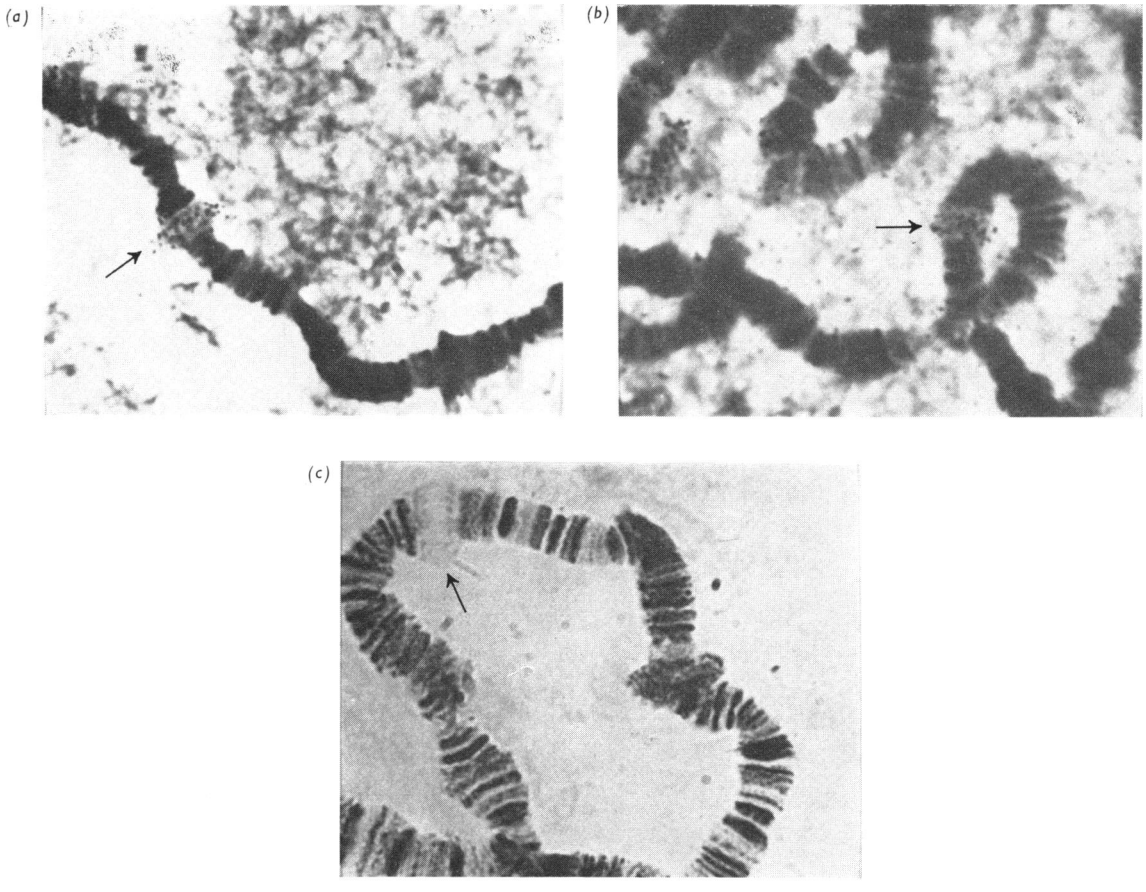
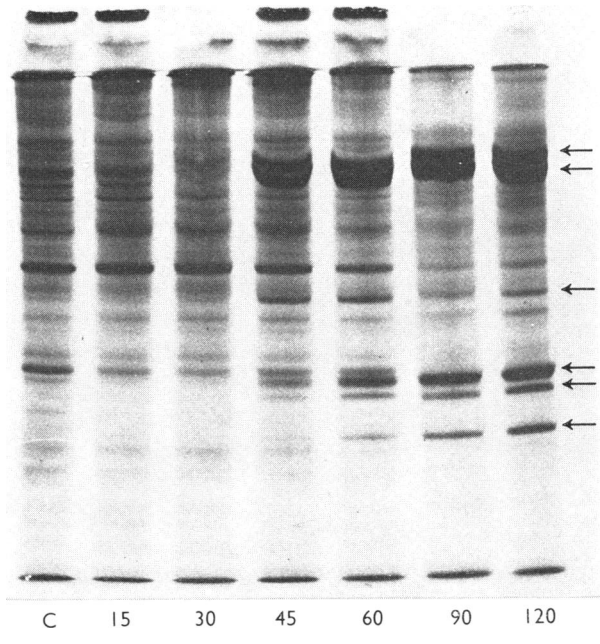


PLATE 2



EXPLANATION OF PLATES 3, 4 AND 5

Radioautographs of [^{35}S]*methionine pulse-labelled proteins, separated by sodium dodecyl sulphate-gel electrophoresis, after puff induction by various treatments*

In all radioautographs the pattern of protein synthesis in control glands (C) and in 2h heat-treated glands (T) is shown for comparison. The numbers indicate the length (in min) of the incubation or the recovery period before the addition of label. (Plate 3) Incubation at 37°C (heat shock); (Plate 4*a*) recovery from 2h N_2 anaerobiosis [in this case, glands were mass-isolated as described by Boyd *et al.* (1968), C_m is the pattern obtained after labelling of control mass-isolated glands]; (Plate 4*b*) incubation in 0.1mM-sodium arsenite; (Plate 5*a*) recovery from a 2h incubation in 1mM-2,4-dinitrophenol; (Plate 5*b*) recovery from a 2h incubation in 50mM-vitamin B-6.

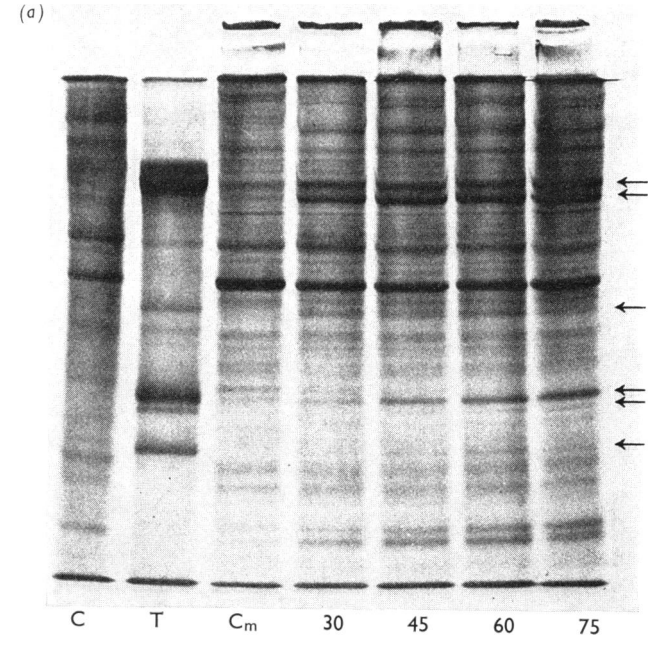


PLATE 4

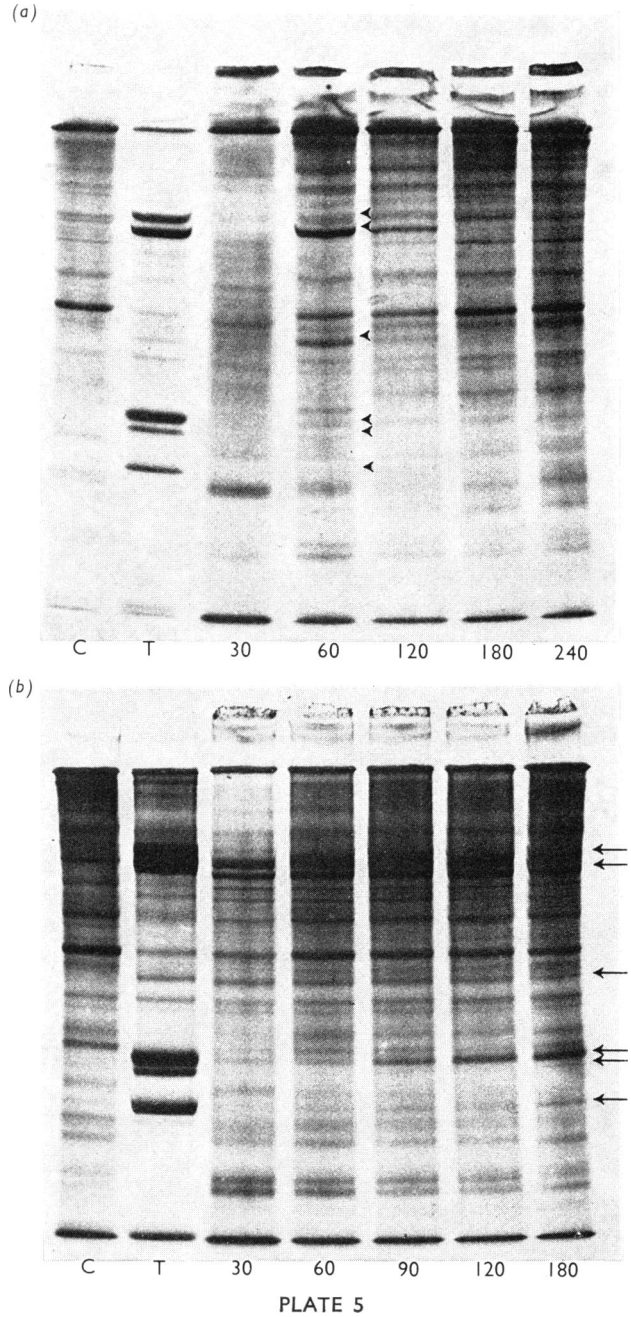
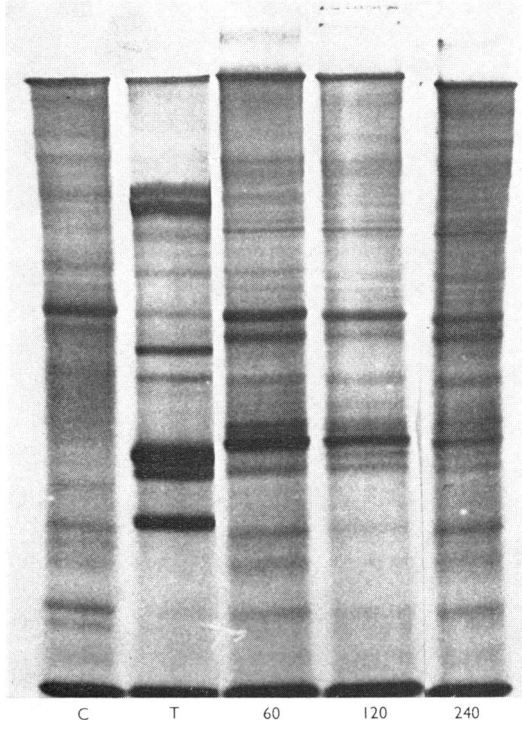


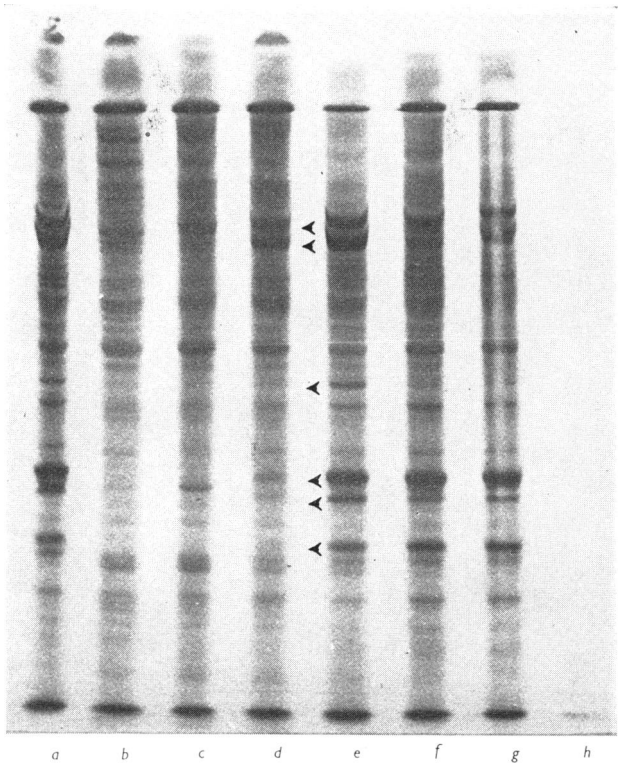
PLATE 5



EXPLANATION OF PLATE 6

Radioautographs of [^{35}S]methionine labelled proteins from extracts of glands incubated with oligomycin

Glands were labelled as described in the Experimental section after the following incubation conditions: Control (C), 2h at 25°C; heat-treated (T), 2h at 37°C; 60, 120 and 240, after 1, 2 and 4h in the presence of 0.25mg of oligomycin/ml respectively.



EXPLANATION OF PLATE 7

Radioautographs of [^{35}S]*methionine pulse-labelled proteins extracted from glands incubated with actinomycin D, chloramphenicol or cycloheximide*

(*a*) Glands were labelled after 2h incubation at 37°C; (*b*) glands were labelled after 2h incubation at 37°C in the presence of 20μg of actinomycin D/ml, actinomycin D was added before the glands were transferred to 37°C; (*c*) as in (*b*), except that actinomycin D was added after a 15min preincubation at 37°C; (*d*) as in (*b*), except that actinomycin D was added after a 30min preincubation at 37°C; (*e*) glands were labelled after a 2h incubation at 37°C in the presence of 100μg of chloramphenicol/ml; (*f*) as in (*e*), except that chloramphenicol was also present during the labelling period; (*g*) as in (*e*), except that chloramphenicol was present only during the labelling period; (*h*) glands were labelled after a 2h incubation at 37°C in the presence of 5μg/ml of cycloheximide.

Under these conditions, if the mRNA coding for the 'heat-shock' bands is unstable, one would expect a transient appearance of the 'heat-shock' bands. This is indeed seen after puff induction with 2,4-dinitrophenol (Plate 5*a*): the six extra bands are clearly seen 60 min after the removal of 2,4-dinitrophenol from the medium, but only faintly after 120 min. However, in the case of vitamin B-6, a slow increase in the amount of additional bands made is seen, even though the puff-inducing stimulus was removed (Plate 5*b*). The reason for this phenomenon is not clear, but it may be due to a slow rate of transport of RNA from the puff area to the cytoplasm. It is clear, however, that also during vitamin B-6 treatment, as well as during all other treatments used here, six additional (as compared with untreated glands) peptide chains are synthesized.

The majority of treatments that interfere with the respiratory mechanism also cause a lowering of the ATP concentration in the cell (Leenders *et al.*, 1974*a*, *b*). This decrease could affect the rate of initiation and thus change the pattern of protein synthesis, independent of any new transcription. To determine whether such a pattern shift does indeed occur, salivary glands were incubated in the presence of oligomycin, which lowers the ATP concentration but does not induce puffs. A change in pattern of protein synthesis does indeed occur, but the typical six bands are not visible (Plate 6). Further, as shown in Plate 7, and in agreement with others (Lewis *et al.*, 1975), the appearance of these bands is strictly dependent on RNA synthesis *de novo* (since it is inhibited by actinomycin D) and cytoplasmic protein synthesis *de novo* (since it is blocked by cycloheximide, but not by chloramphenicol).

Kinetics of induction of the 'heat-shock' bands

From radioautograms such as shown in Plates 3, 4 and 5 it is possible to quantify the relative amount of radioactivity incorporated in each band by measuring the darkness of the film. Clearly such a quantitation cannot yield absolute rates of synthesis: only the relative amount of incorporation of methionine in the 'heat-shock' bands with respect to the total incorporation can be determined. The quantitative changes observed in protein-synthesis patterns under the various puff-inductive conditions are very similar: the rate of synthesis of the 'heat-shock' bands increases rapidly within the first hour. In contrast with previous work (Lewis *et al.*, 1975) no indications were found for a sequential appearance of the bands, rather, all bands appear at the same time

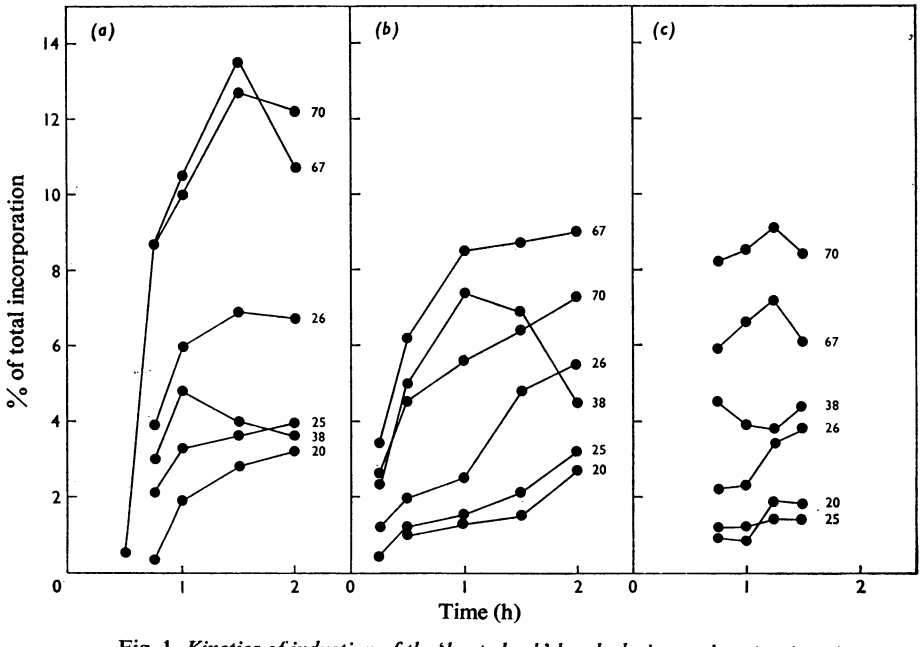


Fig. 1. *Kinetics of induction of the 'heat-shock' bands during various treatments*

The relative amount of label (expressed as % of the total darkening of the film) in the 'heat-shock' bands was calculated as described in the Experimental section from radioautograms as shown in Plates 3, 4 and 5. (*a*) At 37°C; (*b*) in the presence of 0.1 mM-sodium arsenite; (*c*) after release from 2 h of N_2 anaerobiosis. The numbers given in the Figure indicate the molecular weights ($\times 10^{-3}$) of the various peptides.

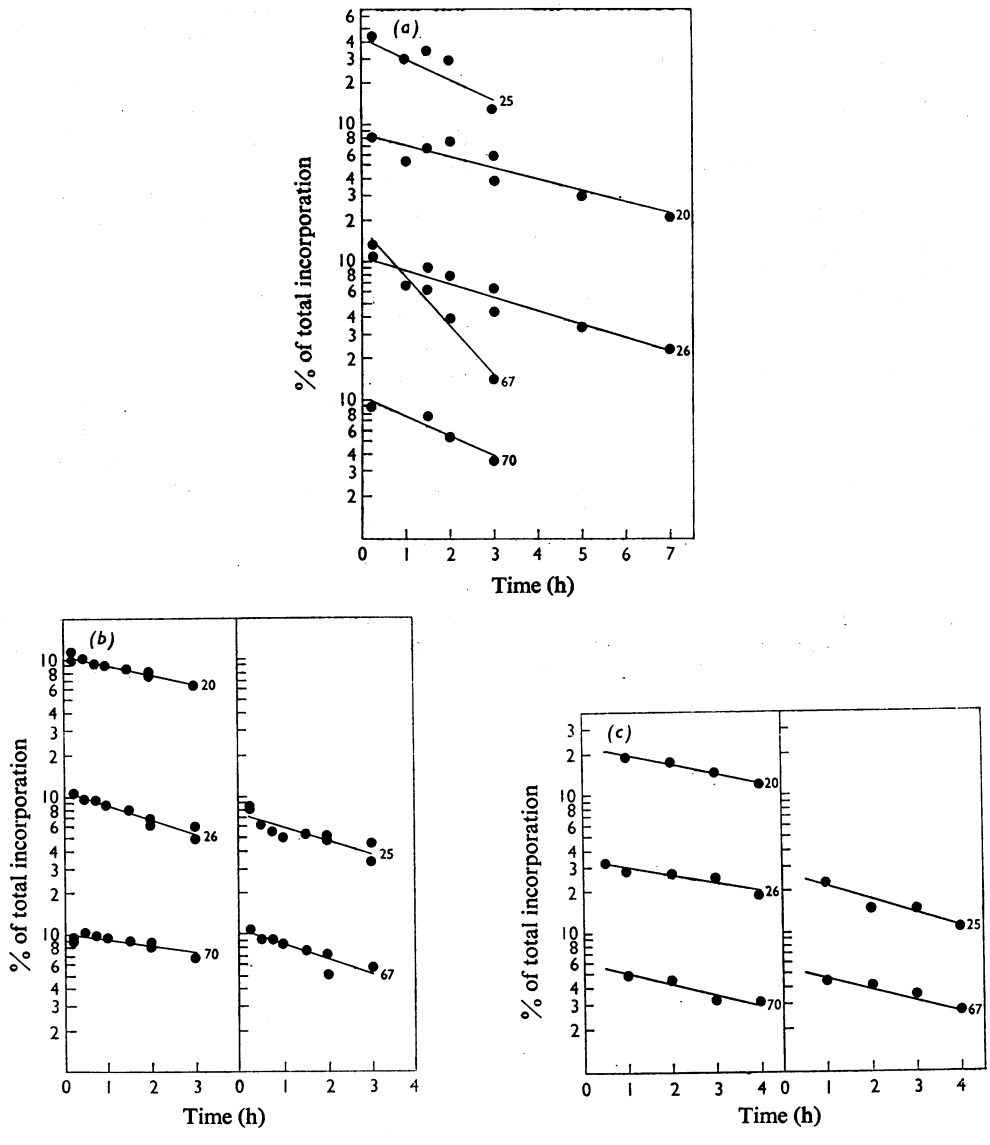


Fig. 2. *Kinetics of decay of the rate of synthesis of 'heat-shock' bands*

The relative amount of label in the 'heat-shock' bands was calculated as described in the Experimental section. (*a*) Glands were incubated for 2h at 37°C and then further incubated at 25°C; (*b*) glands were preincubated for 2h at 37°C, then actinomycin D (20μg/ml) was added and incubation was continued at 37°C; (*c*) glands were preincubated for 2h in 1mM-2,4-dinitrophenol. The numbers given in the Figure indicate the molecular weights ($\times 10^{-3}$) of the various peptides.

(Fig. 1). The rate of synthesis of the bands remains relatively constant after the first hour of induction, except for the 38000mol.wt. band, whose rate of synthesis decreases again after 1h, at least at 37°C or in the presence of arsenite. The relative rate of synthesis of this protein at 37°C appears to be lower than during arsenite treatment or during recovery from N_2 anaerobiosis. On the other hand, the two high mol.wt. proteins (70000 and 67000) are synthesized at a relatively greater rate at 37°C as compared with the other treatments used. The 'heat-shock' proteins account for about 50% of the total protein synthesis at 37°C, but for only 25% during arsenite treatment or after release from N_2 anaerobiosis.

Kinetics of decay of the synthesis of the 'heat-shock' bands

If the incubation temperature of the glands is lowered from 37° to 25°C the temperature puffs rapidly regress. A similar response is seen in the rate of synthesis of the 'heat-shock' bands (Fig. 2*a*): the rate of synthesis of all bands decreases rapidly and with apparent first-order kinetics. The rate of decay of most bands has a half-life of 3h. Only the 67000 mol.wt. band decays faster, with a half-life of about 45min. A decrease in the rate of synthesis is also found after addition of actinomycin D to glands incubated at 37°C (Fig. 2*b*): again the rate of synthesis of the 'heat-shock' bands decreases with first-order kinetics and with a half-life of 3h. After puff induction with 2,4-dinitrophenol the rate of synthesis of the 'heat-shock' proteins decreased with the same apparent half-life (Fig. 2*c*). The mRNA coding for the 'heat-shock' proteins thus appear to be unstable with a half-life of roughly 4h.

Discussion

The data presented in this paper confirm and extend the observations of Lewis *et al.* (1975): namely, all treatments that induce the full set of 'heat-shock' puffs also lead to similar qualitative changes in the pattern of protein synthesis, thus strengthening the hypothesis that these puffed loci contain the genetic information for the protein of these 'heat-shock' bands. Other changes in the protein-synthesis pattern are also observed, for example, an additional high-molecular-weight protein appears during arsenite treatment, but such changes appear to be treatment specific, and do not accompany puff induction in all cases.

As expected, since the size and activity in RNA synthesis of the puffs induced by the various treatments are very similar, there are no large quantitative differences in either the kinetics of induction or the rate of synthesis of the various 'heat-shock' bands between the various treatments, with the possible exception of the 38000mol.wt. band. The decrease in the rate of synthesis of this latter band during either temperature or arsenite treatment is not accompanied by obvious changes in the puffing patterns. From the data presented here, no correlation can be made between the presence of any one puff and the appearance of any one 'heat-shock' band. Not only can a precursor–product relationship between any two bands not be excluded, but a further difficulty is that there are only four major 'heat-shock' puffs in *D. hydei* and at least three small puffs (Berendes *et al.*, 1965). These latter puffs have not been studied intensely, but two of these might also be involved in the synthesis of the 'heat-shock' bands. In *D. melanogaster*-locus 3-87B is the largest puff and the 67000 mol.wt. band the most prominently labelled one (Tissières *et al.*, 1974). Further, RNA isolated from 'heat-shocked' tissue-culture cells hybridized most heavily to this locus (McKenzie *et al.*, 1975; Spradling *et al.*, 1975*a*, *b*). It has thus been suggested that in *D. melanogaster* locus 3-87B codes for the 67000mol.wt. protein. Such a correlation between protein and puff would be strengthened, if the protein patterns are analysed after treatments, which induce only a few of the 'heat-shock' puffs, for example after treatment with antimycin A, which induces the loci 2-32A, 2-36A and 2-48BC, but not 4-81B in *D. hydei*. Unfortunately, antimycin A also inhibits [^{35}S]-methionine incorporation severely and apparently irreversibly.

At 37°C the relative rate of synthesis of the protein in these bands is about twice as high as during the other treatments used here. The data presented by McKenzie *et al.* (1975) suggest that the high rate of synthesis of the 'heat-shock' proteins at 37°C is due to a translational rather than transcriptional control of protein synthesis. If so, the translational control must act negatively on the pre-existing mRNA species rather than positively on mRNA species coding for the 'heat-shock' proteins since the rate of decay of the synthesis of these bands is the same at 25° and at 37°C in the presence of actinomycin D (with the possible exception of the 67000-mol.wt. band). Under the latter conditions, the rate of decrease of synthesis of the bands presumably reflects the rate of decay of the mRNA. Moreover, it cannot be excluded that this translational effect is non-specific; it could be the result of changes in the net rate of initiation of protein synthesis due to an increase in temperature and a decrease in the cellular ATP concentration. Such changes in net initiation rate are expected to result in a change in the protein-synthetic pattern, both theoretically (Lodish, 1974) and also experimentally (Ayuso-Parilla & Parilla, 1975; Plate 6). The lack of suppression of background synthesis at 25°C during arsenite treatment or after N_2 anaerobiosis also suggests that any translational effect at 37°C is correlated with the specific inductive stimulus rather than with the presence of the set of puffs.

Spradling *et al.* (1975*a*, *b*) have found that the half-life of unstable mRNA in an *Aedes albopictus* cell line is 1.2h. A significantly longer functional half-life was found here for the mRNA species coding for the 'heat-shock' bands, namely about 4h. A similar decay rate of the synthesis of the 'heat-shock' proteins was found after removal of the puff-inductive stimulus or in the presence of actinomycin D, except after puff induction with vitamin B-6. In this case the rate of synthesis of the 'heat-shock' proteins increased up to 3h after removal of vitamin B-6 from the medium. The reason for this phenomenon is not clear, but it may be due to a combination of a slow regression of the puffs and a slow rate of transport of the puff RNA from the nucleus. Cytological studies do show a slow

regression of the puff 2-48BC and a slow rate of transport of material from this puff after induction with vitamin B-6 (J. Derksen, personal communication).

The kinetics of induction of the 'heat-shock' bands are very similar to the previously found rate of increase of some mitochondrial enzyme activities (Leenders & Beckers, 1972; Leenders *et al.*, 1974*a*,*b*; Koninkx *et al.*, 1975; Koninkx, 1975; Sin & Leenders, 1975). The data presented here do not therefore exclude the possibility that the 'heat-shock' peptides are part of such enzymes, nor do they offer further support for this suggestion. Further evidence for such a function of the 'heat-shock' proteins must come from a characterization of the peptide chains of these enzymes.

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References

Ashburner, M. (1970) *Chromosoma* **31**, 356–376
Ayuso-Parilla, M. S. & Parilla, R. (1975) *Eur. J. Biochem.* **55**, 593–599
Berendes, H. D., van Breugel, F. M. A. & Holt, Th. K. H. (1965) *Chromosoma* **16**, 35–47
Berendes, H. D. (1966) *Chromosoma* **20**, 32–43
Boyd, J. B., Berendes, H. D. & Boyd, H. (1968) *J. Cell Biol.* **38**, 369–376
Koninkx, J. F. J. G., Leenders, H. J. & Birt, L. M. (1975) *Exp. Cell Res.* **92**, 275–282
Koninkx, J. F. J. G. (1975) *Biochem. J.* **152**, 17–22
Leenders, H. J. & Beckers, P. J. A. (1972) *J. Cell Biol.* **55**, 257–265
Leenders, H. J. & Berendes, H. D. (1972) *Chromosoma* **37**, 433–444
Leenders, H. J., Derksen, J., Maas, P. M. J. M. & Berendes, H. D. (1973) *Chromosoma* **41**, 447–460
Leenders, H. J., Berendes, H. D., Helmsing, P. J., Derksen, J. & Koninkx, J. F. J. G. (1974*a*) *Sub-Cell. Biochem.* **3**, 119–147
Leenders, H. J., Kemp, A., Koninkx, J. F. J. G. & Rosing, J. (1974*b*) *Exp. Cell Res.* **86**, 25–30
Lewis, M., Helmsing, P. J. & Ashburner, M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3604–3608
Lodish, H. F. (1974) *Nature (London)* **251**, 385–388
McKenzie, S. L., Henikoff, S. & Meselson, M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1117–1121
Mitchell, H. K. & Mitchell, A. (1964) *Drosophila Inf. Service* **39**, 135–137
Poels, C. L. M. (1972) *Cell Differ.* **1**, 63–78
Ritossa, F. M. (1962) *Experientia* **18**, 571–572
Ritossa, F. M. (1964) *Exp. Cell Res.* **36**, 601–607
Sin, Y. T. & Leenders, H. J. (1975) *Insect Biochem.* **5**, 447–457
Spradling, A., Hui, H. & Penman, S. (1975*a*) *Cell* **4**, 131–137
Spradling, A., Penman, S. & Pardue, M. L. (1975*b*) *Cell* **4**, 395–404
Tissières, A., Mitchell, H. K. & Tracy, U. M. (1974) *J. Mol. Biol.* **84**, 389–398