dria do accompany aging in invertebrates[15]. In this context, it has been recently noted by Driver and Lamb that old *Drosophila* have impaired energy metabolism and are less energy efficient than young flies[18]. Moreover, since respiratory activity is directly related to the activity of cytochrome oxidase, changes in the activity of this enzyme may stem from changes in the inner mitochondrial membrane. It is thought for example, that temperature plots of membrane bound enzyme activities are reflected by changes in the lipid components of the membrane[19,20]. Possibly, this effect is the result of membrane lipid changes induced by peroxidation of mitochondrial lipids. The fact that lipid peroxidation is related to aging rates is well established[21]. Furthermore, one of the well characterized effects of lipid peroxidation on the mitochondria is an alteration in the physical properties of the phospholipids in the inner membrane bilayer[22]. Thus, age induced lipid peroxidation would be expected to affect mitochondrial enzyme activity and this would be manifested in in vivo respiratory activity. This could explain the altered respiratory rates observed as a function of temperature in this study.

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Heat shock response in the Atlantic sea urchin, *Arbacia punctulata*[1]

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Summary. Synthesis of heat shock proteins of blastulae and gastrulae of the sea urchin, *Arbacia punctulata,* was characterized by analyzing proteins labeled in vivo with [35S]methionine on 2-dimensional polyacrylamide gels. Trypsin was also shown to stimulate synthesis of heat shock proteins.

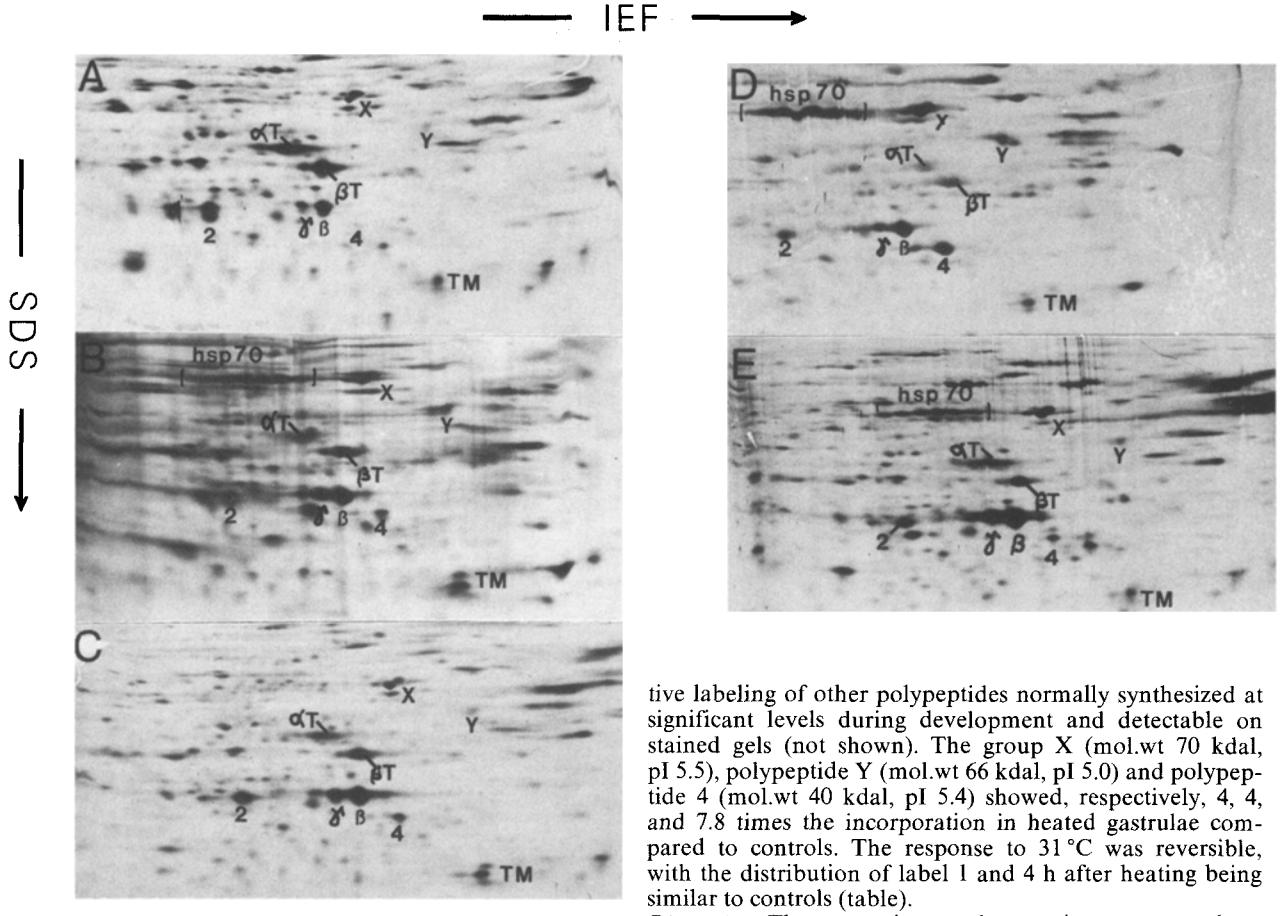
Giudice and coworkers[2,3] have demonstrated that sea urchin embryos resemble other organisms[4] in responding to heat shock by altering the relative synthesis of certain proteins, with the major such heat shock protein (HSP) having a mol.wt of 70 kdal (HSP70). This paper defines this response further by demonstrating isoelectric variants of HSP70 and by quantitating the synthesis of selected polypeptides in response to heat and trypsin.

Materials and methods. Adults and embryos of *Arbacia punctulata* (fertilization $\geq$ 95%) were maintained at 21 °C. Trypsin (150 µg/ml) was added to some embryos after fertilization. Hatched blastulae (8 h after fertilization) and early gastrulae (13 h) were cultured as follows. After 70 min at either 21 °C (control) or 31 °C (heated), control and heated embryos were labeled 20 min at their respective temperatures in artificial sea water (1 ml) containing streptomycin (150 µg) and [35S]methionine ([35S]met, 45 µCi). Non-labeled, heated embryos were quickly cooled to 21 °C after 90 min at 31 °C. Samples of control and heated cultures, taken 1 and 4 h after the heating period, were also labeled 20 min at 21 °C.

Labeled embryos were prepared for electrophoresis[5] with the total hot trichloroacetic acid (TCA)-precipitable cpm and total protein[6] being determined for each sample. Isoelectric focusing (IEF) gels were prepared according to O'Farrell[7] with electrophoresis in the 2nd dimension through 9% separating and 3% stacking gels[8]. The gel slabs were stained, dried, and autoradiographed (Kodak XAR-5) for 10–14 days. Selected spots were cut from dried gels, solubilized, and counted by liquid scintillation spectrometry. [35S]met incorporation into each polypeptide was recorded as the percentage of total hot TCA-precipitable cpm loaded in the IEF dimension. Because this computation assumes 100% recovery in both electrophoretic dimensions, the values obtained probably underestimate the actual percentages.

Results. Heating blastulae and early gastrulae stimulated the synthesis of 5–6 polypeptides (HSP70) with a mol.wt of approximately 70 kdal and isoelectric points (pI) between 5.8 and 6.5. The stimulated synthesis was 6.7 and 9.0 times that of control blastulae and gastrulae, respectively (table). The HSP70 were synthesized slowly, if at all, at 21 °C unless the embryos had been exposed to trypsin (fig., table). Incubation at 31 °C reduced overall protein synthesis about 50%, but not uniformly. Actin and tropomyosin synthesis was much less sensitive to heat than that of tubulins and polypeptide 2 (fig., table).

Heated gastrulae, but not blastulae, showed increased rela-



Autoradiograms of 2D gels of [35S]met-labeled polypeptides from control, heated, and trypsin-treated blastulae and gastrulae. *A* Blastula C; *B* Blastula H; *C* Gastrula C; *D* Gastrula H; *E* Blastula T. (These designations are as in the table legend). β, γ, β-, γ-actins; αT, βT, α-, β-tubulins, TM, tropomyosins.

tive labeling of other polypeptides normally synthesized at significant levels during development and detectable on stained gels (not shown). The group X (mol.wt 70 kdal, pI 5.5), polypeptide Y (mol.wt 66 kdal, pI 5.0) and polypeptide 4 (mol.wt 40 kdal, pI 5.4) showed, respectively, 4, 4, and 7.8 times the incorporation in heated gastrulae compared to controls. The response to 31 °C was reversible, with the distribution of label 1 and 4 h after heating being similar to controls (table).

Discussion. These experiments characterize more completely the HSP synthesized by sea urchin embryos cultured at 31 °C. Among those polypeptides selected for quantitative analysis, it is apparent that HSP70 peptides are similar to those of *Drosophila* and *Dictyostelium* in molecular weight,

The percentage of 35S met incorporated into selected polypeptides in response to heat shock

Embryo type	Polypeptide monitored								
	Actins	α-Tubulin	β-Tubulin	HSP70	X	Y	2	4	Tropomyosins
Blastula C	1.67	0.81	1.81	0.18	0.29	0.08	0.60	0.07	0.16
Blastula H	1.31	0.21	0.35	1.21	0.32	0.06	0.11	0.05	0.16
Blastula H1	2.31	0.26	0.55	0.16	0.37	0.05	0.28	0.04	0.12
Blastula C4	2.65	0.25	0.62	0.10	0.21	0.02	0.35	0.08	0.09
Blastula H4	2.74	0.37	0.78	0.06	0.12	0.03	0.27	0.03	0.09
Blastula T	3.10	0.17	0.26	0.31	0.25	ND	0.25	0.08	ND
Gastrula C	1.15	0.16	0.40	0.19	0.13	0.04	0.28	0.06	0.11
Gastrula H	0.93	0.09	0.15	1.71	0.52	0.16	0.15	0.47	0.14
Gastrula H1	1.99	0.20	0.45	0.21	0.21	0.12	0.22	0.06	0.10
Gastrula C4	1.68	0.23	0.47	0.12	0.07	0.03	0.11	0.09	0.15
Gastrula H4	1.80	0.24	0.57	0.20	0.07	0.07	0.19	0.09	0.14
Blastula H/C	0.80	0.26	0.19	6.7	1.1	0.75	0.18	0.87	1.0
Gastrula H/C	0.80	0.62	0.38	9.0	4.0	4.0	0.53	7.8	1.3

The percentage of total TCA precipitable cpm loaded in the isoelectric focusing dimension recovered for each polypeptide was calculated. The actin values are sums of β- and γ-actins; tubulin values also include isoelectric variants. HSP70 and X-values are sums of the spots indicated on the figure. In all cases but blastula H and gastrula C4, the values are averages of duplicate gels with variation $\leq 15\%$. C, control; H, labeled during the last 20 min of heating; H1, labeled 1 h after returning cultures to 21 °C; H4, labeled 4 h after heating; C4, the corresponding control; T, trypsin treated; H/C, ratio of incorporation in heated embryos to the corresponding control; ND, not determined.

multiplicity of pI, and inducibility[9-11]. The doublet at mol.wt 70 kdal previously observed in sea urchins[2,3] is probably more heterogeneous, including the 5-6 HSP70 and the X-polypeptides characterized here.

The developmental dependence of the stimulated or continued synthesis of other HSP is more marked than noted earlier[2,3]. This discrepancy could result from the quantitative analysis in these experiments, or species or culturing differences. Variation may be more probable in recently hatched blastulae since the ability to synthesize HSP is first apparent then[2]. Also the heat shock response is being superimposed over other developmentally regulated changes in the expression of such polypeptides as 2, X, actins, and tubulins (table). Because normal X-synthesis is greater in blastulae, its continued synthesis at 31 °C does not make it appear to be heat-regulated at that stage.

It is interesting that trypsin induces HSP70 synthesis at 21 °C. Certainly stimuli other than heat induce HSP synthesis[4]. Trypsin has long been known to alter normal embryonic development[12] as well as stimulate DNA synthesis in dissociated cells of sea urchins[13]. Thus patterns of polypeptide synthesis are modified also.

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Differential activation of two monoamine oxidase types by oxygen

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Summary. The stimulation of rat brain monoamine oxidase activity by oxygen is shown to be type-selective, type B being much more strongly stimulated than type A.

The stimulation of monoamine oxidase (MAO EC 1.4.3.4) activity by high oxygen tension, which was observed long ago[2,3], has been found in recent years to be related to the amine substrate used[4-8]. The assumption that MAO may be classified on the basis of the degree of oxygen stimulation[8] (apart from substrate and inhibitor affinities) has come under dispute recently[9], and the suggestion that there is half-sites reactivity of the type described by Seydoux et al.[9] appears to be unnecessary in view of the reports[11-16] that both MAO types show some activity towards most substrates. The present work demonstrates that the phenomenon of oxygen stimulation of MAO activity is not substrate-selective but type-selective, type B MAO being much more strongly stimulated than type A MAO. This phenomenon, however, leads indirectly to some substrate-selectivity as the substrates themselves are to a large extent preferentially oxidized by 1 MAO type, though some are oxidized by both forms of the enzyme.

A rat brain mitochondrial preparation with 1 MAO type selectively inhibited was obtained as described earlier[16], and mitochondrial subfractions were prepared by sucrose density gradient fractionation[17,18]. Initial reaction rates were measured in all cases under atmospheres of air or 100% oxygen. Deamination of tyramine was assayed by measuring the formation of aldehyde according to Green and Haughton[19], as described earlier[17], and that of serotonin or tryptamine was assayed by measuring ammonia formation according to Conway and Byrne[20]. Apparent K_m-values for tyramine were calculated from the double reciprocal plots of initial velocities of MAO activity assayed in duplicate with 6 substrate concentrations under atmospheres of air or 100% oxygen. K_o-values were calculated from double reciprocal plots of initial reaction velocities with varied oxygen tensions at a fixed concentration of tyramine (10 mM). A/B ratios were calculated from the position of the plateau in the per cent inhibition scale in the

Table 1. Influence of air and oxygen on the oxidation of various biogenic amines by rat brain mitochondrial preparations

Active MAO type	Gas phase	MAO activity with Tyramine	Tryptamine	Serotonin
Type A (pargyline-treated rats)	Air	10.2±0.8	6.5±0.7	11.3±0.4
	Oxygen	13.8±1.6 (35)	9.4±0.7 (45)	15.2±0.9 (35)
Type B (chlorgyline-treated rats)	Air	9.8±0.6	2.8±0.5	1.9±0.2
	Oxygen	29.7±2.1 (203)	8.1±0.9 (190)	3.7±0.4 (90)
Both types (untreated rats)	Air	21.0±0.9	9.4±0.6	13.9±0.7
	Oxygen	43.0±1.9 (105)	15.1±1.4 (60)	20.1±1.3 (45)
A/B ratio in untreated rats		55/45	70/30	85/15

The data are averages of 6 determinations. Figures in parentheses denote percent stimulation of MAO activity in presence of 100% oxygen. Final concentration of each substrate amine was 10 mM. Enzyme activity is expressed as nmoles of product formed/100 mg tissue/min ± SD.