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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2 Giudice, G., Roccheri, M., and Di Bernardo, M., Cell Biol. int. Rep. *4* (1980) 69.
3 Roccheri, M., Di Bernardo, M., and Giudice, G., Devl Biol. *83* (1981) 173.
4 Ashburner, M., and Bonner, J., Cell *17* (1970) 241.
5 Tufaro, F., and Brandhorst, B., Devl Biol. *72* (1979) 390.
6 Bradford, M., Analyt. Biochem. *72* (1976) 248.
7 O'Farrell, P., J. biol. Chem. *250* (1975) 4007.
8 Laemmli, U., Nature, Lond. *227* (1970) 680.
9 Loomis, W., and Wheeler, S., Devl Biol. *90* (1982) 412.
10 Mirault, M., Goldschmidt-Clermond, M., Moran, L., Arrigo, A., and Tissières, A., Cold Spring Harb. Symp. quant. Biol. *42* (1978) 819.
11 Sanders, M., J. Cell Biol. *91* (1981) 579.
12 Horstadius, S., J. Embryol. exp. Morph. *1* (1953) 327.
13 Vittorelli, M., Cannizzaro, G., and Giudice, G., Cell Diff. *2* (1973) 279.

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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.

clorgyline dose-response patterns[21]. This is a measure of the ratio of the contributions of the 2 MAO forms in a particular tissue preparation towards the oxidation of a particular amine substrate at the concentration used.

The stimulation of MAO activities of selectively inhibited rat brain mitochondrial preparations by high oxygen tension in the incubation medium is shown in table 1. It was observed that mitochondria showing exclusively activity of type B MAO were more strongly stimulated than those with type A MAO activity. The slight 5-HT oxidation (under 100% oxygen) is more strongly affected by type B MAO preparations than it is by type A MAO. In case of crude mitochondrial preparations of brain tissues of normal, untreated rats, the degree of oxygen stimulation of MAO activity with a particular amine shows a fair correlation with the A/B ratio for the oxidation of that amine. In other words, when the A/B ratio is small, the reaction velocity is remarkably stimulated in oxygen-saturated reaction mixtures.

The difference in the extent of oxygen stimulation of tyramine oxidation by the 2 MAO types can be considered as most significant, because tyramine is a very good substrate for both the forms having K_m-values of the same order[16]. Other variables, such as species and tissue differences, are also excluded with the present systems, thus this difference in the extent of oxygen stimulation can be attributed solely to the type of MAO. Recent studies indicate that both forms of MAO are capable of oxidizing most substrates[12-16], the substrate specificity being determined by the relative K_m- and V_{max}-values of the enzyme forms towards the substrate; it appears that one of the most suitable model substrates for both forms of MAO is tyramine, so that subsequent experiments were mostly done with tyramine as the substrate.

Using mitochondrial subfractions obtained by discontinuous sucrose density gradient centrifugation[17,18], it was observed that the subfractions showed different degrees of oxygen stimulation of tyramine oxidation; these could be predicted from the respective A/B ratios of the subfractions (table 2). With guinea-pig and mouse liver mitochondria, which contain predominantly type A and type B MAO respectively, (the A/B ratios shown in table 3 are approximate values for the oxidation of common substrates kynuramine and tyramine respectively as reported by others[22,23]) it was observed that remarkable stimulation of mouse liver MAO occurred in presence of various amine substrates, in contrast to guinea-pig liver MAO which was moderately stimulated in oxygen-saturated reaction mixtures (table 3).

Some kinetic parameters of the different MAO preparations are summarized in table 4. The preparations showing predominantly type B MAO activity exhibit much higher K_m-values for oxygen (K_o) than those rich in type A MAO activity. The increase in apparent K_m-value for tyramine when incubation was done in 100% oxygen was also much greater with type B rich preparations. The observation that K_o-values of the 2 forms may be different is highly interesting.

Since oxygen is supposed to participate in the 3rd stage of the MAO catalyzed reaction[24,25] which involves reoxidation of the reduced FAD, there does not seem to be much scope for substrate selectivity as this particular step should be identical with all the amine substrates. However, it is not known whether FAD functions in an identical manner in both the MAO types. It is evident from the above results

Table 2. Effects of gas phase on the oxidation of tyramine by MAO of rat brain preparations

Tissue preparations	MAO activity in Air	Oxygen	A/B ratio
Crude mitochondria of			
Adult rat brain	21.0±0.9	43.0±1.9 (105)	55/45
New born rat brain	8.3±0.7	15.8±0.7 (90)	75/25
Mitochondrial subfractions			
Fraction C	5.1±0.8	9.6±1.0 (88)	75/25
Fraction D	9.2±1.1	18.9±1.7 (109)	55/45
Fraction E	6.1±0.6	17.3±1.2 (184)	30/70

The results are the average of 6 determinations. Figures in parentheses denote percent stimulation of MAO activity in presence of 100% oxygen. Enzyme activity is expressed as nmoles of aldehyde formed/100 mg tissue/min ± SD.

Table 3. Effects of gas phase on MAO activity of guinea-pig and mouse liver mitochondria

Mitochondrial preparations	A/B ratio*	Gas phase	MAO activity with Tyramine	Tryptamine	Serotonin
Guinea-pig liver	70/30	Air	79.6±3.1	26.7±2.2	70.4±5.3
		Oxygen	127.3±6.7 (60)	44.1±3.8 (65)	109.1±4.1 (55)
Mouse liver	5/95	Air	11.9±1.4	2.6±0.3	4.3±0.2
		Oxygen	58.3±3.6 (390)	8.1±0.9 (210)	8.7±0.7 (103)

The results are the average of 6 determinations. Figures in parentheses denote percent stimulation of MAO activity in presence of 100% oxygen. Enzyme activity is expressed as nmoles of product formed/100 mg tissue/min ± SD. *As reported by Das and Guha[22] and Squires[23].

Table 4. Some kinetic parameters of MAO of various tissues

Mitochondrial preparation	K_m for tyramine (μM) Air	Oxygen (100%)	K_m for oxygen (μM)
Mouse liver	454	2000	335
Guinea-pig liver	160	218	67
Pargyline treated rat brain (type A active)	125	220	80
Clorgyline treated rat brain (type B active)	110	570	402

Average values of 4 determinations are given.

that the degree of MAO stimulation by high oxygen tension is largely determined by the type of MAO participating in the reaction and that apart from the substrate specificities and inhibitor sensitivities, this appears to be another characteristic that distinguishes the 2 MAO types. The lower K_o-value of type A MAO may, however, not really be due to a higher affinity of this form for oxygen; it may rather be caused by some kind of permeability barrier near the type A active site on account of its lipid microenvironment[26,27].

Among the substrates tested, benzylamine seems to be the only exception which, though oxidized by type B MAO, is not remarkably stimulated by high oxygen tension; this may be due to some difference in the reaction mechanism involved in benzylamine oxidation[28]. Moreover, the exact mechanism of the oxidation of reduced FAD is not yet known. It is, however, known that FAD can activate oxygen in different ways[29] and hence the reaction between reduced FAD and oxygen may play an important role so far as the differential effects of oxygen tension on MAO types are concerned. It seems that answers to these problems may help in understanding the phenomenon of oxygen activation of MAO types.

1 Pargyline and clorgyline were gift samples from Abbott Laboratories, USA and May and Baker Ltd, England respectively.
2 Kohn, H.I., Biochem. J., *31* (1937) 1693.
3 Philpot, F.J., Biochem. J. *31* (1937) 856.
4 Novick, W.J., Biochem. Pharmac. *15* (1966) 1009.
5 Jain, M., Sands, F., and Von Korff, R.W., Analyt. Biochem. *52* (1973) 542.
6 Achee, F.M., Togulga, G., and Gabay, S., J. Neurochem. *22* (1974) 651.
7 Nakagawa, K., Folia pharmac. jap. *73* (1977) 297.
8 Fowler, C.J., and Callingham, B.A., Biochem. Pharmac. *27* (1978) 1995.
9 Fowler, C.J., and Oreland, L., Biochem. Pharmac. *29* (1980) 2225.
10 Seydoux, F., Malhotra, O.P., and Bernhard, S.A., C.R.C. Crit. Rev. Biochem. *2* (1974) 227.
11 Mantle, T.J., Houslay, M.D., Garrett, N.J., and Tipton, K.F., J. Pharm. Pharmac. *28* (1976) 667.
12 Ekstedt, B., Biochem. Pharmac. *25* (1976) 1133.
13 Suzuki, O., Katsumata, Y., and Oya, M., in: Monoamine oxidase: Structure, function and altered functions, p. 197. Eds T.P. Singer, R.W. Von Korff and D.L. Murphy. Academic Press, New York 1979.
14 Parkinson, D., Lyles, G.A., Brown, B.J., and Callingham, B.A., J. Pharm. Pharmac. *32* (1980) 844.
15 Peers, E.M., Lyles, G.A., and Callingham, B.A., Biochem. Pharmac. *29* (1980) 1097.
16 Mitra, C., and Guha, S.R., Biochem. Pharmac. *29* (1980) 1213.
17 Mitra, C., and Guha, S.R., Biochem. Pharmac. *27* (1978) 2455.
18 Mitra, C., and Guha, S.R., Biochem. Pharmac. *28* (1979) 1135.
19 Green, A.L., and Haughton, T.M., Biochem. J. *78* (1961) 172.
20 Conway, E.J., and Byrne, A., Biochem. J. *27* (1933) 419.
21 Goridis, C., and Neff, N.H., Br. J. Pharmac. *43* (1971) 814.
22 Das, P.K., and Guha, S.R., Biochem. Pharmac. *29* (1980) 2049.
23 Squires, R.F., Adv. Biochem. Psychopharmac. *5* (1972) 355.
24 Tipton, K.F., Eur. J. Biochem. *5* (1968) 316.
25 Yasunobu, K.T., and Oi, S., Adv. Biochem. Psychopharmac. *5* (1972) 91.
26 Houslay, M.D., J. Pharm. Pharmac. *29* (1977) 664.
27 Houslay, M.D., Biochem. Pharmac. *29* (1980) 3211.
28 Roth, J.A., in: Monoamine Oxidase: Structure, function and altered functions, p. 153. Eds T.P. Singer, R.W. Von Korff and D.L. Murphy. Academic Press, New York 1979.
29 Hemmerich, P., and Wessiak, A., in: Biochemical and clinical aspects of oxygen, p. 491. Ed. W.S. Caughey. Academic Press, New York 1979.

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A preferential uptake of cholesterol by the brain tissue of the housefly, *Musca domestica* L.

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Summary. Cholesterol is taken up by the brain tissue of larvae of *Musca domestica* in preference to β-sitosterol. The uptake reaches a maximum value when both sterols are available to the insect each as 0.01% of the diet.

Insects are unable to synthesize sterols de novo and the need for sterol for normal growth and development in many species can be satisfied by cholesterol[2]. A number of plant feeding insects are able to metabolize phytosterols[3]. In insects unable to carry out such a conversion much of their requirement for cholesterol can be met with closely related sterols called sparing sterols[4]. When *Eurycotis floridana* were fed with cholesterol and a sparing sterol (cholestanol), the cholesterol to cholestanol ratio was observed to be greater in the insect than in the diet. The ratio of cholesterol to cholestanol was not the same in all the tissues but was particularly high in the nervous tissue of both nymphs and adult insects[5]. Analysis of the sterols in houseflies, *Musca domestica* fed on diets containing a mixture of sterols also showed that these insects preferentially utilized cholesterol or the sterol most closely resembling cholesterol in structure[6]. Houseflies cannot convert β-sitosterol to cholesterol like some other insect species such as the Mexican bean beetle[8], the milkweed bug[9], and the khapra beetle[10], and although the phytosterol supports larval growth in houseflies, it does so less effectively than cholesterol[7]. It may therefore be considered to be a 'sparing sterol' for the housefly, and the present experiments were conducted to examine whether the preferential accumulation of cholesterol in the nervous tissue of the insect occurred in the presence of a sparing sterol in the diet.

This paper reports the distribution of sterols in the various tissues of the housefly, *Musca domestica*, when the concentration of larval dietary cholesterol was altered in the presence of β-sitosterol (0.01 or 0.02% of the diet). Carrier-free [4-^{14}C] cholesterol (sp. act. 61 mCi/mM) and β-[22,23(n)^3-H] sitosterol (sp. act. 58 Ci/mM) were obtained from the Radio-chemical Centre, Amersham, England. The purity of [^{14}C] cholesterol and β-[^{3}H] sitosterol was checked by TLC and radio-gas liquid chromatography. Reference β-sitosterol and its derivatives were obtained from the M.R.C. Steroids Reference Collection, London, U.K. The larvae of houseflies were reared under aseptic condition on diets containing the desired proportion of sterols as described previously[11,12]. The dissection of larval tissue and the procedures used for the extraction of their lipids are reported in detail elsewhere[12,13]. An aliquot of the lipid extract was dried under nitrogen and the residue saponified. Sterols were isolated from the other unsaponifiable