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.
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0014-4754/83/030270-03$1.50 + 0.20/0

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

lipids by means of TLC on silica gel-G using a solvent system hexane:diethylether (95:5 v/v). A conventional gas chromatograph equipped with a flame ionization detector and a fraction collector (splitter ratio 1:9.7) was used to separate sialated sterols at 270 °C on a 1.52 m glass column packed with OV-17 (1:33 w/w) on diatomite CQ (100–120 mesh). The split samples were absorbed on silica gel and eluted with diethyl ether, evaporated to dryness and dissolved in 10.0 ml scintillation fluid; radioactivity was assayed in a Packard Tricarb scintillation system. The [^{14}C] and [^{3}H]-radioactive counts were corrected for quenching using the external standard channel ratio method.

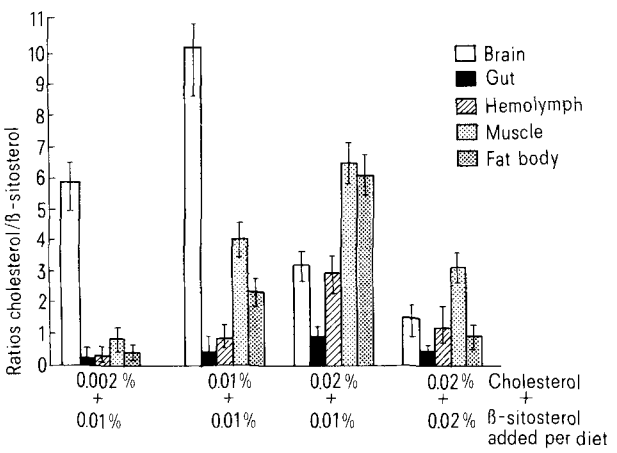
No conversion of β-sitosterol to cholesterol was observed during larval development of insects fed on labeled β-sitosterol. As shown in the table, the brain tissue contained a higher proportion of cholesterol to β-sitosterol than any other larval tissues examined when the dietary cholesterol was 0.002% and β-sitosterol 0.01%, although the total concentration of sterols in whole insects was similar to that reported in larvae reared on 0.02% of cholesterol in the diet[11]. As shown in the figure, the ratio of the 2 sterols was less in the lipid extract of brain tissue from insects reared on diets containing 0.02% of both cholesterol and β-sitosterol than it was when 0.002, 0.01, and 0.02% of cholesterol with 0.01% of sitosterol were included in the diets. A maximum value was observed in the ratio of cholesterol to β-sitosterol in the brain tissue of larvae when dietary concentration of cholesterol and β-sitosterol each were 0.01% of the diet, and further addition of dietary cholesterol did not increase the proportion of cholesterol in the larval brain tissues. The results suggest that brain tissues of houseflies, like those of *E. floridana*, have a mechanism for preferentially accumulating cholesterol.

These results are reminiscent of results obtained with low levels of choline and β-methylcholine in the housefly[14,15]. These studies suggested a preferential uptake of choline into the nervous system, where it was accumulated in the form of phosphatidylcholine[16,17]. House flies are unable to synthesize either cholesterol or β-sitosterol, the incorporation of 2 sterols into various tissues should be dependent upon their relative concentration in the diet, absorption from the gut, efficiency of transport and their specific requirements for the development of the insect. The results do not suggest interference of β-sitosterol with the absorption of cholesterol from the gut since the level of total sterols in whole insects remained similar on alteration of their dietary cholesterol. The preferential accumulation of cholesterol by the brain tissue of the insects has been observed in houseflies when reared on 0.002% of dietary cholesterol[12] and in *E. floridana* when 95% of the cholesterol requirement was spared by cholestanol[4]. The brain-ring gland complex of diptera is known to produce hydroxylated steroids from cholesterol as intermediate for ecdysone biosynthesis[18]. It is likely, therefore, that there is a specific requirement for cholesterol for the brain tissue, and this is essential for the growth and development of insect. Evidently β-sitosterol may substitute for cholesterol in all tissues of the insect with some degree of stereochemical flexibility. However, replacement of cholesterol by a sparing sterol in the brain tissue may not fully satisfy the structural and metabolic requirement of insects.

Relative contents of cholesterol and β-sitosterol in various larval tissues of *Musca domestica* reared on diets containing labeled cholesterol (0.002% of diet) and β-sitosterol (0.01% of diet)

Tissues	Cholesterol (nmoles/mg wet wt) A	β-Sitosterol (nmoles/mg wet wt) B	A/B
Diet[a]	0.05	0.26	0.20
Brain	2.79 ± 0.57	0.47 ± 0.13[d]	5.94
Fat body	0.09 ± 0.03	0.23 ± 0.08[c]	0.39
Composite gut fraction	0.31 ± 0.06	1.20 ± 0.25[d]	0.26
Muscle	0.24 ± 0.05	0.33 ± 0.22[b]	0.73
Cuticle	0.08 ± 0.02	0.13 ± 0.05[c]	0.62
Hemolymph (nmoles/μl)	0.09 ± 0.03	0.36 ± 0.07[d]	0.25
Whole larvae	0.34 ± 0.10	0.78 ± 0.11[d]	0.44

Sterols were separated by GLC from the lipid extracts and the radioactivity of the trapped effluent was counted. The results are expressed as means ± SD of 4 separate experiments. [a]μmoles sterols/g wet wt of diet. [b]Not significantly different from A ($p < 0.250$); [c]significantly different from A ($p < 0.025$); [d]significantly different from A ($p < 0.005$).



The ratios of cholesterol to β-sitosterol in various tissues of the larvae fed on various concentrations of cholesterol with β-sitosterol. Both sterols are expressed as percentage of the diets. Lipid sterols were extracted as described in text. Results are expressed as means ± SD for 4 separate experiments.

1 Acknowledgments. The author thanks R.G. Bridges, A.R.C. Unit, Department of Zoology, Cambridge, for his valuable suggestions.

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