limited only in the supranuclear zone as in the previous stage. During fourth and fifth instars, the cells of the mid-gut epithelium, cells of hepatic caecae and the epithelial cells of the hind-gut showed heavy impregnation. Cells of fore-gut epithelium and nerve cells did not indicate any increase in the ascorbic concentration. Heavy impregnation of the spinning gland cells was now found mostly towards the lumen surface of the cells. The fat body and blood cell also showed considerable concentrations of ascorbic acid in these latter instars. A comparative investigation of different instars showed that there was a gradual increase in the amount of ascorbic acid in different tissues as the larva grew, except in the case of nerve cells. Estimation of ascorbic acid during different instars based on the extracts of whole larvae also indicated such gradual increase (Table 1).

Table 1

Instars	mg/g
First instar larva	0·08
Second instar larva	0·35
Third instar larva	0·49
Fourth instar larva	0·71
Fifth instar larva	0·82

From both histological examination and extract estimation, it is evident that high concentration of ascorbic acid is found in the larval life. This ascorbic acid does not appear to be purely of dietary origin, as in the first instar larva, which had not started feeding, an appreciable quantity of ascorbic acid was found in the tissues. The gradual increase in the amount of ascorbic acid from first instar to the last suggests that ascorbic acid is required during the process of metamorphosis during the formation of adult organs.

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MICROBIOLOGY

Inhibition of Yeast Growth by Streptomycin

For the isolation of a pure culture from yeast cultures heavily contaminated with bacteria, sub-cultures were made into media containing penicillin and streptomycin. With certain yeasts we observed that yeast growth as well as bacterial growth was inhibited. Preliminary observations suggested that the streptomycin was the inhibitory agent. This observation contradicts earlier opinion which considered fungi, including yeasts, to be insensitive to the action of streptomycin[1,2]. Based on this opinion of insensitivity to streptomycin, some media designed for the isolation of pathogenic[3] and saprophytic[4] yeasts include streptomycin, at a concentration of 40 μg/ml., to inhibit bacterial growth.

However, partial inhibition of yeast growth by streptomycin at the high concentration of 100–10,000 μg/ml. (ref. 5), and also partial inhibition of a variant of *Torula utilis* by 13 μg/ml. of streptomycin[6], have been reported. This communication describes experiments which were designed to measure quantitatively the streptomycin-sensitivities of yeast strains from the National Collection of Yeast Cultures which appropriate screening had suggested to be sensitive to this agent.

The streptomycin-sensitivities of the yeast strains were assessed in M.Y.G.P. (ref. 7) except for the osmophilic strains which were assayed in osmophilic medium (malt extract 0·3 per cent, yeast extract 0·3 per cent, sucrose 40 per cent, dextrose 5 per cent, peptone 0·5 per cent). The final *p*H of both types of medium was 6·9–7·0. Streptomycin sulphate was added aseptically to the medium just prior to inoculation. As the nature of the medium has been demonstrated to have a significant effect on the activity of streptomycin towards yeasts[5], a complex medium was deliberately chosen for assay so that inhibition of yeast growth in the assay system would then reflect the probability of inhibition of yeast growth occurring during laboratory isolations. Assays were performed in 10 ml. amounts of medium, and each tube was inoculated with a standard loopful of a 3-day yeast culture (4·5–11 × 10^4 yeast cells per tube). Incubation was at 25° C for 72 h on an automatic shaker (3 cm amplitude, 80 cycles per min). The degree of growth at the end of this period was assessed visually.

Table 1. Sensitivity of Yeasts to Streptomycin

Yeast	N.C.Y.C. No.	Growth in presence of streptomycin sulphate (μg/ml.) 0	10	20	50	100
Candida utilis	193	+	+	+	+	+
Endomycopsis fibuliger	13	+	+	Sl†	–	–
Hansenula anomala (*O*)*	522	+	+	+	+	+
Hansenula subpelliculosa (*O*)	558	+	+	+	+	+
Saccharomyces acidifaciens	464	+	+	+	–	–
Saccharomyces carlsbergensis	511	+	+	+	Sl	–
Saccharomyces carlsbergensis	519	+	+	+	–	–
Saccharomyces carlsbergensis	520	+	+	+	+	–
Saccharomyces cerevisiae	83	+	+	+	+	Sl
Saccharomyces elegans	128	+	+	+	–	–
Saccharomyces ludwigii	364	+	+	+	–	–
Saccharomyces rosei	492	+	+	–	–	–
Saccharomyces rouxii (*O*)	381	+	+	+	+	–
Saccharomyces rouxii (*O*)	581	+	+	+	+	+
Schizosaccharomyces octosporus (*O*)	427	+	+	–	–	–

* (*O*) Denotes osmophilic strain.
†Sl denotes slight growth.

Table 2. Effect of *p*H on Inhibitory Action of Streptomycin towards Yeasts

Yeast	N.C.Y.C. No.	*p*H	Growth in presence of streptomycin sulphate (μg/ml.) 0	10	20	50	100
Saccharomyces elegans	128	4·7–5·0	+	+	+	+	Sl
		5·7–6·0	+	+	+	–	–
Saccharomyces acidifaciens	464	4·7–5·0	+	+	+	–	–
		5·7–6·0	+	+	+	–	–
Saccharomyces rosei	492	4·7–5·0	+	+	Sl	–	–
		5·7–6·0	+	+	Sl	–	–
Saccharomyces carlsbergensis	519	4·7–5·0	+	+	+	+	–
		5·7–6·0	+	+	+	Sl	–

The results summarized in Table 1 appear to be the first demonstration that streptomycin at such low concentrations can completely inhibit the growth of a variety of yeasts during the incubation period. Furthermore, the degrees of sensitivity of the various species tested suggest that the level of streptomycin incorporated into media for isolation of yeasts may inhibit some yeast strains as well as bacteria. Media used for the isolation of yeasts are of lower *p*H than the assay media, but Table 2 reveals that reduction of *p*H to 5·7–6·0 has little effect on the degree of sensitivity of these yeast strains to streptomycin, and even at *p*H 4·7–5·0 some yeast strains could still be inhibited by the concentration of streptomycin in these media.

In the light of these observations, therefore, it would appear unwise to incorporate streptomycin routinely in any medium designed for the isolation of yeasts.

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