

Both citric and malic acid were added to fermentations to determine if these acids stimulated carotenogenesis. In addition, the residue of the molasses after precipitation of acids and also the carbohydrate components of the residue were tested. Approximately 43 per cent carbohydrate remained in the citrus molasses residue after organic acid separation. The carbohydrates were about 50 per cent sucrose and 50 per cent reducing sugars.

Table 1 shows that the combination of organic acid fraction or citric acid with the molasses residue or sucrose resulted in yields approximating that given by unfractionated citrus molasses. Either glucose or fructose at a concentration of 1.5 per cent could substitute for sucrose, but at higher concentrations this resulted in inhibition of carotenogenesis. Malic, gluconic, and 2-ketogluconic acid could not replace citric acid and gave only slight stimulation.

Table 1. STIMULATION OF CAROTENOGENESIS BY CITRUS MOLASSES AND VARIOUS FRACTIONS

Type	Adjuvant*	Amount (per cent)	Dry mycelium g/100 ml.	In solids mg/g	Carotene yield Per volume mg/100 ml.
None	—	—	4.79	9.9	46.6
Citrus molasses		5.0	6.72	17.3	115.0
Organic acid fraction †		2.0	4.67	17.3	80.7
Citric acid		0.075	4.77	16.7	79.2
Sucrose		3.0	5.45	14.9	80.4
Glucose		1.5	7.43	7.2	53.5
Glucose		3.0	7.72	5.9	45.9
Fructose		3.0	7.54	5.4	41.0
Organic acids + residue ‡		2 + 5	6.02	17.3	103.9
Citric acid + residue		0.075 + 5	6.31	16.4	103.1
Citric acid + sucrose		0.075 + 3	4.93	20.4	100.2
Citric acid + glucose		0.075 + 1.5	6.73	14.4	96.2
Citric acid + glucose		0.075 + 3	7.41	6.9	50.7
Citric acid + fructose		0.075 + 3	7.56	6.3	47.7
Organic acids + sucrose		2 + 3	5.65	21.1	117.6

* In addition to the adjuvant, the fermentation medium contained cottonseed embryo meal, 5 per cent; corn, 2.5 per cent; cotton-seed oil, 5 per cent; deodorized kerosene, 5 per cent; non-ionic detergent, 0.12 per cent; thiamine hydrochloride, 0.2 mg per cent; tap water; pH not adjusted.

† One ml. of the organic acid fraction was equivalent to 2.5 ml. of citrus molasses.

‡ Residue is the aqueous fraction remaining after separation of organic acids from citrus molasses; 1 ml. residue was equivalent to 1 ml. citrus molasses.

Inhibition of metabolic processes by glucose is well documented^{3,4} and has become known as the "glucose effect". Neidhart and Magasanik^{5,6} formulated the concept that the glucose effect represented catabolite repression; that is, "Catabolites formed rapidly from glucose accumulate in the cell and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds". However, glucose inhibition of carotenogenesis does not appear to be the result of catabolic inhibition as defined by Neidhart and Magasanik since carotene biosynthesis is an anabolic process.

Two hypotheses may be advanced to explain the present data. (a) Part of the biosynthetic pathway of carotene synthesis may be under repressor control⁷. If a potential internal inducer is used in biosynthetic processes that also involve catabolic products produced from a readily metabolized substrate such as glucose, then insufficient internal inducer is available to act on the repressed carotene pathway. When the carbon source is a less readily metabolized compound such as sucrose, the metabolite pool decreases and the internal inducer becomes available to de-repress carotenoid synthesis. (b) The second hypothesis assumes that the biosynthetic pathway of carotene synthesis is under continuous influence of an internal inducer but that in the presence of a readily metabolizable carbon source such as glucose, sufficient carbon is available to produce an "internal inducer-destroying enzyme" and the system becomes repressed. If sucrose were a less readily available carbon source because of slower uptake, or because of lower turnover number or concentration of invertase or sucrose phosphorylase, insufficient carbon would be available for synthesis of the enzyme. These hypotheses will be investigated.

The function of citric acid in enhancing yields is probably non-specific and would appear to be that of a precursor to early metabolites in the carotene biosynthetic sequence.

ZDISLAW PAZOLA
ALEX CIEGLER
HARLOW H. HALL

Northern Regional Research Laboratory,
U.S. Department of Agriculture,
Peoria, Illinois.

¹ Ciegler, A., Nelson, G. E. N., and Hall, H. H., *App. Microbiol.*, **11**, 128 (1963).

² Jorysch, D., Sarris, P., and Marcus, S., *Food Technol.*, **16**, 90 (1962).

³ Epps, H. M. R., and Gale, E. F., *Biochem. J.*, **36**, 619 (1942).

⁴ Magasanik, B., in *Cold Spr. Harb. Symp. Quant. Biol.*, edit. by Frisch, L. **26** (Waverly Press, Baltimore, 1961).

⁵ Neidhart, F. C., and Magasanik, B., *Nature*, **178**, 801 (1956).

⁶ Neidhart, F. C., and Magasanik, B., *J. Bacteriol.*, **73**, 253 (1957).

⁷ Reyes, P., Chichester, C. O., and Nakayama, T. O. M., *Biochim. Biophys. Acta*, **90**, 578 (1964).

'Endogenous' Alcohol in Body Fluids

THE question as to whether or not ethanol is a normal constituent of body fluids has attracted the attention of many workers, and their findings have been ably reviewed by Harger and Forney¹ and Ward Smith².

Many methods used have been of doubtful accuracy and/or specificity at the levels of ethanol found, and the method of Curry, Walker and Simpson³ has been modified to study the problem.

A Pye 104 gas-liquid chromatograph, fitted with a flame ionization detector and a PEG 400 on 'Celite' column, is used with an amplifier alternation setting of 20, 10 µl. of a 1 + 5 dilution of the sample (blood or urine) with a 0.25 mg per cent solution of *n*-propanol in water is injected.

Due to baseline instability, an integrator is not used as in the unmodified method, and quantitation is achieved by the ratios of the peak heights of the ethanol and *n*-propanol peaks: this means that each individual operator must establish his own factor.

The peak height ratios are linear against ethanol concentrations over the range 0.05–1.0 mg per cent, and it is possible to detect with certainty the presence of 0.03 mg per cent of ethanol. The accuracy found varies from ± 5 per cent at the 1 mg per cent level to ± 25 per cent at the 0.05 mg per cent level.

Preliminary results of a survey of the occurrence of ethanol in the blood and urine of normal persons who have not taken alcohol indicate that the amounts present are less than 0.1 mg per cent.

G. W. WALKER
A. S. CURRY

East Midland Forensic Science Laboratory,
Nottingham.

¹ Harger, R. N., and Forney, R. B., *Progress in Chemical Toxicology*, edit. by Stolman, A., **1** (Academic Press, London, 1963).

² Ward Smith, H., *Methods of Forensic Science*, edit. by Curry, A. S., **4** (Interscience Publishers, New York, 1965).

³ Curry, A. S., Walker, G. W., and Simpson, G. S. (in the press).

BIOLOGY

Genetical Homogeneity and the Stability of the Mating-type Factors of 'Fairy Rings' of *Marasmius oreades*

EXAMINATIONS have been made of the distribution of multiple-allelomorphic mating-type factors (m.t.f.) carried by basidiospores and produced by adjacent carpophores of higher fungi. The spatial distribution patterns found have been interpreted to provide evidence concerning the disposition of the mycelium giving rise to the sporophores and its genetical homogeneity or heterogeneity¹.

Many fungi give rise to a 'fairy ring' type of growth. It is generally assumed that such a mycelium has a central