(Russia), Stade Paladi (Barbados), Atlas 46 (U.S.A.)). Chloroplasts were isolated from DDT-treated plants, before chlorosis began to develop, by using a medium giving chloroplast preparations exhibiting high rates of photophosphorylation (M. Plesnicar, personal communication). In all cases the effect of DDT on photosynthesis correlated with the susceptibility or resistance of the barley to DDT in greenhouse trials. In these studies chloroplasts from susceptible barley showed a parallel effect on all the investigated aspects of photosynthesis, including 2,6-dichlorophenol-indophenol and ferricyanide photoreductions and cyclic and non-cyclic photophosphorylations. The distinction between cyclic and non-cyclic photophosphorylations evident in earlier work appears to be a result of the different method of chloroplast isolation and treatment before assay of photosynthetic activities.

Most of the chloroplast preparations isolated from DDT-treated susceptible barleys showed inhibition of electron-transport activity as measured by ferricyanide photoreduction concomitant with non-cyclic photophosphorylation; however, two varieties showed no decrease in electron transport as measured by this method, even though both cyclic and non-cyclic photophosphorylations, and 2,6-dichlorophenol-indophenol and ferricyanide photoreductions measured without concomitant photophosphorylation, were inhibited. The reason for these two distinct types of effect is not clear, although the existence of a barley variety with reaction to DDT controlled independently by two genes is known (G. A. Wiebe, personal communication); hence if both genes are segregating independently three genotypes of susceptible barley differing in reaction to DDT might exist.

Other work has been concerned with the search for the 'target site' affected by DDT in the photosynthetic lamellar membrane. Chloroplast thylakoid proteins have been isolated from both susceptible and resistant types of a single variety of barley (Zephyr), the two types of barley being identical but for the distinction in their reaction to DDT. Details of the sedimentation properties and amino acid compositions of the thylakoid proteins will be presented.

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The Effect of Isonicotinyl Hydrazide on the Greening of Maize and Barley

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A pathway has been proposed for the formation of acetyl-CoA in chloroplasts (Rogers, Shah & Goodwin, 1968; Shah & Rogers, 1969; Rogers & Goodwin, 1970) involving the sequence $\text{CO}_2 \rightarrow \text{glycollate} \rightarrow \text{glyoxylate} \rightarrow \text{glycine} \rightarrow \text{serine} \rightarrow \text{pyruvate}$. Part of the evidence for this sequence came from experiments involving inhibitors affecting reactions in the pathway, and the present communication reports some additional results obtained by using one of the inhibitors.

Isonicotinyl hydrazide (isoniazid) inhibits conversions requiring pyridoxal phosphate as cofactor. In the above sequence it might therefore be expected to affect each of the steps in the conversion of glyoxylate into pyruvate. In *Chlorella* (Pritchard, Whittingham & Griffin, 1963) and *Pisum sativum* (Marker & Whittingham, 1966) isonicotinyl hydrazide predominately inhibits the conversion of glycine into serine.

We have investigated the effect of isonicotinyl hydrazide on development of chlorophyll and carotenoid during illumination of segments of etiolated maize and barley shoots immersed in solutions of various inhibitor and metabolite concentrations. Chlorophyll and carotenoid were assayed by the method described by Kirk (1968). Maize shoots were used within 3 days of the leaves unrolling, barley shoots when they were approximately 5 in tall.

It was found that 10 mM-isonicotinyl hydrazide inhibited pigment synthesis by some 60% in maize and some 80% in barley. In maize inhibition of greening in the presence of isonicotinyl hydrazide was not overcome by addition of glycollate or glycine, but was restored by serine and by pyruvate. Results were similar in barley with the difference that only pyruvate would restore pigment formation. Isonicotinyl hydrazide thus inhibits pigment

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formation in barley at the serine $\rightarrow$ pyruvate stage in addition to the earlier step(s) as in maize, indicating differences in strength of binding of pyridoxal phosphate to the enzymes in the two plant species studied.

These results are in agreement with an earlier observation (Shah & Rogers, 1969) that, whereas isonicotinyl hydrazide inhibited the incorporation of radioactivity from $[2-^{14}\text{C}]$ glyoxylate, $[2-^{14}\text{C}]$ glycine and $[^{14}\text{C}]$ formate into β -carotene of illuminated etiolated maize seedlings, it did not affect incorporation from $[\text{U}-^{14}\text{C}]$ serine. In a further series of experiments related to the latter observations we have shown that incorporation of radioactivity from $\text{L}-[3-^{14}\text{C}]$ serine into β -carotene of illuminated etiolated maize seedlings was extremely low but could be considerably enhanced by supplying the plants at the same time with glycine and formate or with isonicotinyl hydrazide and glycine. The studies indicate that serine supplied to intact plants may be subject to considerable interconversion into glycine before incorporation into pigments. Radioactivity at C-3 of the serine may be diluted out in this process by unlabelled C_1 fragments present *in situ*. Inhibition of this interconversion by the presence of isonicotinyl hydrazide resulted in a 40-fold enhancement of incorporation of C-3 of the added serine into carotenoids, whereas there was little effect on incorporation of radioactivity into sterols.

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A New Type of Bile Salt

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Bile of the very large freshwater teleost *Arapaima gigas* of the family Osteoglossidae was kindly given to us by Dr P. H. Greenwood. The bile salts showed on t.l.c. at least four major components, one corresponding in R_f to taurocholate. Dioxan-

trichloroacetic acid cleavage and separation on Celite gave two crystalline bile alcohols, 'arapaimol-A' and 'arapaimol-B', both showing an intense purple response in the Hammarsten (HCl) test. I.r. and n.m.r. spectroscopy showed that they had the same steroid nucleus; mass spectra indicated formulae $\text{C}_{27}\text{H}_{48}\text{O}_5$ and $\text{C}_{27}\text{H}_{48}\text{O}_6$ and a steroid nucleus containing four hydroxyl groups. Arapaimol-A with lead tetra-acetate consumed about 3g-atoms of oxygen/mol; it did not give an acetonide. N.m.r. spectra suggested $-\text{CH}(\text{CH}_3)\cdot\text{CH}_2\cdot\text{OH}$ and $-\text{CH}(\text{CH}_2\cdot\text{OH})_2$ for the terminal side-chain group of the molecules of arapaimol-A and -B respectively, and indicated that, if hydroxyl groups were at C-3, C-7 and C-12, the fourth hydroxyl group might be at C-1, C-2 or C-6. All these results pointed to the partial synthesis of $2\beta,3\alpha,7\alpha,12\alpha$ -tetrahydroxycholestan-24-oic acids.

Methyl $7\alpha,12\alpha$ -diacetox-4 β -bromo-3-oxo-5 β -cholestan-24-oate with sodium acetate-acetic acid gave a mixture containing methyl $2\beta,7\alpha,12\alpha$ -triacetox-3-oxo-5 β -cholestan-24-oate (I), as expected from the work of Satoh, Takahashi, Aoki & Hagitani (1969). Reduction of compound (I) with sodium borohydride, followed by hydrolysis, readily gave $2\beta,3\alpha,7\alpha,12\alpha$ -tetrahydroxy-5 β -cholestan-24-oic acid (II), whose i.r. spectrum showed all the bands between 9.0 and $15.0\mu\text{m}$ of the arapaimols. The n.m.r. spectra of (II) showed the same chemical shifts for the C-18 and C-19 protons as found for the arapaimols. We conclude that arapaimols-A and -B are $5\beta,25\zeta$ -cholestane- $2\beta,3\alpha,7\alpha,12\alpha,26$ -pentol and 5β -cholestane- $2\beta,3\alpha,7\alpha,12\alpha,26,27$ -hexol respectively.

After alkaline hydrolysis of the bile salts we obtained 'arapaimic acid', believed from i.r., n.m.r. and mass-spectral observations to be $2\beta,3\alpha,7\alpha,12\alpha$ -tetrahydroxy-5 $\beta,25\zeta$ -cholestan-26-oic acid, and Dr I. G. Anderson, by g.l.c., confirmed the presence of cholic acid.

2β -Hydroxylation has not previously been observed in any bile salt, but is a feature of insect steroid hormones.

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Regulation of Enzymes Converting L-Mandelate into Benzoate in Bacterium N.C.I.B. 8250

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The conversion of L-mandelate into benzoate in bacterium N.C.I.B. 8250 involves the enzymes L-mandelate dehydrogenase, benzoylformate