substituent at the 3'-position of the pyrimidone or imidazolidinone ring. The effect of such substitution on schistosomicidal action is shown in table 2. In the pyrimidone series, the unsubstituted compound, the 3-methoxymethyl, the 3-ethyl and the 3-n-propyl derivatives were the most active, whereas the 3-ethyl and 3-acetyl were the best of the imidazolidinone series. The inactivity of the 3-acetylpyrimidone and the 3-n-propylimidazolidinone made any correlation for the 2 series difficult to conceive.

In all tests, the lethality of the compound was judged by the number of dead or dying schistosomes encapsulated within the substance of the host liver, compared to the total number of worms found in the liver and portal system after perfusion and examination of a liver squash preparation.

The most active compounds have been given to infected mice as single oral doses ranging from 300 to 1000 mg/kg. The percentage kill of schistosomes was linear with respect to $\log_{10}$ dose, and the following ED_{50} results were obtained (compound I): 1-(5-nitro-2-thenylideneamino)-tetrahydro-2(1H)-pyrimidone, 334 mg/kg with 95% confidence limits of 312 and 357: 1-(5-nitro-2-thenylideneamino)-3-ethyl-2-imidazolidinone, 450 mg/kg (95% limits 363 and 557); 1-(5-nitrothiazolyl)-2-imidazolidinone (niridazole), 443 mg/kg (95% limits 387 and 507). Thus, the nitrothiophenes compared favourably with niridazole under these conditions. In the course of these studies, it was found that none of the mice (8/group) exhibited any overt toxic symptoms after single oral doses of 1000 mg/kg. In a separate test groups of 8 mice also tolerated 200 mg/kg given twice daily for 4 consecutive days. All of these mice harboured S. mansoni in varying numbers and were generally in better condition than undosed mice when examined. However, LD_{50} values have not been established for either healthy or parasitized mice.

The most active pyrimidone (compound I) has also been tested against S. mansoni harboured in Syrian hamsters. A dose regime of 300 mg/kg daily for 4 consecutive days produced a kill of 29% (39 of a total of 134 worms recovered from 6 hamsters were dead), while a single dose of 750 mg/kg gave an average of 27% kill in another group of 6 hamsters. Preliminary investigations of the metabolism of compound I in mice and hamsters indicated that biotransformation and excretion was more rapid and extensive in the hamsters, which may explain the lowered effectiveness in that species.

These findings, a) that certain nitrothiophene compounds possess schistosomicidal activity, and b) that a methyleneimine bridging group gives a correct configuration for activity, both extend and modify the concept of Robinson, Bueding and Fisher[3] as to the structural limitations imposed upon nitroheterocyclic compounds if they are to have antischistosomal action.

Development of photochemical activity during greening of heat-stressed etiolated seedlings of Zea mays

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Summary. When etiolated maize seedlings were subjected to a heat stress of 40 or 45 °C for 16 h, and subsequently illuminated, photoreduction of ferricyanide and noncyclic photophosphorylation by chloroplasts isolated therefrom were retarded, and the lag in the appearance of these photochemical reactions was extended.

Introduction. The accumulation of photosynthetic pigments and the development of photosynthetic activity in chloroplasts isolated from greening etiolated plants have been investigated[1–7]. In previous communications, we reported that the rates of formation of chlorophylls[8,9], and protochlorophyll[10], and the rates of cyclic photophosphorylation with phenazine methosulfate and of ATP hydrolysis[11] of such chloroplasts were retarded if the etiolated plants had been subjected to high temperatures before greening. In this report, the development of 2 other photochemical activities of chloroplasts i.e. reduction of ferricyanide and noncyclic photophosphorylation with ferricyanide during greening of heat stressed dark-grown maize seedlings is described.

Materials and methods. Seeds of maize (Zea mays Linn. cv NS1) were germinated at 25 °C in darkness on sand. The resulting seedlings were daily supplied with distilled water. On the 4th day after sowing, different lots of seedlings were subjected to temperatures of 40 or 45 °C for 16 h in darkness. The seedlings were then returned to 25 °C and illuminated at 3000 lux intensity supplied by small fluorescent tubes.

At intervals of 4, 6 or 8 h after the onset of illumination, chloroplasts were extracted by the method of Howes and Stern[12] from leaves harvested randomly from each treatment. Light reactions were carried out in a glass-sided water bath which was illuminated on each side by 2 300 W reflector spot lamps. Temperature was maintained at 24 °C by means of an electric cooling coil immersed in the water bath. Reactions proceeded for 2 min in the light (80,000 lux) or dark and were terminated by turning the light off, where appropriate, and adding trichloroacetic acid to a final concentration of 3% (w/v). After centrifugation, aliquots of the supernatants were assayed for ferricyanide reduction and noncyclic photophosphorylation.

The standard reaction mixture contained in μmoles: tris (pH 8.0), 135; $MgCl_2$, 24; ADP, 12; NaK phosphate (pH 8.0), 36; BSA, 0.135; $K_3Fe(CN)_6$, 4.5; chloroplasts containing 75–150 μg chlorophyll; and water to a final volume of 9 ml.

1 J. M. Anderson and N. K. Boardman, Aust. J. Biol. Sci. *17*, 93 (1964).
2 A. D. Dodge and C. P. Wittingham, Ann. Bot. *30*, 711 (1966).
3 G. I. Raitsina, T. D. Lebedeva and A. S. Vecher, Dokl. Akad. Nauk B. SSSR *12*, 731 (1968).
4 A. O. Glydenholm and F. R. Whatley, New Phytol. *67*, 461 (1968).
5 C. D. Howes, Ph. D. Thesis, Amherst, Univ. Massachusetts (1969).
6 L. Bogorad, Proc. Aust. Acad. Sci. *15* (1969).
7 C. D. Howes and A. I. Stern, Plant Physiol. *51*, 386 (1973).
8 I. C. Onwueme and A. O. Lawanson, Planta, Berlin *110*, 81 (1973).
9 I. C. Onwueme and A. O. Lawanson, Phyton *33*, 69 (1975).
10 A. O. Lawanson and I. C. Onwueme, Z. Pflanzenphysiol. *69*, 461 (1973).
11 A. O. Lawanson, Phyton *34*, 51 (1976).
12 C. D. Howes and A. I. Stern, Plant Physiol. *44*, 1515 (1969).

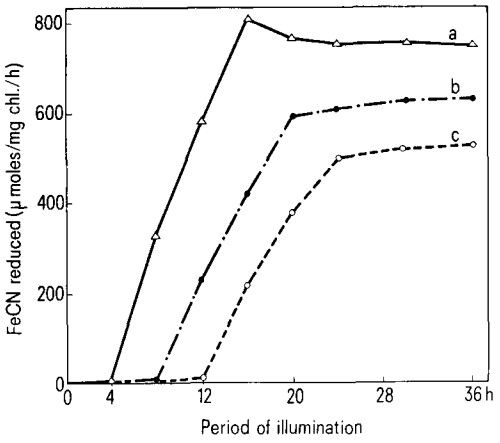

Fig. 1. Time-course of net ferricyanide reduction (light minus dark) by chloroplasts isolated from heat-stressed and unstressed maize seedlings during greening. Etiolated seedlings were exposed to 40 or 45 °C for 16 h in the dark, and subsequently illuminated. *a* Control (unstressed, 25 °C); *b* 40 °C prior heat stress for 16 h; *c* 45 °C prior stress. S. D. varied between 1.1 and 3.4 in all figures.

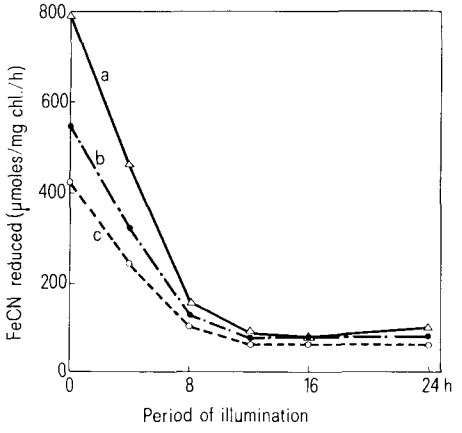

Fig. 2. Time-course of ferricyanide reduction in the dark by chloroplasts isolated from heat-stressed and unstressed maize seedlings during greening. Etiolated seedlings were exposed to 40 or 45 °C for 16 h in the dark, and subsequently illuminated. *a* Control (unstressed, 25 °C); *b* 40 °C prior heat stress for 16 h; *c* 45 °C prior stress.

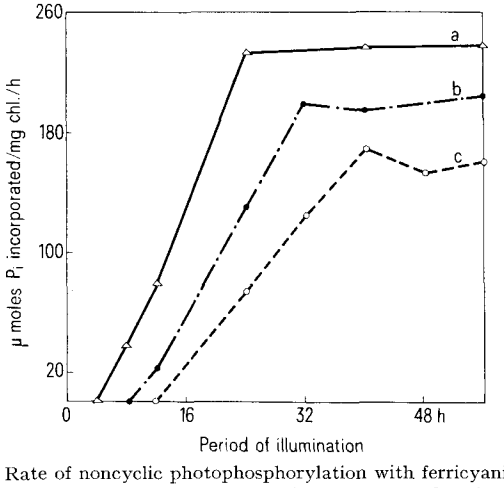

Fig. 3. Rate of noncyclic photophosphorylation with ferricyanide in chloroplasts isolated from heat-stressed and unstressed maize seedlings during greening. Etiolated seedlings were exposed to 40 or 45 °C for 16 h in the dark, and subsequently illuminated. *a* Control (unstressed, 25 °C); *b* 40 °C prior heat stress for 16 h; *c* 45 °C prior stress.

The reduction of ferricyanide was measured by the colorimetric procedure of Avron and Shavit[13]. Chlorophyll was determined by the method of Arnon[14]. The incorporation of inorganic phosphate into ADP was measured by estimating the decrease in the amount of inorganic phosphate. Inorganic phosphate was determined by the colorimetric method of Chen et al.[15].

Results and discussion. Net photoreduction of ferricyanide was observed in chloroplasts isolated from the control seedlings after approximately 4 h of illumination (figure 1). The rates of net ferricyanide photoreduction increased sharply during the next 12 h and then fell slightly to levels observed with mature chloroplasts. This pattern is in agrrement with values reported by other workers[1, 3, 7]. When the seedlings were subjected to temperatures of 40 or 45 °C for 16 h (figure 1), the lag in net photoreduction of ferricyanide was extended to 8 and 12 h respectively. Thereafter the rates increased, reaching a maximum not at 16 h of illumination as in the control but at 20 and 24 h respectively. Maximal values of net ferricyanide photoreduction in the seedlings stressed at 40 and 45 °C for 16 h were lower than the value observed for control seedlings. Unlike in the control seedlings, the rates of net photoreduction in the stressed seedlings did not fall but remained constant after attaining a maximum. Dark reduction of ferricyanide by chloroplasts isolated from control seedlings was initially very high, but fell sharply during the first 8 h of chloroplast development, and stayed very low thereafter (figure 2). This pattern is similar to previously reported patterns[1, 7]. Chloroplasts isolated from the stressed seedlings also reduced ferricyanide initially in the dark but at a rate lower than that of the control. Also, initial dark reduction was lower at the higher temperature of stress. Here again, the rates fell sharply during the first 8 h of development and remained low thereafter. This decrease in reducing power available for biosynthesis at early stages of plastid development must handicap the establishment of the developing seedling.

It is concluded from the above that the retarding effect of prior heat stress on net photoreduction of ferricyanide is largely due to its retarding effect on the actual photochemical reaction. Noncyclic photophosphorylation with ferricyanide was not detected in chloroplasts isolated from the control seedlings until after 4 h of illumination (figure 3). Thereafter the rates increased gradually until 24 h and remained constant for the remaining part of the experiment. This pattern is similar to patterns described by previous workers[4, 5, 7, 16]. Chloroplasts isolated from seedlings which had been previously subjected to temperatures of 40 and 45 °C showed an extended lag of 8 and 12 h respectively in ferricyanide photophosphorylation. Rates of photophosphorylation increased gradually in these stressed seedlings, reaching a maximum at 32 and 40 h of development respectively. The maximal values attained were, however, lower than those observed for the control. Such kinetics indicate that chloroplasts isolated from greening, previously heat-stressed seedlings experience a delay in the acquisition of photosynthetic competence.

These results support the earlier suggestion[11] that prior heat stress retards the energy-transforming process in photosynthesis.

13 M. Avron and N. Shavit, Analyt. Biochem. *6*, 549 (1963).
14 D. I. Arnon, Plant Physiol. *24*, 1 (1949).
15 P. S. Chen, Jr, T. Y. Toribara and H. Warner, Analyt. Chem. *28*, 1756 (1956).
16 T. Oku and K. Sugahara, Mem. Fac. Sci. Kyushu Unv. Ser. E (Biol.) *5*, 49 (1969).