(95:7:5)], which from radioisotope studies is probably structurally closely related to DHES, seemed considerably increased. Alternatively, a new alkaloid with identical chromatographic characteristics had been superimposed. The procedure was repeated and 2.4 mg alkaloid (R_f 0.15), which represented approximately 5% of the total alkaloids, was obtained. Field desorption mass spectrometry showed m/z 565, interpreted as the addition of 16 mass units (1 oxygen) to the molecular ion of DHES (m/z 549)[6]. The electron impact mass spectrum showed, in addition, m/z 269 (base peak) which corresponds to the dihydrolysergamide fragment of DHES. The spectrum also showed an important ion (m/z 170) and a weak ion (m/z 154); the latter could represent a fragment of the ergoline nucleus[7], or a proline-containing fragment of the cyclic tripeptide of DHES[6] in which case m/z 170 would represent the OH-prolyl analogue. DL-hydroxy [2-^{14}C] proline (10 μCi) was administered to a 12-day 10-ml culture of *S. sorghi*. Autoradiography of a chromatogram of the alkaloids subsequently produced showed intense radioactivity exactly coincident with the new alkaloid. A similar result was obtained when the radioisotope was given at 5, 8 and 10 days after inoculation. Radioisotopic and mass spectral evidence thus indicated that *S. sorghi* can use OH-Pro to biosynthesize the novel alkaloid 9′-hydroxy-dihydroergosine (OH-DHES), following the conventional nomenclature[8]. The yield was relatively low but a pro$^-$ auxotroph might be expected to give improved yield[9]. Other proline analogues were tested at concentrations of 6–12 mg ml^{-1} for their ability to become incorporated, but only cis-4-OH-Pro gave rise to a new alkaloid having a slightly lower chromatographic R_f-value than the OH-DHES derived from the allo-form. D-proline, allo-4-OH-D-proline, L-azetidine-2-carboxylic acid, 3,4 dehydro DL-proline, DL-pipecolic acid, L-pipecolic acid, OH-L-pipecolic acid, L-baikain, thioproline and S-piperazic acid were not incorporated.

Addition of OH-Pro (1–10 mg ml^{-1}) to cultures after growth at the 12-, 14-, 16- or 18-day stage did not affect total alkaloid yield. Similarly, when OH-Pro was administered (0–0.5 mg ml^{-1}) during growth at 5, 8 or 10 days alkaloid yield was not affected and up to 1.5 mg ml^{-1} at the same stages did not affect biomass accumulation. Total alkaloid yield was only diminished when > 0.5 mg ml^{-1} was given at 5 days; at less than 0.5 mg ml^{-1} there was a dose dependant effect on the proportion of OH-DHES formed but at greater concentrations the proportion of OH-DHES was not increased. On agar media containing OH-Pro (0.4 mg ml^{-1}) colony growth was reduced by 50%. Maximum growth suppression occurred at 4 mg ml^{-1} but complete fungistasis was not achieved even at 10 mg ml^{-1}. Since in this fungus alkaloid synthesis commences only after growth has ceased, optimum directed biosynthesis using OH-Pro could conveniently be achieved by administration to surface cultures at about day 12.

Clearly, in ergot fungi the multi-enzyme complex forming the cyclic tripeptide alkaloid moiety is not always specific (table) and this now applies to all 3 amino acid components. However, it does not necessarily mean that analogues can be accepted for all of the 3 amino acids by any one fungus. In the present biosynthetic studies *S. sorghi* would not accept iso-leucine or nor-leucine to replace leucine, and a *Claviceps purpurea* strain typically producing ergotamine[11] nevertheless failed to accept OH-Pro although, as in *S. sorghi*, it was completely taken up by the mycelium. Also OH-Pro did not substitute for proline in the biosynthesis of the tremorgenic mycotoxin verruculogen when tested in the system described elsewhere[10]. *S. sorghi* appears therefore to be unique in accepting OH-Pro in the biosynthesis of a fungal secondary metabolite, the only others containing 4-OH-Pro are the *Amanita* toxins which can be considered to be biosynthesized initially from proline, oxygen being inserted later.

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In vivo inhibition of citrate cleavage enzyme by polychlorinated biphenyls[1]

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Summary. Polychlorinated biphenyls (Aroclor 1254, PCB) administered in the diet (0.01%, w/v) to rats inhibited citrate cleavage enzyme. The results suggest that the decreased activity might in part account for decreased fatty acid synthesis in the livers of PCB-treated rats.

Alterations in lipid metabolism by polychlorinated biphenyls have been reported by several investigators[2-5]. Nagai and coworkers[6] found that the oral administration of PCB reduced the free fatty acids and triglyceride in the skin lipids of the rat. Incorporation of acetate into triglyceride and free fatty acids was decreased. Kling et al.[7] previously reported that PCB, in vitro, had no effect on acetyl CoA carboxylase, the enzyme which catalyzes the rate limiting step in the biosynthesis of fatty acids or on fatty acid synthase. Citrate cleavage enzyme was inhibited in vitro by polychlorinated biphenyls. The inhibition was noncompetitive. The enzyme occupies a key position in the biosynthesis of fatty acids. Firstly, it directly provides the precursor acetyl CoA. Secondly, it indirectly participates in the for-

mation of the reducing equivalents in the form of NADPH. It was therefore of interest to determine whether or not the enzyme was inhibited in vivo by polychlorinated biphenyls, thereby constituting a cause of the altered biosynthesis of fatty acids observed upon PCB administration. The results of the investigation are presented in this article.

Materials and methods. Male and female Wistar rats (150–200 g) were fed the control diet (dextrose, 67% (w/w); casein, 22% (w/w); Hubbel-Mendel-Wickman salts, vitamin mix and minerals 6.0% (w/w)) with and without Aroclor 1254 (PCB) at 100 and 500 ppm, for 30 days. The animals were sacrificed and livers removed and analyzed as described below. Citrate cleavage enzyme was purified and assayed by the method of Srere[8]. Protein was determined by the method of Lowry. Deoxyribonucleic acid was determined by the method of Burton[9].

In vivo effect of Aroclor 1254 (PCB) on citrate cleavage enzyme

	μmoles/min g tissue	Inhibition (%)	μmoles/min/ total liver*	Inhibition (%)
Control	0.156 ± 0.049	–	0.651 ± 0.106	–
PCB, 100 ppm	0.048 ± 0.014	69	0.246 ± 0.082	62

Rats were administered PCB (0.01%, w/w) in the diet for 30 days. The controls received the same diet without PCB. Citrate cleavage enzyme was purified and assayed by the method of Srere[8]. The extent of purification (12.7-fold) was the same for control and PCB-treated livers. No significant change in liver DNA or protein was found. Values are averages of 9–12 determinations ± SD.

* The μmoles/min/total liver were calculated by multiplying the observed absorbance/mg protein by the number of μmoles DPNH/unit absorbance by the mg protein/total liver. 1 absorbance unit equals 0.218 μmoles DPNH.

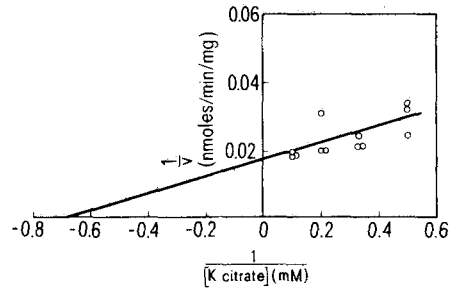

Figure 1. A Lineweaver-Burk reciprocal plot of citrate cleavage enzyme activity. The enzyme was isolated, purified and assayed by the method of Srere[8] from livers of rats fed the control diet as described under 'materials and methods'. The curve was fitted by the least-square method.

Results and discussion. It can be seen in the table that PCB in vivo at 0.01% (w/w) inhibited citrate cleavage enzyme 69%. Similar inhibition was observed at 0.05% (w/w) PCB. PCB inhibition of citrate cleavage enzyme was independent of citrate concentration (2, 3, 5 and 10 mM) eliciting 58 ± 3, 67 ± 4, 60 ± 4, and 67 ± 3% inhibition respectively. Lineweaver-Burk plots of the activity of enzyme from control and PCB-treated rat livers are shown in figures 1 and 2. The apparent K_m for the latter enzyme is lower than that of the control, but the difference was not significant. V_{max} of the enzyme from PCB treated liver (19.2 nmoles/min/mg) is lower than that of the controls (58.8 nmoles/min/mg). It is apparent from the data that citrate cleavage enzyme is inhibited in vivo by PCB. The weights of the rat livers were control 4.0 ± 0.7 g and PCB-treated 5.1 ± 0.6 g. The specific activity of the control enzyme extracts (0.009 μmoles/min/mg protein) was in the range of the values reported by Srere[8]. The observed decrease in the activity of PCB-treated livers cannot be accounted for by the differences in rat liver weights. As can be seen in the table, there is a decrease in total activity per g of liver when PCB was added to the control diet. A similar decrease is observed when the data are converted to activity per total liver. It should be noted that the rats were maintained on a strict diet of known composition, since activity is influenced by diet. Srere[8] does not indicate any control of the diet in his experiments. Differences in our values may be due to dietary variations.The finding is compatible with the previous suggestion made regarding its role in the observed decrease in fatty acid synthesis in liver in vitro and in vivo after treatment of rats with PCB. Inhibition of citrate cleavage enzyme would lead to reduced fatty acid synthesis. The reduced NADPH concentration, moreover would lead to changes in the redox state of the pyridine nucleotides, which ultimately would lead to alterations in energy metabolism, especially ATP formation. The latter would contribute to the gross metabolic trauma observed with prolonged treatment of rats with PCB. These results in addition to our previous finding that PCB inhibits other key metabolic enzymes (Kling et al.[7], Dzogbefia et al.[10]) strongly support the idea that the primary action of PCB is to alter the primary metabolic system of cells and thereby causing the gross changes observed in morphology and constitutent content of body fluids and organs.

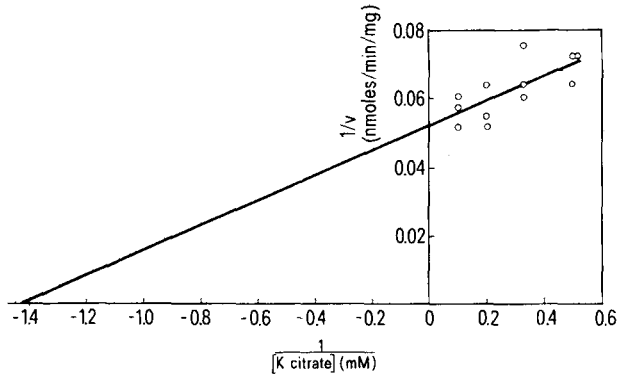

Figure 2. A Lineweaver-Burk reciprocal plot of citrate cleavage enzyme activity. The enzyme was isolated, purified and assayed by the method of Srere[8] from livers of rats fed Aroclor 1254 (0.01%, w/w) in the diet as described under 'materials and methods'. The curve was fitted by the least-square method.

1 This research was supported by a grant from the National Institutes of Health (ES-00040). Aroclor 1254 was a gift of Monsanto Company, St. Louis, Missouri, USA.

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