illumination were harvested after 30 days, dried at 65 °C for 24 h (about 2.3 g dry wt), subjected to the decarboxylation procedure and extracted continuously with 100 ml absolute ethanol under reflux for 1 h. The solvent was removed in vacuo, and the residue taken up in a small amount of ethanol and analyzed by TLC. Comparison with authentic samples of cannabinoids, $\Delta^{1(6)}$-THC **3**, Δ^{1}-THC, cannabidiol, cannabinol, revealed the presence of a FBS-positive compound with the same R_f-value as $\Delta^{1(6)}$-THC **3** in 3 solvent systems (petroleum ether/ether 4:1; petroleum ether/CH_2Cl_2 1:1; $CHCl_3$). The cannabinoid was purified by chromatography on Sephadex LH-20 (column 1.5×90 cm; eluent: petroleum ether/$CHCl_3$/ethanol 10:10:1) as described earlier[10]. The major compound obtained in a yield of 150 µg (6.5×10^{-3}% of dry wt) was shown to be identical to $\Delta^{1(6)}$-THC **3** by mass spectroscopy and gas liquid chromatography on SE-30 and OV-17.

Growth kinetics of the callus cultures were studied on B5-3 medium (fig. 2). The formation of acids **1** and/or **2**, determined as $\Delta^{1(6)}$-THC **3** correlated closely with the exponential growth phase of the cultures and the concentration of **3** declined sharply as growth stagnation.

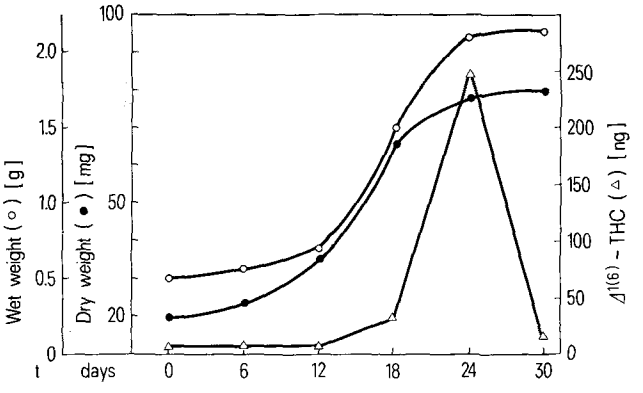

Figure 2. Correlation of growth period (days), wet weight (○) and dry weight (●) of callus cultures of *C. sativa* with the concentration of $\Delta^{1(6)}$-THC (△) **3**, taken as a measure for acids **1** and/or **2**. All values are averaged from 2 determinations.

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Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two-month period

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Summary. The feeding of a high-sucrose diet to normal rats for a period of 2 months increased serum and tissue cholesterol and triglycerides very significantly. Simultaneous feeding of the unsaturated oils of onion or garlic with the sucrose diet counterated these effects of sucrose. However, along with the lipid-reducing effects, a small but significant tissue-protein reducing effect of the oils were also observed. The hypolipidemic action of the oils may be related to their action on both lipid and protein synthesis.

Certain medicinal effects of onion and garlic and their volatile unsaturated principles have been observed before[2-4]. In 2 separate studies it was shown that aqueous extracts of these vegetables could counteract the hyperlipidemic effects of sucrose in normal rabbits[5,6]. Sucrose, which is a food with a high calorie-yield, has been observed to be responsible for increasing certain enzymes, such as glycerokinase, alpha-glycerophosphate dehydrogenase[7] and lipogenic enzymes[8]. Plasma glycerides are increased to varying degrees by sucrose depending on age, obesity and sex[9,10]. Over-consumption of sucrose leads to increased conversion of both glucose and fructose to triglyceride fatty acids[11,12]. Hyperlipidemia is a high risk factor in heart diseases[13,14]. It is found that the incidence of heart diseases is low in countries where onion and garlic are widely used; the hypolipidemic actions of onion and garlic are pertinent in this context. Temple[15] observed that the hypocholesterolemic action of garlic is due to its sulfur compounds. Gas-chromatographic analysis of steam-volatile fractions (oils) of onion and garlic[16] showed that they are composed of allyl propyldisulphide (C_3H_5–S–S–C_3H_7) and diallyl disulphide (C_3H_5–S–S–C_3H_5) respectively. The present study was designed to investigate the hypolipidemic actions of these two unsaturated oils in sucrose-fed rats.

Materials and methods. Allyl propyl disulphide and diallyl disulphide were prepared according to a published modification[17] of the method of Platenius[18] from fresh onion and garlic respectively. Male Wistar rats (average weight 150 g) were used for the experiments. They were divided into 4 groups of 6 each. 1 group was maintained ad libitum on a rat diet supplied by Pfizer (Kaduna, Nigeria). The composition of the diet was carbohydrate 73%, protein 16%, fat 3%, fiber 5%, minerals 2% and vitamin supplements 1%. This group was kept as a control group. The other 3 groups i.e. groups 2, 3 and 4 were given a sucrose-rich diet[19] ad libitum. This diet was composed of 73% sucrose, 20% milk powder, 5% fiber (Millet husk) and 2% salt mixture enriched with vitamins. The 2nd group of rats were kept as a sucrose control group. The 3rd group of rats were given the unsaturated onion oil (100 mg/kg/day) and the 4th group was given the unsaturated garlic oil, in the same dose as above, as a saline suspension through stomach tubes. Feeding of the sucrose rich diet and the 2 unsaturated oils was continued for a period of 2 months. At the end of the 60 th day the rats were sacrificed and their blood, livers, and kidneys were collected for various estimations. Blood sugar was estimated by the method of Asatoor and King[20]. Serum albumin and total protein were determined by the method

of Reinhold[21], liver and kidney proteins were determined by the method of Lowry et al.[22], serum, liver and kidney triglyceride glycerol by the method of Burton[23], serum, liver and kidney cholesterol by the method of Zlatkis et al.[24], and liver total lipids by the method of Koch-Weser et al.[25]. Statistical analysis of the results were made according to Student's t-test.

Results. The results are given in the table. The values obtained from the sucrose-fed group are compared with those of the normal group, and the values for the groups fed sucrose plus onion oil or garlic oil are compared with those of the sucrose-fed group.

Effects on blood sugar. In the sucrose-fed group or in the sucrose plus oil-fed groups there was no significant change in the blood sugar.

Effects on lipid levels. Sucrose feeding increased the total cholesterol levels in serum, liver and kidneys significantly ($p < 0.01$–$p < 0.001$). The increase was 31% in serum, 46% in liver and 35% in kidneys. Feeding of onion or garlic unsaturated oils along with sucrose counteracted the cholesterol-increasing effect of sucrose effectively, and the levels of cholesterol were maintained at nearly normal levels in serum, liver and kidneys. The cholesterol-reducing effects of the oils are highly significant ($p < 0.001$). Sucrose feeding raised triglyceride levels in serum, liver and kidneys significantly ($p < 0.001$). The increase was to about 2.5 times the normal values in serum, liver and kidneys. Both onion and garlic unsaturated oils counteracted the triglyceride raising effect of sucrose effectively, and maintained the triglyceride levels within the normal range in liver and kidneys and at about 50% of the sucrose-fed values in serum. The triglyceride lowering effects of the oils are highly significant ($p < 0.001$). Sucrose feeding also increased the liver total lipids. The increase was 48% and is significant ($p < 0.002$). Onion and garlic unsaturated oils counteracted this effect of sucrose and maintained the total lipids within the normal range. The lipid-reducing effects of the oils are highly significant ($p < 0.01$ for onion oil and $p < 0.001$ for garlic oil).

Effects on protein levels. Neither sucrose nor sucrose plus the unsaturated oils had any effect on serum total proteins or on the abibumin to globulin ratio. Similarly, sucrose feeding showed no significant change in liver total proteins. However, sucrose plus the oils decreased the liver total proteins to subnormal levels; the decrease was 22% for the onion oil and 24% for the garlic oil group. With onion oil the decrease was just significant ($p < 0.05$), but with garlic oil the decrease was highly significant ($p < 0.01$). Neither sucrose nor sucrose plus onion oil had any effect on kidney total proteins. However, sucrose plus garlic oil had a significant protein-reducing effect on the kidneys ($p < 0.02$).

In short, sucrose feeding increases the serum and tissue lipids, and the unsaturated oils of onion and garlic counteracted this effect. In addition, these oils also have a protein-reducing effect, which is pronounced for liver proteins.

Discussion. The observed lipid-reducing-action of the oils indicates the potential medicinal value of onion and garlic. The organic disulphides found in the 2 oils are good acceptors of hydrogen and their biological actions may be ascribed partly to their reactions with thiol group substances and partly to that with reduced pyridine nucleotides, eg. NADPH. It is established that organic disulphides can inactivate thiol group (−S H) substances as a result of thiol-disulphide exchange reactions[26–29] and that they can easily oxidize NADPH[30]. The reaction mechanisms are possibly as follows:

$$\text{O–SH} + \text{R–S–S–R} \rightarrow \text{O–S–S–R} + \text{R–SH}$$

$$\text{C}_3\text{H}_5\text{–S–S–R} + 2\,\text{NADPH} + 2\,\text{H}^+ \rightarrow \text{C}_3\text{H}_7\text{–SH} + \text{R–SH} + 2\,\text{NADP}^+$$

Where R is C_3H_5 or C_3H_7 and O–SH stands for any thiol group compound. As such reactions could inactivate thiol group enzymes, eg. CoA and HMG CoA reductase, and also oxidize NADPH, and all these are necessary for lipid synthesis, the daily intake of onion or garlic or their oils may reduce lipid synthesis in the body[31,32].

The aminoacid cysteine is inactivated by organic disulphides[29,33] and such reactions of the disulphides present in the above oils may partly inhibit protein synthesis also. However, this effect of the oils is not much pronounced, and if they are used only in small quantitites, they may not affect the general health, but only control lipid accumulation. A definite conclusion regarding the mechanism of action of the 2 oils in sucrose-fed rats can be drawn only after the determination of individual enzymes, proteins and NADPH in the test animals.

This and previous experiments[5,6] show that either the extracts or the oils of onion and garlic could counteract the lipid-increasing effect of sucrose very effectively. As sucrose and the above vegetables are important ingredients of our food, and also as the adverse effects of the former are counteracted by the disulphides of the latter, the present results serve as a guide line for adjusting the use of

Effects of unsaturated oils of onion and garlic on sucrose fed rats

Groups of rats	Normal	Sucrose fed	Sucrose + onion oil fed	Sucrose + garlic oil fed
Blood sugar (g/l)	0.87 ± 0.13	0.96 ± 0.15	0.77 ± 0.23	0.96 ± 0.09
Serum				
Total cholesterol (g/l)	0.84 ± 0.11	1.11 ± 0.08[d]	0.79 ± 0.07[e]	0.80 ± 0.05[e]
Triglyceride glycerol (g/l)	0.20 ± 0.03	0.51 ± 0.04[e]	0.27 ± 0.03[e]	0.24 ± 0.02[e]
Albumin (g/l)	54.0 ± 5.0	50.0 ± 7.0	51.0 ± 6.0	50.0 ± 6.0
Total protein (g/l)	72.0 ± 5.0	75.0 ± 5.0	80.0 ± 5.0	75.0 ± 2.0
Albumin/globulin ratio	3.80 ± 2.1	2.2 ± 1.0	2.1 ± 0.9	2.1 ± 0.6
Liver (values mg/g wet wt)				
Total cholesterol	7.4 ± 1.0	10.8 ± 0.6[e]	6.1 ± 0.7[e]	6.7 ± 0.8[e]
Triglyceride glycerol	3.0 ± 0.2	7.5 ± 0.5[e]	3.0 ± 0.1[e]	2.8 ± 0.1[e]
Total lipids	29.2 ± 5.0	43.2 ± 4.7[d]	31.6 ± 3.2[c]	30.4 ± 2.3[e]
Total protein	122.0 ± 7.5	118.0 ± 13.4	92.0 ± 12.9[a]	89.8 ± 5.7[c]
Kidney (values mg/g wet wt)				
Total cholesterol	0.9 ± 0.1	1.22 ± 0.2[c]	0.8 ± 0.05[c]	0.79 ± 0.04[e]
Triglyceridеglycerol	0.4 ± 0.05	1.1 ± 0.1[e]	0.39 ± 0.04[c]	0.42 ± 0.05[e]
Total protein	91.0 ± 3.5	91.2 ± 5.3	87.0 ± 4.9	82.8 ± 1.8[b]

Mean values ± SD. Student's t-test. Significant changes are shown: [a]$p < 0.05$; [b]$p < 0.02$; [c]$p < 0.01$; [d]$p < 0.002$; [e]$p < 0.001$.

each in our daily food. The findings of Sainani et al.[34] also support our observations and evidence is thus accumulating that the use of these vegetables or their oils may help one as a precautionary measure against hyperlipidemia which may lead to atherosclerosis and heart diseases.

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An arachidonic acid-specific lipoxygenase from the gorgonian coral *Pseudoplexaura porosa*[1]

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Summary. A novel lipoxygenase was partially purified from the gorgonian coral *Pseudoplexaura porosa* and was found to be specific for arachidonic acid. This soluble enzyme catalyzed the formation of 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) from arachidonic acid.

Lipoxygenases [E.C. 1.13.11.12] have been isolated and characterized from both plant and mammalian sources[2-5]. While the role of lipoxygenases in plants remains unexplained, lipoxygenase activity in mammals is usually associated with tissues that actively metabolize prostaglandins, thromboxanes and leukotrienes[4,6]. This is the first description of lipoxygenase activity in an invertebrate species.

Reports of prostaglandins in certain gorgonian corals[7,8] have stimulated speculation regarding the function of prostaglandins in these marine invertebrates[9]. The enzyme, prostaglandin endoperoxide synthetase, has been reported to be present in a large number of marine invertebrates[10], but characterized only in *Plexaura homomalla*[11]. Morse et al.[10] used an arachidonate-dependent epinephrine oxidation assay to test for the presence of enzymes that initiate prostaglandin biosynthesis, and concluded that this enzyme was common to many gorgonians. We found this method to be very unreliable for the detection of prostaglandin synthetase in gorgonian corals and doubt its validity as the sole test for prostaglandin synthetase activity in any tissue[12]. Examination of arachidonic acid metabolism in *Pseudoplexaura porosa* showed the absence of prostaglandin biosynthesis, but did show the presence of an active lipoxygenase. We now report on the specificity of this lipoxygenase towards arachidonic acid.

Experimental. Fatty acids of 99% purity and soybean lipoxygenase were purchased from Sigma Chemical Co. Bistrimethylsilyl-trifluoroacetamide (Regisil) was purchased from Regis Chemical Co. TLC was performed on EM silica gel 60 F-254 precoated plates (Brinkmann Instruments) in solvent systems A: benzene/dioxane/acetic acid (95:5:2) and B: hexane/ethyl ether/acetic acid (60:40:1). Lipoxygenase products were visualized with I_2 and with a peroxide specific spray reagent[13]. The hydroperoxide, 15-HPETE, was prepared with soybean lipoxygenase and purified by the procedure of Funk et al.[14]. The hydroperoxide product of the *Ps. porosa* lipoxygenase reaction was prepared by incubation of 140 µg of partially purified enzyme with 500 µM arachidonic acid added as a solution in 30 µl of methanol in a total volume of 5.0 ml of 50 mM Tris · HCl, pH 8.0 at 25 °C for 15 min. The solution was acidified to pH 4 with 0.2 M citric acid and extracted 3 times with 10 ml of ice-cold water, dried over sodium sulfate, evaporated to dryness in vacuo, and dissolved in 1.0 ml of absolute ethanol. Aliquots of 5 µl were applied to TLC plates and developed in solvent systems A and B. The single product band migrating with $R_f = 0.16$ in solvent system A and $R_f = 0.26$ in solvent system B was reactive toward the peroxide specific spray reagent[13]. Soybean lipoxygenase and *Ps. porosa* derived hydroperoxides were converted to