Enzymatic activities of rabbit kidney mitochondria previously incubated with 0.5 m*M* or 2 m*M* lead acetate

	Control	Lead acetate (0.5 m*M*)	Lead acetate (2 m*M*)
Succinate oxidase [a]	174 ± 15	100 ± 12	9 ± 4
Succinate dehydrogenase [b]	153 ± 7	150 ± 12	152 ± 13
NADH cytochrome-c reductase [b]	228 ± 18	42 ± 16	19 ± 7
Citochrome oxidase [a]	1900 ± 150	1500 ± 206	850 ± 162
Glutamate dehydrogenase [b]	102 ± 6	36 ± 7	21 ± 6
Monoamine oxidase [a]	6.2 ± 0.8	6.5 ± 0.5	6.2 ± 0.6

The results given are means ± S.D. [a] Enzymatic activities are expressed as nmoles of O_2 consumed/min/mg protein. [b] Enzymatic activities are expressed as nmoles of substrate oxidized or reduced/min/mg protein.

in vitro effects of lead on some enzymes contained in the inner membrane and cristae (succinate oxidase, succinate dehydrogenase, NADH cytochrome-c reductase, and cytochrome oxidase), in the matrix (glutamate dehydrogenase), or in the outer membrane (monoamine oxidase) of mitochondria[6].

Material and methods. Mitochondria were isolated from rabbit kidney homogenate by centrifugation in a solution containing 210 m*M* mannitol, 70 m*M* sucrose, 1 m*M* *Tris*-HCl buffer (pH 7.2) and 0.1 m*M* disodium EDTA[7], and incubated for 30 min at 37 °C in the same mannitol-sucrose-*Tris* solution containing either 0.5 or 2 m*M* lead acetate without EDTA. Mitochondria were then centrifuged at 12,000 *g* for 15 min, and washed twice with the mannitol-sucrose-*Tris* solution without lead or EDTA added. Finally, the mitochondrial pellets were assayed for succinate oxidase[8], succinate dehydrogenase[9], NADH cytochrome-c reductase[10], cytochrome oxidase[11], glutamate dehydrogenase[12], and monoamine oxidase[13] activities, and protein content[14].

Results and discussion. Succinate oxidase system showed a strong reduction of enzymatic activity (Table). Also NADH cytochrome-c reductase and cytochrome oxidase were markedly reduced, while succinate dehydrogenase was normal. It seems likely, then, that succinate oxidase system is altered in vitro because the sequence of electron-transfer reactions is inhibited in cytochromes by lead.

Monoamine oxidase was unchanged, while glutamate dehydrogenase activity was significantly diminished; this is in agreement with the reduction of glutamate dehydrogenase found in homogenates of kidney tissue of lead-intoxicated guinea-pigs[15].

Riassunto. Dopo incubazione con acetato di piombo (2 m*M* o 0.5 m*M*) e successivo lavaggio i mitocondri delle cellule renali del coniglio mostrano una significativa diminuzione dell'attività della succinato-ossidasi, della NADH-citocromo c-reduttasi, della citocromo-ossidasi e della glutammato-deidrogenasi, mentre la succinato-deidrogenasi e la monoamino-ossidasi non risultano modificate.

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Lipid Lowering Effect of Allicin (Diallyl Disulphide-Oxide) on Long Term Feeding to Normal Rats

Many therapeutic virtues have been ascribed to garlic among which its use in diabetes and in diseases of bacterial origin and cardiac ailments have received particular attention[1-5]. A decoction of garlic with milk is used in small doses for heart disease[5]. The active principles present in garlic have been found to be mainly the sulphur containing compounds. The essential oil of garlic contains 6% allyl propyl disulphide and 60% diallyl disulphide[6]. The latter is produced from allicin, an antibiotic during steam distillation of garlic juice[7]. Allicin is identified as diallyl disulphide-oxide (C_3H_5–S–$\overset{\overset{O}{|}}{S}$–$C_3H_5$) by CAVALLITO et al.[8,9]. It is present in garlic to the extent of 0.15%. In a preliminary study the authors have found that feeding of garlic juice to normal rats for a period of 2 months significantly reduced the lipid levels in serum and liver[10]. It has also been observed that daily administration of garlic juice to hypercholesterolaemic human beings

(dose 0.5 ml/kg) for a period of 2 months lowered the serum cholesterol level very considerably[11]. TEMPLE[12] reported that daily administration of 2 water soluble fractions of fresh garlic and 2 synthetic polysulphides resembling those in garlic oil, to rabbits fed cholesterol, brought about a less increase in free cholesterol in all treated rabbits than for the controls. This experiment suggests that the cholesterol lowering effect of garlic is due to its polysulphide compounds. In order to study the effect of allicin, a polysulphide which has been ascribed with the property of combining with –SH group compounds[7–9], the present work has been undertaken and the results on long term feeding of this active principle to normal rats have been presented.

Material and methods. 4-month-old young male wistar rats of average weight (130 g) were divided into 2 groups of 6 animals each. They were fed ad libitum with normal laboratory diet (Hind lever rat feed) supplied by Hindustan Lever Limited. Allicin was prepared from fresh garlic cloves according to the method of CAVALLITO and BAILEY[8]. Its capacity to combine with cysteine was demonstrated by the method of CAVALLITO et al.[9]. One group of rats was kept as control and distilled water (2 ml/rat) was orally administered to them every day. Freshly prepared allicin (Dose 100 mg/kg/day) was dissolved in distilled water (2 ml/rat) and orally administered to the second group of rats. The increase in weights of each group and also their food consumption were recorded. After 2 months feeding the rats starved for 18 h, were sacrificed by decapitation. Blood was collected from the jugular vein and various estimations were made on their serum and liver. Lipid from liver was extracted by the method of ENTENMAN[13]. Total lipid content of liver and serum was determined gravimetrically using chloroform methanol extract as described by SPERRY and BRAND[14]. Total cholesterol was estimated by the method of CARR and DREKTER[15], free cholesterol by the method of SCHOENHEIMER and SPERRY[16,17], phospholipid by the method of ACKERMAN and TORO[18], and triglycerides by the method of VAN HANDEL-ZILVERSMIT[19]. Protein was estimated by LOWRY's method[20] using Folin Ciocalteu reagent[21]. The protein values were calculated using a standard checked by Kjeldahl nitrogen determination.

Results. Significant results are recorded in the Table. Allicin produced no significant effects on the protein levels of serum and liver. However it significantly reduced the lipid levels of serum and liver of normal rats on long term administration. The increase in weights and food consumption of both the groups of rats were more or less the same. The lipid lowering effect of allicin is more pronounced on the liver than on the serum and it is largely due to the decrease in the level of triglycerides and free cholesterol. Serum lipid components which are in a less dynamic state than the liver lipids are also lowered in tune with the lipid lowering action of allicin on serum. All the values are significantly different from the controls.

Discussion. Liver is the main site of formation of lipids and the blood levels of lipids are controlled primarily by their production and utilization in liver. Therefore the effect of allicin may be on certain process of synthesis or

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Effect of Allicin obtained from *A. sativum* Linn on lipid levels of liver and serum of normal rats after 2 months treatment (dose 100 mg/kg/day)

	Liver weight (100 g wet tissue)				
Rats	Total lipids [c] (g)	Phospholipid [a] (g)	Triglyceride glycerol [c] (mg)	Total cholesterol [a] (mg)	Free cholesterol [b] (mg)
Normal group	4.75 ± 0.15	3.4 ± 0.15	410.0 ± 15.0	282.0 ± 15.0	165.0 ± 8.0
Allicin group	3.25 ± 0.10	2.8 ± 0.12	305.0 ± 12.0	230.0 ± 10.0	125.0 ± 6.0
	Serum (mg/100 ml)				
Normal group	240.0 ± 20.0 [a]	135.0 ± 12.0 [a]	25.0 ± 2.0 [a]	55.0 ± 5.0 [a]	25.0 ± 3.0 [a]
Allicin group	180.0 ± 12.0	90.0 ± 8.0	18.0 ± 1.5	40.0 ± 3.0	15.0 ± 1.5
	Weight increase (g/100 g body wt.)			Food consumption (g/100 g body wt./day)	
Normal group	22.0 ± 2.0			12.5 ± 1.5	
Allicin group	19.5 ± 1.5			11.5 ± 1.0	

Values are mean of 6 animals ± S.E. Student's *t*-test; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$. Significantly different from the control.

break down of lipids in the liver. Although useful information may be obtained by the analysis of blood lipoproteins and free fatty acids it is considerably more common to determine the levels of cholesterol, phospholipid and total lipid. It is recognized that these three chemical classes of lipids may each be made up of components from many lipid complexes of plasma. Since the blood levels of these lipids and proteins are often of clinical significance and since they are controlled by their levels in liver they are estimated in the livers and serum of test animals. The biosynthesis of triglycerides and phospholipids are dependent on the production of D-1, 2 diglyceride which occurs primarily in the liver and also which requires the participation of CoA. Allicin is ascribed with the property of combining with –SH group, the functional part of CoA which is necessary for the biosynthesis of fatty acids, cholesterol, triglycerides and phospholipids. The lipid lowering effect of allicin may therefore be attributed to its capacity to inactivate –SH group compounds. The cholesterol lowering effect of allicin is more pronounced on the free cholesterol levels than on the total cholesterol levels. The present results are in agreement with the findings of TEMPLE[12] who studied the cholesterol lowering effects of polysulphides resembling those found in garlic oil. The noted decrease in the level of free cholesterol may suggest that its esterification is accelerated and thus the transport and utilization of lipids enhanced. From a quantitative stand point, the serum cholesterol largely if not exclusively arises from the hepatic synthesis. Hence the primary effect of allicin may be on the liver. In a recent paper PRASANNAN[22] has reported that the liver lipid of rats reaches its peak level when they are 5–6 months old. In our study as we used rats of this age group we can state that allicin in some way prevents this fat accumulation in liver. From the results of the present experiment we may presume that the biosynthesis of cholesterol and other lipid components were inhibited in rats fed allicin as this compound can inactivate –SH groups. This may explain the therapeutic values of garlic which is used in the treatment of heart disease and arteriosclerosis. Detailed study on the mechanism of action of allicin is under progress.

Zusammenfassung. Experimenteller Nachweis eines hypolipidämischen Effektes des Knoblauch-Inhaltstoffes Allicin nach Langzeit-Fütterungsversuchen mit Ratten, wobei die Allicin-Wirkung sich stärker auf die Leber als auf das Blutserum auswirkt.

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Cycloheximide: A Specific Inhibitor of Protein Synthesis and Intercellular Ion Transport in Plant Roots

In a recent publication[1], we have provided evidence suggesting that cycloheximide (CHM) specifically inhibits ion transport from an external solution through the root into the root xylem, from where ions are delivered to the shoot. This kind of transport requires movement of ions in the symplasmic continuum which, by way of the plasmodesmata, extends from cell to cell in the root parenchyma[2]. By contrast, ion accumulation in the vacuoles of the root cells, which is largely under the control of membrane transport (plasmalemma, tonoplast), is not impaired by CHM. Since incorporation of ^{14}C-leucine into protein, but not ^{14}C-leucine uptake into the root is also strongly inhibited by CHM, we concluded that concurrent protein synthesis is a basic requirement of symplasmic transport. These earlier results are summarized by the following tabulation, in which the values given represent % of the controls obtained in the absence of CHM:

	CHM 1	[µg × ml^{-1}] 10
Ion transport through the root	60	10
Ion accumulation in the root	98	105
^{14}C-leucine incorporation into protein	61	38
^{14}C-leucine uptake by the root	100	113
Respiratory O_2 uptake by the root cells	104	95

It has been argued repeatedly, however, that such results have to be considered with extreme care, because antibiotics such as CHM may inhibit protein synthesis rather indirectly and unspecifically, e.g. via impairing energy transferring systems[3–5]. The negative effect of CHM on O_2 uptake shown in the above tabulation is not sufficient evidence to rule out an (uncoupling) effect on oxidative phosphorylation. In principle, using inhibitors which may under certain circumstances, but not generally, exert specific effects in systems as complex as the intact plant root, it is not sufficient to rely on the description of inhibitor effects in the literature. A number of control experiments must be performed with the given material and experimental conditions. To support the above conclusion on the specific action of CHM in barley roots, we have compared the effects of CHM on O_2 uptake and levels of ATP and ADP in the roots with those of the well-known uncoupler CCCP (carbonylcyanide m-chlorophenyl-hydrazone).

Roots from barley plants grown for 6 days in the dark at 25 °C in Hoagland's culture solution were harvested, rinsed and kept for 2 h in aerated solutions as usually used for the ion uptake and transport experiments (i.e. 5 m*M* KCl + 0.1 m*M* $CaSO_4$; cf. ref. [1]) with CHM added as indicated in the Table. At the end of this period, the tissue was rapidly frozen in liquid N_2 and transferred to a

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