Multiple Inhibitory Effects of Garlic Extracts on Cholesterol Biosynthesis in Hepatocytes

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Exposure of primary rat hepatocytes and human HepG2 cells to water-soluble garlic extracts resulted in the concentration-dependent inhibition of cholesterol biosynthesis at several different enzymatic steps. At low concentrations, sterol biosynthesis from [^{14}C]acetate was decreased in rat hepatocytes by 23% with an IC_{50} (half-maximal inhibition) value of 90 μg/mL and in HepG2 cells by 28% with an IC_{50} value of 35 μg/mL. This inhibition was exerted at the level of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) as indicated by direct enzymatic measurements and the absence of inhibition if [^{14}C]mevalonate was used as a precursor. At high concentrations (above 0.5 mg/mL), inhibition of cholesterol biosynthesis was not only seen at an early step where it increased considerably with dose, but also at later steps resulting in the accumulation of the precursors lanosterol and 7-dehydrocholesterol. No desmosterol was formed which, however, was a major precursor accumulating in the presence of triparanol. Thus, the accumulation of sterol precursors seems to be of less therapeutic significance during consumption of garlic, because it requires concentrations one or two orders of magnitude above those affecting HMG-CoA reductase. Alliin, the main sulfur-containing compound of garlic, was without effect itself. If converted to allicin, it resulted in similar changes of the sterol pattern. This suggested that the latter compound might contribute to the inhibition at the late steps. In contrast, nicotinic acid and particularly adenosine caused moderate inhibition of HMG-CoA reductase activity and of cholesterol biosynthesis suggesting that these compounds participate, at least in part, in the early inhibition of sterol synthesis by garlic extracts.
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Long-term exposure of rats to water-soluble garlic extracts has been reported to result in decreased serum cholesterol and triglyceride levels (1). Similar observations were reported for rabbits with and without feeding cholesterol (2,3) as well as for other species (see Ref. 4). Recently, significant reduction in serum cholesterol after long-term intake of garlic powder tablets has been found for humans in double-blind crossover studies (5,6). Little is known as yet about the mechanism(s) and the active principle(s) responsible for these effects.

Primary cultures of rat hepatocytes provide a suitable model system for studies on the physiological regulation and pharmacological modulation of cholesterol biosynthesis and metabolism (7–13). Likewise, HepG2 cells have been used for characterizing hepatic cholesterol metabolism and its inhibition (14–16). Using rat hepatocyte cultures we have recently described inhibition of cholesterol biosynthesis by water-soluble garlic extracts (17). In the present study, we have carefully analyzed the concentration dependence of these effects and report on the interaction at different points during the biosynthetic pathway of cholesterol depending upon the concentration of the garlic extracts. In addition, the effect of garlic extracts and of some constituents on cholesterol biosynthesis in rat hepatocytes was compared with that in HepG2 cells.

MATERIALS AND METHODS

Materials. The garlic powder used (corresponding to Kwai®/Sapec®, Lichtwer Pharma, Berlin, Germany) is prepared immediately after harvesting of garlic cloves from central China. The garlic of this region is characterized by a very high content of sulfur-containing components. The water is removed from the fresh garlic cloves by careful air drying. The other components (such as alliin and the enzyme alliinase) are maintained in concentrated form in the garlic powder. The powder is standardized to an alliin content of 1.3% (18,19) and a capacity for liberation of allicin of 0.6%. The garlic powder, as well as pure, synthetic alliin (18) were provided by Lichtwer Pharma GmbH. These substances were kept dry at 4°C. Mevastatin, triparanol and ketoconazole were kind gifts from Dr. H.J. Kempen (TNO, Leiden, Netherlands).

[U-^{14}C]Acetate sodium-salt (1.9 GBq/mmol, 51.3 mCi/mmol) and RS-[2-^{14}C]mevalonic acid dibenzylethylenediamine salt (2.1 GBq/mmol, 56.7 mCi/mmol) for cholesterol biosynthesis, as well as [1-^{14}C]oleic acid (1.95 GBq/mmol, 52.6 mCi/mmol) and [4-^{14}C]cholesterol (2.2 GBq/mmol, 59.4 mCi/mmol) for the determination of enzyme activities were obtained from Amersham/Buchler (Braunschweig, Germany).

Collagenase (0.225 U/mg) was from Boehringer (Mannheim, Germany) and was used only from batches which proved suitable for the isolation of hepatocytes in our laboratory. Newborn calf serum was obtained from Sebio (Walchsing, Germany) or Serva (Heidelberg, Germany); Williams Medium E was from Flow Laboratories (Meckenheim, Germany). The Extrelut® 20-columns were from Merck (Darmstadt, Germany). All other chemicals were from Boehringer, Merck or Sigma (Munich, Germany).

Animals. Male Sprague-Dawley rats (220–270 g) were used as hepatocyte donors. They were kept in a controlled 12-h light/dark cycle on a standardized diet of Alma® H 1003 (Botzenhardt, Kempten, Germany) and tap water *ad libitum.*

Isolation and cultivation of rat hepatocytes. Rat hepatocytes were isolated according to the two-step collagenase perfusion technique previously described (20). Viability of the cells averaging to 93.2 ± 3.1% was routinely checked by staining with trypan blue.

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Abbreviations: HMG-CoA reductase, hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34); IC_{50}, the concentration for half-maximal inhibition; LDH, lactate dehydrogenase (EC 1.1.1.27); SI-TLC, silver-ion thin-layer chromatography.

Dedication: This article is dedicated to Prof. Dr. D. Mecke on the occasion of his 60th birthday.

The isolated hepatocytes were suspended in Williams Medium E containing 10% newborn calf serum, 2 mM glutamine, penicillin (50 units/mL), streptomycin (50 μg/mL) and 10^{-7} M dexamethasone (17). They were seeded in 2 mL of culture medium at a density of 1.25×10^5 cells/cm^2 into Petri dishes (ϕ 60 mm) precoated with a thin film of collagen (21) and were incubated at 37°C, 90% humidity and 7% CO_2. From 2 h on, serum-free medium was used.

Cultivation of HepG2 cells. The human hepatoma cell line HepG2 was maintained as monolayers as described (22). Confluent cultures were detached with 0.2% trypsin, suspended in medium containing 0.2% trypsin inhibitor and split 1:3 once a week. The cells were used between passages 40–70 (our laboratory). Incubations with garlic extracts were performed in the same serum-free medium used for hepatocytes.

Extraction of garlic powder. Garlic powder stored under dry conditions was extracted with doubly distilled water as described (17), carefully (<30°C) evaporated to dryness and redissolved in Williams Medium E (5 mL/g powder; initial weight). This stock solution was further diluted with Williams Medium E. All concentration data relate to the initial amount of garlic powder per extraction volume, since the amount of the dissolved material (approximately 80%) was not determined. Control media were made using doubly distilled water instead of the garlic extract.

Pure alliin was dissolved directly in Williams Medium E (1 mg/mL) and sterilized by filtration. Dilutions were always made with Williams Medium E. For preincubation with alliinase, alliin (1.66 mg/mL) was directly dissolved in a garlic extract of 5 mg/mL) and was incubated at room temperature for 20 min. These conditions should lead to the complete conversion of all added alliin to allicin (18). The mixture was then diluted 1:100 with Williams Medium E.

Incubation of the primary cultures with the extracts. Eighteen hours after inoculation of the hepatocyte cultures the medium was removed and fresh medium containing [^{14}C]acetate (18.5 KBq/mL; 0.5 μCi/mL), together with the appropriate dilutions of the garlic extracts or compounds to be tested, was added. After incubation at 37°C for 2 h, the medium was removed and the cell layer was washed twice with saline and scraped into 2 mL distilled H_2O. The cells were homogenized by sonication (20 s, grade 3, Ref. 20). In several experiments [^{14}C]-acetate was replaced by [^{14}C]mevalonate (9 KBq/mL, 0.24 μCi/mL).

Determination of acetate incorporation into and separation of nonsaponifiable neutral lipids. The incorporation of [^{14}C]acetate into nonsaponifiable neutral lipids was determined as described using Extrelut®-columns (large-pore kieselgur) for efficient separation (17). The neutral lipophilic nonsaponifiable substances were eluted with *n*-heptane (17). The precursors such as [^{14}C]acetate or [^{14}C]-mevalonate are retained on the column to more than 99%. For measurements of incorporation, the eluate was collected directly in scintillation vials and measured in the scintillation counter after addition of 10 mL of Ultima Gold® (Packard, Merident, CT). The yield of the elution step was 92% (86–95%). Recovery was determined using [^{3}H]cholesterol added to the initial samples. For determination of the newly synthesized sterol pattern, the eluate was evaporated to dryness in a vacuum concentrator (Desaga, Heidelberg, Germany), taken up in 50 μL chloroform and applied to silver-ion thin-layer chromatography (SI-TLC) plates which were developed once using a mixture of *n*-heptane/ethyl acetate (2:1, vol/vol) according to Pill *et al.* (23). With [^{14}C]mevalonate, a similar protocol was used.

Determination of cytotoxicity. Cytotoxicity of garlic extracts and inhibitors was determined by means of the leakage of lactate dehydrogenase (LDH) relative to the total activity of LDH after lysing the hepatocytes with 0.1% Triton X-100 as described (17).

Determination of enzyme activities. For the determination of enzyme activities, a liver homogenate (1:10 wt/vol) in 0.1 M potassium phosphate buffer (pH 7.4) containing 4 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid and 2 mM dithiothreitol was prepared with a Dounce homogenizer (Braun Melrungen AG, Melrungen, Germany) from the liver of starved (24 h) rats and diluted 1:8. Either the $10000 \times g$ supernatant or the microsomal fraction was used for the measurements. For testing the garlic extracts, the extracts were added before the dilution step at such a concentration that the proper dilution was reached together with that of the enzyme activities. Enzyme activities were determined as follows: hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) according to Shapiro *et al.* (24) and fatty acid synthase according to Nepokroeff *et al.* (25). Protein was determined following the procedure of Lowry *et al.* (26).

Statistical evaluation. The IC_{50} values (the concentration for half-maximal inhibition) were determined from the dose response curves by use of curve fitting programs on a PC. The data were evaluated statistically using Student's *t*-test. Data are given as means ± SD.

RESULTS

Incubation of primary rat hepatocyte cultures with water-soluble extracts of garlic inhibited the incorporation of [^{14}C]acetate into the sterol fraction extracted from hepatocyte homogenates. Figure 1 illustrates the effect of concentration. Obviously, there is a strong inhibition above 10 mg/mL, although cytotoxic effects were not found below 125 mg/mL within the incubation period of 2 h (Table 1). Between 0.25 and 5 mg/mL, a plateau was observed which was characterized by a mean inhibition of about 23% (Fig. 1). At lower concentrations the inhibition decreased continuously and an IC_{50} value of approximately 90 μg/mL could be determined. In the presence of the well known inhibitor mevastatin, biosynthesis of nonsaponifiable lipids was inhibited by more than 90% at concentrations above 10^{-6} M.

Similar inhibitory effects by garlic extracts were seen with cultures of HepG2 cells. The range of cytotoxic concentrations was about the same (Table 1), while inhibition of sterol biosynthesis was somewhat more sensitive with an IC_{50} value of approximately 35 μg/mL and a maximal inhibition at the plateau phase of about 28% (Fig. 2). At high concentrations, inhibition with HepG2 cells was less pronounced than with rat hepatocytes.

If the radioactively labeled acetate was replaced by labeled mevalonate, the inhibitory effect of the garlic extracts, as well as that of mevastatin largely disappeared in both types of cells (Table 2) indicating that inhibition

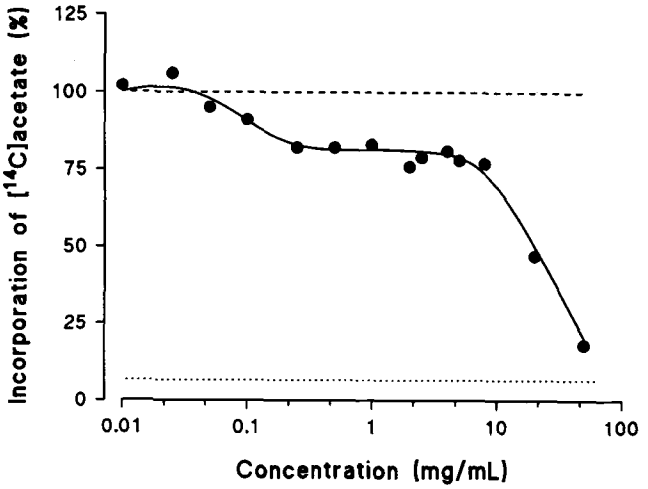

FIG. 1. Dependence of the incorporation of [^{14}C]acetate into nonsaponifiable lipids on the concentration of water-soluble extracts of garlic powder in cultured rat hepatocytes. The dashed line indicates the control level, the dotted line the incorporation in the presence of mevastatin (10^{-6} M). Data represent means ± SD of three to five independent determinations.

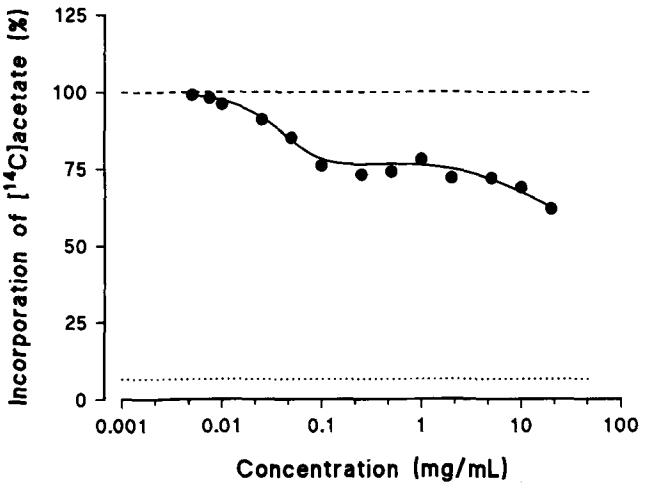

FIG. 2. Dependence of the incorporation of [^{14}C]acetate into nonsaponifiable lipids on the concentration of water-soluble extracts of garlic powder in HepG2 cells. The dashed line indicates the control level, the dotted line the incorporation in the presence of mevastatin (10^{-6} M). Data represent means ± SD of three to four independent determinations.

TABLE 1

Cytotoxicity of Water-Soluble Garlic Extracts on Cultured Rat Hepatocytes and HepG2 Cells

Concentration of garlic extracts (mg/mL)	LDH leakage (% total activity)[a]	
	Rat hepatocytes	HepG2
Control	5 ± 3	2 ± 1
1	5 ± 2	2 ± 1
10	6 ± 1	1 ± 1
50	6 ± 3	3 ± 2
100	12 ± 4	4 ± 2
125	31 ± 7[b]	25 ± 6[b]
150	84 ± 6[b]	67 ± 8[b]
200	100 ± 0[b]	93 ± 6[b]

[a]Data represent means ± SD of triplicate determinations of lactate dehydrogenase (EC 1.1.1.27) (LDH) leakage.
[b]Significant cytotoxicity, $P > 0.001$.

TABLE 2

Inhibition of the Biosynthesis of Nonsaponifiable Lipids from [^{14}C]Acetate or [^{14}C]Mevalonate by Garlic Extracts and Mevastatin in Rat Hepatocytes and HepG2 Cells

Inhibiting material	Inhibition (%)[a]	
	[^{14}C]Acetate	[^{14}C]Mevalonate
Hepatocytes[b]		
Garlic extract[c]	10 ± 3	1 ± 1[d]
Mevastatin[e]	84 ± 7	13 ± 3[f]
HepG2[g]		
Garlic extract[c]	23 ± 6	3 ± 2[f]
Mevastatin[e]	81 ± 8	15 ± 4[f]

[a]Values represent means ± SD of triplicate determinations.
[b]Incorporation of radioactivity for controls was 11.6 ± 1.3 dpm/μg protein and 8.3 ± 1.0 dpm/μg protein for [^{14}C]acetate and [^{14}C]mevalonate, respectively.
[c](100 μg/mL).
[d]Significantly different from acetate: $P < 0.01$.
[e]10^{-5} M.
[f]Significantly different from acetate: $P < 0.001$.
[g]Incorporation of radioactivity for controls was 16.7 ± 2.3 dpm/μg protein and 13.1 ± 1.6 dpm/μg protein for [^{14}C]acetate and [^{14}C]mevalonate, respectively.

occurs at some enzymatic step prior to the formation of mevalonate.

It is known that mevastatin exerts its inhibitory effect on the level of HMG-CoA reductase activity, and preliminary evidence suggested that the same holds true for garlic extracts (17). Therefore, the direct effect of garlic extracts on some enzymes of cholesterol and fatty acid metabolism was tested in detail using liver homogenates from fasted rats. For HMG-CoA reductase, a concentration-dependent inhibition was found starting at concentrations as low as 25 μg/mL (Fig. 3). Above 500 μg/mL inhibition plateaued at about 15% of total enzymatic activity. In the presence of 10^{-5} M mevastatin, almost complete inhibition of the enzyme activity was noted (not shown). Fatty acid synthase was also inhibited (Fig. 3). However, no significant inhibition was found below 100 μg/mL, while maximal inhibition by slightly more than 15% was reached above 1 mg/mL.

The effect of several compounds present in the garlic powder, namely alliin, nicotinic acid and adenosine, on sterol biosynthesis is shown in Figure 4. There was only slight inhibition at high concentrations of alliin (but not at low concentrations, *c.f.* Table 3). Nicotinic acid was shown to be somewhat more potent. Interestingly, adenosine inhibited sterol synthesis to a much greater extent and showed a biphasic concentration dependence similar to that shown by the extracts. After preincubation of alliin with alliinase (from a garlic extract of low concentration), *i.e.* after conversion to allicin (18), a slight inhibition was observed in HepG2 cells only (Table 3) indicating that allicin but not alliin might, under certain conditions, be an active principle. In accordance with this assumption, alliin did not significantly inhibit HMG-CoA reductase

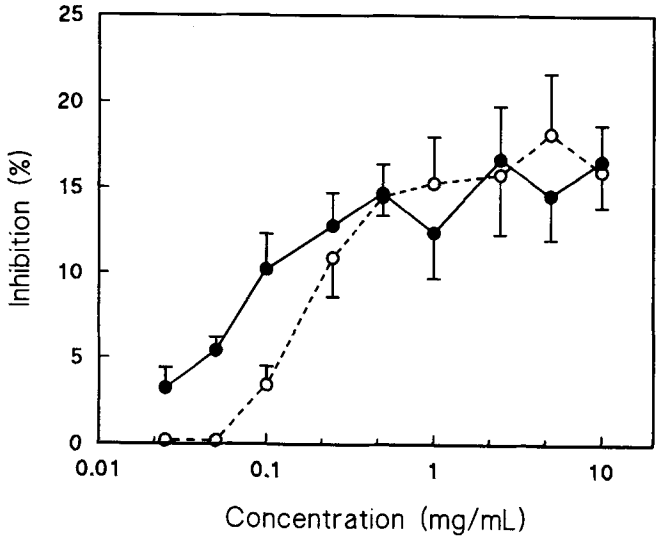

FIG. 3. Dependence of the inhibition of HMG-CoA reductase (● — ●) and fatty acid synthase (O--O) on the concentration of water-soluble garlic extracts. Values represent means ± SD of triplicate determinations.

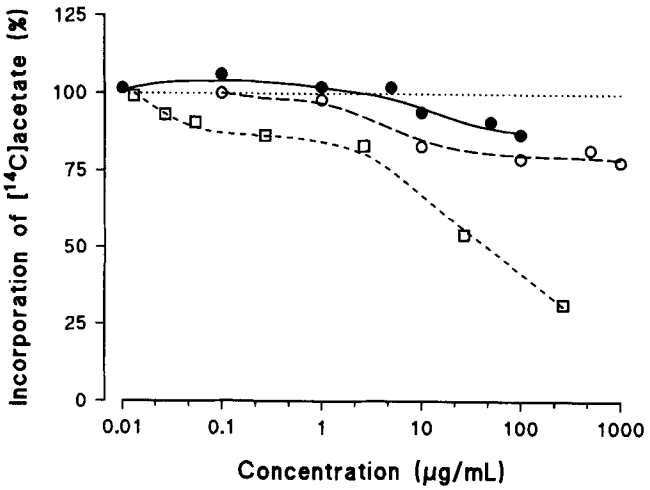

FIG. 4. Dependence of the incorporation of [^{14}C]acetate into nonsaponifiable lipids on the concentration of alliin (● — ●), nicotinic acid (O- - -O) and adenosine (□--□) in cultured rat hepatocytes. The dotted line indicates the control level. Data represent means of duplicate determinations.

in liver homogenates (Fig. 5). Nicotinic acid caused about 10% inhibition above 10 μg/mL. Interestingly, adenosine inhibited HMG-CoA reductase with even greater efficiency (Fig. 5).

When the sterol pattern produced by the rat hepatocytes was analyzed by SI-TLC, cholesterol was shown to be the main sterol formed (Fig. 6, A and B). The precursors lanosterol and 7-dehydrocholesterol amounted to 8 and 4%, respectively. In the presence of garlic extracts at concentrations above 0.5 mg/mL, a shift in the proportions of the sterols could be observed (Fig. 6A). Lanosterol and, to some extent, 7-dehydrocholesterol, increased at the expense of cholesterol indicating an inhibition at several steps during the conversion of lanosterol to cholesterol. The percentage of 7-dehydrocholesterol never exceeded

TABLE 3

Effect of Alliin with and without Preincubation with Alliinase on the Biosynthesis of Nonsaponifiable Lipids from [^{14}C]Acetate in Rat Hepatocytes and HepG2 Cells

	Incorporation of [^{14}C]acetate into nonsaponifiable lipids[a]			
	Hepatocytes		HepG2	
Compound	dpm/μg Protein	%	dpm/μg Protein	%
None	12.3 ± 1.9	100	15.8 ± 1.1	100
Alliin[b]	11.6 ± 1.3	94	15.0 ± 0.9	95
Alliin (preincubated)[c]	11.4 ± 1.2	93	14.1 ± 0.9[d]	89

[a]Values represent means ± SD of duplicate determinations from four different cultures.
[b]10^{-4} M.
[c]Aliin (10^{-4} M) was preincubated with alliinase from a garlic extract (50 μg/mL) as described in Materials and Methods.
[d]Statistical significance with respective controls: $P < 0.01$.

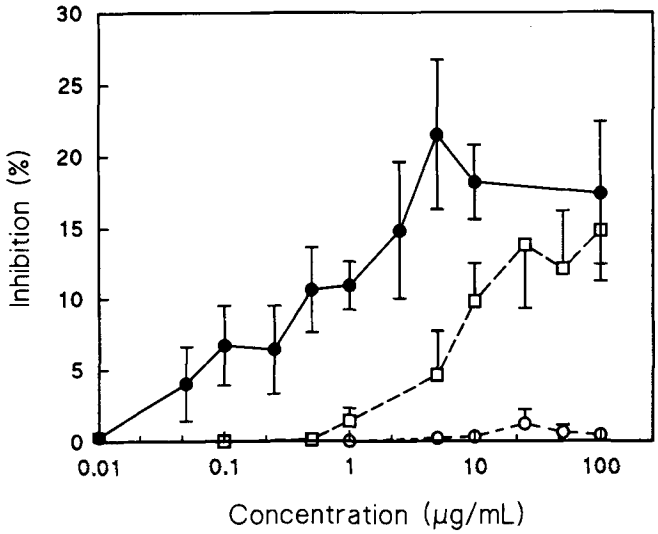

FIG. 5. Dependence of the inhibition of HMG-CoA reductase on the concentration of alliin (O--O), nicotinic acid (□— —□) and adenosine (● — ●). Values represent means ± SD of triplicate determinations.

25% (relative proportion), because at higher concentrations inhibition of lanosterol metabolism predominated. No desmosterol was formed at all concentrations used [an indication to the contrary in a previous abstract (27) was due to inadvertent mislabeling of the respective figure]. With HepG2 cells similar results were obtained (Fig. 6B, Table 4), but pronounced accumulation of lanosterol required even higher concentrations of the extracts than with rat hepatocytes. The proportion of 7-dehydrocholesterol never exceeded 14%. Other (partially unidentified) precursors, presumably dihydrolanosterol (5α-lanost-8-ene-3β-ol), were observed in slightly greater percentages.

Direct addition of several garlic compounds revealed that neither alliin and nicotinic acid nor adenosine (Fig. 7) caused a shift in the composition of the sterol fraction produced by rat hepatocytes. However, when alliin was converted to allicin by preincubation with alliinase, a similar shift in the proportion of cholesterol, lanosterol and

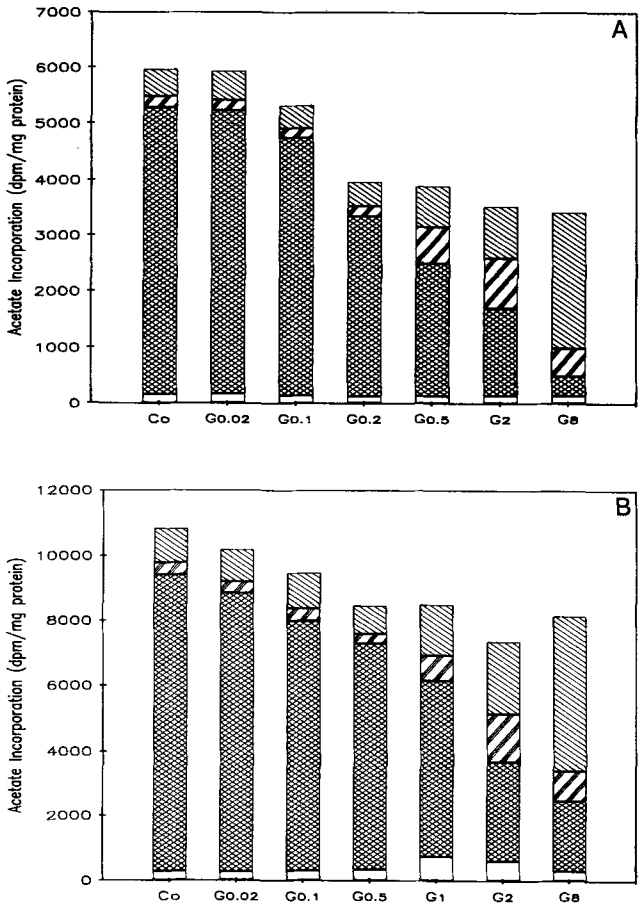

FIG. 6. Influence of different concentrations of garlic extracts on the incorporation of [^{14}C]acetate into cholesterol and cholesterol precursors in (A) rat hepatocytes and (B) HepG2 cells. Co, control; G0.02 to G8, garlic extracts ranging from 0.02 to 8 mg/mL (cross-hatched bars) cholesterol, (left-hatched bars) lanosterol, (striped bars) 7-dehydrocholesterol, (open bars) other sterols.

7-dehydrocholesterol was found as with the garlic extracts (Fig. 7). Similar effects were observed with HepG2 cells (Table 4) or after incubation with mevalonate (not shown). In contrast, mevastatin did not change sterol composition in rat hepatocytes, but caused a considerable reduction of acetate incorporation into the sterol fraction only (Fig. 7). Triparanol and ketoconazole caused marked changes in the sterol composition that were considerably different from those observed with the garlic extracts. With the latter inhibitors, large proportions of desmosterol were formed (Fig. 7).

TABLE 4

Effect of Alliin with and without Preincubation with Alliinase on the Incorporation of [^{14}C]Acetate into Cholesterol and Cholsterol Precursors in HepG2 Cells

Sterol	Incorporation of [^{14}C]acetate into cholesterol and precursor sterols (% of total)[a]		
	Control	Alliin[b]	Alliin (inc.)[c]
Lanosterol	7.7 ± 1.4	9.2 ± 1.6	25.8 ± 2.7[d]
Dihydrolanosterol	0.5 ± 0.3	0.4 ± 0.2	6.1 ± 1.6[d]
7-Dehydrocholesterol	3.9 ± 0.8	5.9 ± 1.5	10.7 ± 1.7[d]
Cholesterol	87.1 ± 5.3	83.7 ± 7.0	55.9 ± 3.8[d]
Others	0.8 ± 0.4	0.8 ± 0.5	11.5 ± 2.1[d]

[a]Values are expressed as percentage of radioactivity in relation to the total incorporation in each group and represent means ± SD from three to four experiments.
[b]10^{-4} M.
[c]Alliin (10^{-4} M) was preincubated (inc.) with alliinase from a garlic extract (50 μg/mL) as described in Materials and Methods.
[d]Statistical significance with control: $P < 0.01$.

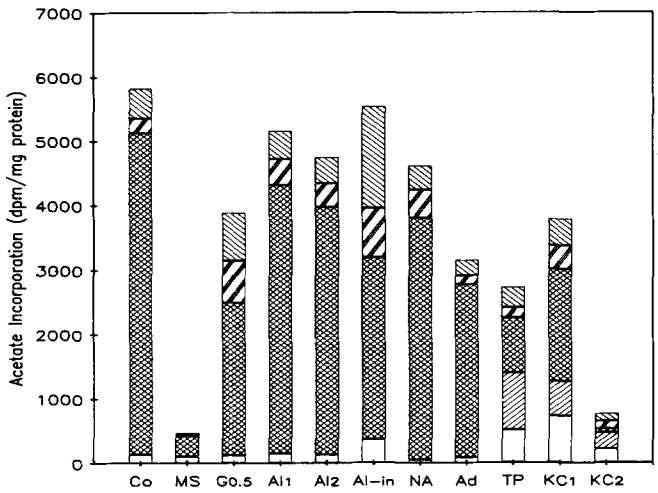

FIG. 7. Influence of different inhibitors and garlic compounds on the incorporation of [^{14}C]acetate into cholesterol and cholesterol precursors in rat hepatocytes. Co, control; MS, mevastatin; G0.5, garlic extract (0.5 mg/mL); AL1, alliin (10^{-4} M); AL2, alliin (10^{-3} M); AL-in, alliin (10^{-3} M) preincubated with alliinase; NA, nicotinic acid (1 mg/mL); Ad, adenosine (20 μg/mL); TP, triparanol (10^{-5} M); KC1, ketoconazole (10^{-5} M); KC2, ketoconazole (10^{-4} M). (cross-hatched bars) Cholesterol, (left-hatched bars) lanosterol, (striped bars) 7-dehydrocholesterol, (right-hatched bars) desmosterol, (open bars) other sterols.

DISCUSSION

In the present study we report on multiple inhibitory effects of garlic extracts at several different steps (early and late) in the cholesterol biosynthetic pathway. The extent of inhibition depended strongly on the concentration of the extracts.

Firstly, inhibition of total sterol biosynthesis was recognized as a biphasic phenomenon caused mainly on the level of HMG-CoA reductase. The fact that rat hepatocytes and human HepG2 cells responded very similarly may indicate that the results are relevant for humans consuming fresh garlic or equally potent garlic preparations. Indeed, the degree of inhibition observed at low concentrations (25-30%) nicely reflects the decrease in cholesterol levels described for rat serum (1) as well as human serum (4,5). The high concentrations studied herein, which cause much stronger inhibition comparable to that by mevastatin, are not likely to be reached even during excessive consumption of garlic preparations.

Studies in which acetate was replaced by mevalonate and direct measurement of enzyme activities suggested that the garlic extracts exert their lowering effect essen-

tially through inhibition of HMG-CoA reductase. A similar inhibition with water-soluble extracts had previously been observed with the chicken liver enzyme (28). Which compounds contained in the extracts are responsible for this inhibition is not known. As shown in this study, nicotinic acid and adenosine may be among the candidates. The relative concentration of nicotinic acid in garlic extracts (approximately 4 μg/mL, estimated from Ref. 29), however, seems too low to account for the total inhibition observed with the garlic extracts. Adenosine, on the other hand, the concentration of which in garlic is higher (approximately 10 μg/mL, estimated from Ref. 29) seems to contribute to the inhibition of cholesterol biosynthesis in an indirect manner, since it is unlikely to enter the hepatocytes. Whether garlic extracts raise the intracellular adenosine level remains to be investigated. The contribution of alliin and its product allicin to the inhibition at the level of HMG-CoA reductase appears of minor importance, although the effect of allicin in HepG2 cells was significant. Thus, it seems most likely that other still unknown components may also be involved in the inhibition. Hepatocyte cultures may aid in further defining these active principles and their mode of action.

Secondly, exposure of rat hepatocytes or HepG2 cells to higher concentrations of garlic extracts resulted in a pronounced shift in the composition of the sterol fraction indicating inhibition at later stages of cholesterol biosynthesis. This inhibition led to the pronounced accumulation of lanosterol and, to a lesser extent, of 7-dehydrocholesterol. The latter compound represents 6-9% of total sterols in subcellular membranes of normal rats (30). The accumulation of lanosterol is comparable to the effect of ketoconazole, a known antimycotic, and of buthiobate which both have been found to inhibit the conversion of lanosterol to demethyllanosterol (14,15,31). Whether the same enzymatic step is inhibited by the garlic extracts remains to be investigated. On the other hand, the accumulation of 7-dehydrocholesterol seems much less than with other inhibitors such as *trans*-1,4-*bis*(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride (32) and 4-(2-[1-(4-chlorocinnamyl)piperazin4-yl]ethyl)benzoic acid (10). This indicates that inhibition of 14α-demethylation by the garlic extracts might be much more pronounced than that of side-chain saturation. Desmosterol, which is known to cause adverse effects as a consequence of massive accumulation, was not formed in the presence of garlic extracts. Thus, side-chain saturation again appears less inhibited. In contrast, triparanol in our hepatocyte cultures gave rise to desmosterol which is in agreement with the known accumulation of desmosterol *in vivo*, particularly in the skin (33,34), as well as *in vitro* (15). Although the interference of garlic compounds with late stages of cholesterol biosynthesis is very interesting from the molecular point of view, it seems to be of minor therapeutic significance, because it requires concentrations one or two orders of magnitude above those affecting the early stage. These concentrations seem too high to be reached during normal and even long-term consumption of garlic preparations.

Neither nicotinic acid nor adenosine seem to be involved in the inhibition at late stages of cholesterol biosynthesis. However, there is some indication that allicin at high concentrations might be involved in this inhibition in both types of cells. Whether this effect is specific for this compound or is observed also with other garlic compounds derived from alliin, which contain sulfur- and allyl-groups, is currently being investigated.

It is concluded that defined compounds present in water-soluble extracts from garlic inhibit the biosynthesis of cholesterol in hepatocytes thus contributing to the reduction of serum cholesterol. The observed gap between the low concentrations inhibiting the early steps and the high concentrations required to inhibit the late steps in the synthetic pathway may aid in lowering cholesterol biosynthesis without accumulating undesirable cholesterol precursors.

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