Preface

Sales of dietary supplements have skyrocketed over the past few years. Despite widespread interest in and use of these products, information about the safety and efficacy of dietary supplements in humans is generally sparse compared with the information available about prescription drugs. Herbalists and laypersons have used herbs for centuries, but most nonherbal dietary supplements came into vogue only within the past few decades, further limiting the information available about these products. The companion volume, *Toxicology and Clinical Pharmacology of Herbal Products* addressed herbal dietary supplements, whereas *Dietary Supplements: Toxicology and Clinical Pharmacology* focuses on nonherbal dietary supplements. Supplements were chosen for inclusion based on their popularity, toxicity, and the quantity and quality of information available. Some supplements described here are no longer available as dietary supplements (e.g., gamma-hydroxybutyric acid and related substances, L-tryptophan), but are available through various channels, either legal or illegal. Others are advertised as dietary supplements, although the Food and Drug Administration does not view them as such (e.g., hydrazine sulfate).

The aim of this book is to present, in both comprehensive and summary formats, objective information on nonherbal dietary supplements from the most reliable sources, with an emphasis on information not readily available elsewhere (i.e., detailed descriptions of case reports of adverse effects, pharmacokinetics, and chemical and biofluid analysis). It is not designed to be a prescriber's handbook; the intended audience is both forensic and health care professionals, particularly researchers and clinicians interested in more detailed information than is available in most "herbal" or "natural product" references.

Although information about dietary supplements is widely available on the Internet, it is usually provided by product distributors and is designed to sell products rather than provide objective information about product efficacy and toxicity. Even reviews of dietary supplements in journals, newsletters, books, and electronic databases can be biased or incorrect. In compiling information to be included in *Dietary Supplements: Toxicology and Clinical Pharmacology,* emphasis was placed on original studies published in reputable, peer-reviewed journals. Older studies as well as more current literature were utilized for completeness. Where appropriate, information was obtained from meta-analyses, systematic reviews, or other high-quality reviews such as those written by recognized experts. Case reports of adverse effects and interactions, although anecdotal

in nature, were used to identify and describe uncommon, but potentially serious, adverse events that may not have been noted in controlled studies because of small sample size or short duration. The detail in which studies in this section is described is a function of the popularity of the supplement, the extent to which study results conflict with each other and with advertised efficacy claims, the attention recent supplement studies have received in both lay and medical news, and other factors described in the text.

This volume begins with an updated discussion of the legal aspects of dietary supplements. Each of the following supplement monographs includes a review of the product's history, current promoted uses, sources and chemical composition, and descriptions of available products, which are kept general owing to the myriad of ever-changing products on the market. Product quality is also discussed in this section. For those supplements that are endogenous to humans, the physiologic role is then described. The pharmacologic effects of the products, divided into in vitro/animal data and clinical studies, are reviewed. The in vitro/animal data included were chosen to provide an explanation for the product's clinical effects in humans and to show the rationale for clinical studies. It should be noted that because of the nature of dietary supplement claims (*see* Part I, Legal/Regulatory Aspects), some promoted product uses might not have been studied in humans; conversely, known pharmacologic and therapeutic effects might not be promoted commercially. As a result, in most chapters there is a mismatch between the information in the Current Promoted Uses and Clinical Studies sections.

The Pharmacokinetics section covers absorption, tissue distribution, elimination, and body fluid concentrations. Such pharmacokinetic information is not usually included in other sources and may be useful in forensic investigations, or in the clinical setting when the product is used in patients with renal or hepatic insufficiency. A section on Adverse Effects and Toxicity follows, which includes detailed information on case reports of adverse reactions to the supplement. The Interactions section discusses interactions between the supplement and drugs or foods, as well as the effects of drugs on endogenous levels of the supplement if it is an endogenous compound. The Reproduction section is generally limited, owing to lack of information. Methods of Chemical and Biofluid Analysis are included for forensic professionals and for those investigating the product in clinical studies. Each monograph ends with a discussion of Regulatory Status of the product. The amount of information included in each of these sections varies according to availability.

At the end of each monograph is a summary presenting key information in bulleted form. A table at the end of the book summarizes supplement toxicities and adverse effects, drug interactions, and use in special populations (e.g., pregnancy and lactation, renal and hepatic impairment). The source of this information (animal data, in vitro effects, clinical trials, case reports, and theoretical concerns) are given. This section is intended for quick reference, and readers should refer to the chapter for more detailed discussion.

Adverse reactions to dietary supplements appear to be uncommon compared with those attributed to prescription drugs. This may be a function of health care and forensic professionals' unfamiliarity with a product's pharmacology and toxicology or assumption that a product is "natural" and therefore safe. Thus, an adverse reaction may go

unrecognized or be attributed to a prescription medication. It is hoped that the information in *Dietary Supplements: Toxicology and Clinical Pharmacology* will be used to solve clinical or forensic problems involving dietary supplements, to promote dialog between health care professionals and patients, and to stimulate intellectual curiosity about these products, fostering further research on their therapeutic and adverse effects.

Melanie Johns Cupp

Chapter 2

Chitosan

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2.1. History

Chitosan was purportedly first discovered in 1859 by Rouget while he was experimenting with chemical and thermal manipulation of the natural fiber chitin (Au Natural Herbals, 2001). Since then, chitosan has been used as a pharmaceutical excipient in sustained release dosage forms, as an immunostimulant, and to promote wound healing (Colombo and Sciutto, 1996). Chitosan's ability to bind to a variety of substances including acids, lipophilic substances, and minerals (Jing et al., 1997) has enabled it to be used for water purification for more than 30 yr. Chitosan has been sold in Europe and Japan for the past 20 yr as a nonprescription product to inhibit fat absorption (Au Natural Herbals, 2001). Chitosan was first marketed as a dietary supplement in the United States in the late 1990s.

2.2. Chemical Structure

See Fig. 2-1 for the chemical structure of chitosan.

2.3. Current Promoted Uses

Because of its ability to inhibit fat absorption, chitosan is promoted primarily for use in obesity and hyperlipidemia.

2.4. Sources and Chemical Composition

Chitosan is a fiber product that is obtained from deacetylated chitin. Chitin is a naturally occurring substance found in the shells of crustaceans, invertebrates, and fungi (Sciutto and Colombo, 1995). Chitin is a linear polysaccharide comprised of *N*-acetyl-D-glucosamine chains [β(1–4)-2-acetamide-2-deoxy-D-glucose]. It is similar to

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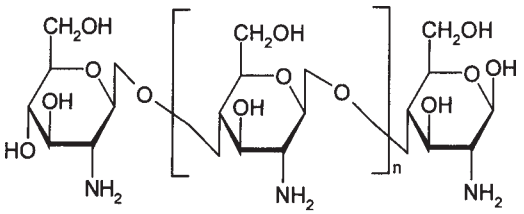

Fig. 1. Chemical structure of chitosan.

unramified cellulose and is insoluble in water. After deacetylation with sodium hydroxide, the resulting chitosan becomes soluble in acid, such as is present in the stomach. Once solubilized, the chitosan forms a gel-like substance that binds lipids in the gastrointestinal tract, resulting in their fecal elimination (Macchi, 1996).

A new form of chitosan has been extracted and purified. This electrostatically charged chitosan is poorly absorbed systemically but is able to bind lipids and prevent their digestion. The positively charged amino groups on the chitosan molecule bind to the negatively charged carboxylic groups of free fatty acids. This electromagnetic bond seems to be stronger than those observed in other dietary fibers. Additionally, hydrophobic bonds are also formed between chitosan and neutral fats such as cholesterol and triglycerides (Macchi, 1996).

2.5. Products Available

Chitosan is available in dietary supplements sold under several proprietary names. Products may be combined with a starch excipient. Additionally, chitosan may be found in combination with other substances, such as appetite suppressants, stimulants, chromium picolinate, carnitine, or amino acids in products marketed for weight loss (Candlish, 1999).

2.6. Animal Data

Muzzarelli (1999) reviewed animal data pertinent to chitosan's therapeutic effects. In hypercholesterolemic, apolipoprotein E-deficient mice, which develop high blood cholesterol levels and atherosclerosis without scientific manipulation, 20 wk of dietary chitosan resulted in a 52% reduction in serum cholesterol. Additionally, the area of plaque in the aortic arch was 50% lower in treated mice compared with control mice. On the other hand, the chitosan-fed mice experienced increased growth, with an overall 65% weight gain; the control mice experienced growth retardation.

In dogs given 2 wk of oral chitosan, total cholesterol levels decreased to 77% of baseline by day 7 of therapy and then to 54% of baseline by day 14. Levels returned to baseline on day 28. Other dogs treated with chitin or celluloses experienced no reduction in cholesterol (Muzzarelli, 1999).

Another study examined the hyperglycemic and hypolidemic effects of chitosan in normal and diabetic mice. Diabetic mice consisted of obese mice with hyperinsulinemia (KK-Ay) and lean mice with hypoinsulinemia [neonatal streptozocin-induced diabetic mice (NSZ)]. After 4 wk of a 5% chitosan diet, no change in body

weight was observed in any of the mice groups. In the normal mice, decreased levels of blood glucose, cholesterol, and triglycerides were observed. In NSZ mice, significant reductions in blood glucose and cholesterol were also observed; however, no reductions were observed in the KK-Ay mice.

2.7. Clinical Studies

Several studies have examined the effects of the new electrostatically charged, polycationic chitosan on lipids and body weight (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Colombo and Sciutto, 1996; Veneroni et al., 1996; Macchi, 1996;). An additional study with findings not supporting chitosan's efficacy in reducing weight and serum lipids does not identify the product as being the new electostatically charged form (Pittler et al., 1999). Most of the chitosan studies examined the effects of a hypocaloric diet (1000–1100 kcal/d) plus chitosan (1 gm twice daily before main meals) vs the same hypocaloric diet plus placebo in mildly obese (10–25% overweight) subjects (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Colombo and Sciutto, 1996; Veneroni et al., 1996). These double-blind, placebo-controlled trials show statistically significant reductions in body weight, percent overweight, total and LDL cholesterol, and triglycerides and an increase in HDL in all treatment groups compared to baseline; however, between-group comparisons favor the groups receiving chitosan (Colombo and Sciutto, 1996; Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Veneroni et al., 1996). Three studies also examined chitosan's effects on systolic and diastolic blood pressure and found that both diastolic (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Macchi, 1996) and systolic (Sciutto and Colombo, 1995; Macchi, 1996) blood pressure were reduced with chitosan. One of these studies examined the effect on respiratory rate and found it to be reduced in both treatment groups, with a statistically greater effect in the chitosan group (Giustina and Ventura, 1995).

One double-blind, placebo-controlled study of 30 moderately obese (25% overweight) subjects examined the effects of chitosan 1 g twice daily before main meals plus a hypocaloric diet (1200 kcal/d), placebo plus a hypocaloric diet, and chitosan plus an unrestricted diet. The chitosan plus hypocaloric diet and chitosan plus unrestricted diet groups showed a statistically significant decease in body weight, body mass index (BMI), body fat, and skinfold thickness compared with baseline ($p < 0.0001$, Wilcoxon matched pairs, signed rank test). The diet plus placebo group also showed a statistically significant decrease in weight ($p < 0.0001$), BMI, body fat, and skinfold thickness ($p < 0.001$). The decrease was greatest in the chitosan plus hypocaloric diet group. Additionally, these reductions were greater in the chitosan plus unrestricted diet group than in the placebo plus hypocaloric diet group. For example, the average weight loss was 4 kg ($p < 0.0001$) in the chitosan plus hypocaloric diet group, 2.6 kg ($p < 0.0001$) in the placebo plus hypocaloric diet group, and 2.8 kg ($p < 0.0001$) in the chitosan plus unrestricted diet group.

Statistically significant reductions in total cholesterol and triglycerides were seen in all groups, with the greatest reductions observed in the chitosan groups. The average reduction in total cholesterol was 26 mg/dL ($p < 0.0001$) in the chitosan plus hypocaloric diet group, 15 mg/dL ($p < 0.01$) in the placebo plus hypocaloric diet group,

and 28 mg/dL ($p < 0.0001$) in the chitosan plus unrestricted diet group. The average reduction in triglycerides was 27 mg/dL ($p < 0.01$) in the chitosan plus hypocaloric diet group, 26 mg/dL ($p < 0.0001$) in the placebo plus hypocaloric diet group, and 27 mg/dL ($p < 0.0001$) in the chitosan plus unrestricted diet group. A statistically significant increase in HDL cholesterol (11 mg/dl, $p < 0.001$) was seen only in the chitosan plus unrestricted diet group.

Statistically significant reductions in systolic blood pressure (6 mmHg, $p < 0.01$ and 11 mmgHg, $p < 0.0001$) and diastolic blood pressure (7 mmHg, $p < 0.001$ and 9 mmHg, $p < 0.001$) were seen in the chitosan plus hypocaloric diet and in the chitosan plus unrestricted diet groups, respectively, but not in the placebo plus hypocaloric diet group. Heart rate did not change significantly during the study (Macchi, 1996).

A randomized, double blind, placebo-controlled study examined the effect of chitosan vs placebo in 34 overweight subjects maintaining their normal diet. After 4 wk of treatment, weight, BMI, blood pressure, total cholesterol, and triglycerides were not different in subjects receiving chitosan compared with those receiving placebo. For example, baseline and 4-wk weights in the chitosan group were 71.8 ± 8.4 kg and 72.6 ± 8.6 kg, respectively; values in the placebo group were 76.4 ± 9.5 kg and 77.9 ± 10.8 kg (NS). Baseline and 4-wk total cholesterols in the chitosan group were 5.77 ± 0.87 mmol/l and 5.32 ± 0.93 mmol/L, respectively; values in the placebo group were 5.36 ± 0.92 mmol/L and 5.64 ± 1.31 mmol/L (NS). Similar patterns were seen in the other outcome measures (Pittler et al., 1999).

A discussion in a metaanalysis conducted by two authors from the previous study criticizes the studies of chitosan in conjunction with a hypocaloric diet. Although the metaanalysis of the included studies (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Colombo and Sciutto, 1996; Macchi, 1996; Veneroni et al., 1996) indicated that the weighted mean difference between chitosan and placebo groups is 3.28 kg (95% CI, 1.5–5.1), the authors suggest that some other mechanism might be involved in the weight loss in those studies. This is postulated because the extra 3.28 kg weight loss in the chitosan groups would require a fecal fat loss of more than 100 g/d. This amount of fat would not normally be consumed from a hypocaloric diet of 1000–1200 kcal/d. Further criticism reveals that the articles were not cited in searchable databases, but rather were provided from one manufacturer and appeared in the same journal, although the journal is peer-reviewed and of accepted standing in its field (Ernst and Pittler, 1998).

Chitosan has also been studied in the treatment of the sequelae of chronic renal failure in hemodialysis patients. In an unblinded study (Jing et al., 1997), 40 hemodialysis patients were administered 10 tablets containing chitosan (Kitosan Shokuhin Kogyo) 45 mg three times daily for 12 wk. The chitosan used in the study had a molecular weight of 27,000 daltons and was 89% deacetylated. At baseline and every 4 wk thereafter, the patients' blood pressure, weight, serum creatinine, blood urea nitrogen (BUN), lipids, hemoglobin, and electrolytes were measured. Means for the outcome measures were compared with those of a control group that did not receive chitosan. Data analysis was performed using repeated *t*-tests. Statistical significance was accepted at the $p < 0.05$ level. At weeks 8 and 12, total cholesterol and lipoprotein were significantly lower in the treatment group. At weeks 4, 8, and 12, hemoglobin

was higher and BUN and creatinine were significantly lower in the treatment group. Subjective improvements in sleep, appetite, physical strength, halitosis, and itching were noted. Because chitosan is not capable of binding to creatinine in vitro, it was hypothesized that the effects of chitosan on creatinine and hemoglobin were secondary to its binding "uremic toxins" in the gastrointestinal tract, leading to improvement in residual renal function. Symptom improvement could also be attributed to binding of these toxins and nitrogenous wastes in the gut. Placebo-controlled trials are needed to confirm the results of this study and to assess the long-term safety and pharmacokinetics of chitosan in this population.

2.8. Pharmacokinetics

Experimental evidence suggests that chitosan is partially digested and absorbed owing to the acidic environment of the stomach, enzymes present in saliva and gastric juice, and bacterial enzymes in the large intestine. Oral intake of 1 g/day of chitosan increases the serum concentration of *N*-acetyl-D-glucosamine. Serum levels remain high 48 h after ingestion (Muzzarelli, 1999).

2.9. Adverse Effects and Toxicity

Chitosan is generally considered to be nontoxic, and, unlike medication used to treat hyperlipidemia, chitosan has no activity on enzymes involved in cholesterol biosynthesis (Muzzarelli, 1999). In clinical studies, very few adverse effects have been reported. Adverse effects have been mild and transient and have consisted of mild nausea (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Veneroni et al., 1996), flatulence (Colombo and Sciutto, 1996), throat irritation, itching (Jing et al., 1997), constipation rarely requiring laxatives (Macchi, 1996), and soft fatty stool (Veneroni et al., 1996). Adverse effects did not occur more frequently in patients receiving chitosan than in those receiving placebo (Giustina and Ventura, 1995; Sciutto and Coluombo, 1995; Colombo and Sciutto, 1996; Veneroni et al., 1996).

2.10. Interactions

Theoretically, there has been concern that chitosan could bind fat-soluble vitamins and deprive the body of these essential nutrients. It has been suggested that vitamin supplements should be taken at a separate time from chitosan in order to avoid any potential interaction (Muzzarelli, 1999). Absorption of vitamins E (Muzzarelli, 1999; Pittler et al., 1999), D, and A and β-carotene is not altered by chitosan intake (Pittler et al., 1999). One study found that vitamin K levels were significantly higher after 4 wk of treatment with chitosan but were not outside the normal range (Pittler et al., 1999). Theoretically, this could lead to a decrease in the efficacy of warfarin.

It was also once thought that chitosan might interfere with intestinal absorption of trace metal ions such as iron and zinc (Muzzarelli, 1999) and mineral salts (Colombo and Sciutto, 1996). Studies have shown, however, that absorption of these substances is not inhibited by chitosan administration (Giustina and Ventura, 1995; Sciutto and Colombo, 1995; Colombo and Sciutto, 1996; 1999; Veneroni et al., 1996; Muzzarelli, Pittler et al., 1999).

2.11. Reproduction

Chitosan's effects on fertility, pregnancy, and lactation are not known.

2.12. Chemical and Biofluid Analysis

Chitosan is a mixture of chitooligosaccharides that to our knowledge have not been measured in human tissue (e.g., blood or urine). Lopatin and colleagues (1985) described a technique involving liquid chromatography-mass spectrometry (LC-MS) for characterizing *N*-acetylchitooligosaccharide derivatives that may be adaptable to measuring concentrations in blood, if desired. However, some modifications of the method will be needed since it was developed for use in analyzing crab shells.

The initial step involves hydrolysis of the shell to release the chitosan. Chitosan (10 g) is dissolved in 0.5% acetic acid (1000 mL), and then 30 mg of lyophically dried chitinases complex (from *Streptomyces kurssanovii*) is added to the solution. The mixture is then partially purified by metal-ion affinity chromatography. The solution is stirred for 4 h at 45°C, an additional 40 mg of enzyme complex is added and allowed to mix for an additional 16 h at 37°C. This is followed by heating to denature the enzymes. Finally, once cool, the solution is filtered through a Diasorb C-16 (1 × 10 cm) column, and the filtrate is lyophilized to dryness. (It should be noted that this hydrolysis step may not be necessary when measuring concentrations in human blood or urine.) The lyophilized residue is then dissolved in 50 mL of water; 10 mL of methanol and 20 mL of acetic anhydride are added to acetylate the chitooligosaccharides. The reaction is allowed to proceed for 16 h, and then the residue is concentrated under vacuum until the acetic acid odor disappears. The resulting residue [*N*-acetylchitooligosaccharides ($GlcNAc_{2-7}$)] is dissolved in 30 mL of water, and 2 g of Amberlit MB-3 resin is added and stirred for 15 min. The solution is then dried to a powder.

The sample is initially fractionated on a Sephadex G-25 sf (3 × 70 cm) column with water as the mobile phase and a flow rate of 225 mL/h. Ten mL fractions (10 mL) are collected, concentrated and identified by reversed-phase chromatography.

For the chromatographic separation, a LiChrospher 100 RP-18 column (4 × 250 mm) is used. Water (0.5 mL/min) is used as the mobile phase and detection is via an ultraviolet (UV) detector set at 206 nm. The samples are readily separated with a maximum run time of 60 min for $GlcNAc_7$. For positive peak identification, mass spectra are obtained on a time/flow biochemical mass spectrometer with plasma desorption by fission products of californium-252. The *N*-acetylchitooligosaccharides are dissolved in 0.1% trifluoroacetic acid and then dried prior to introduction into the mass spectrometer. Once the peak identities have been conclusively verified, it may be possible to omit the mass spectrometric detection and simply quantitate the samples via the LC-UV chromatography step.

As an alternative method, Chang and associates (1979) have developed an indirect method for quantitating chitooligosaccharides using an amino acid analyzer. The preparative stages of this assay are also somewhat complex, as with the LC method just described. Also, this method has not been used to quantitate the oligosaccharides in biologic tissues.

2.13. Regulatory Status

Chitosan is regulated as a dietary supplement in the United States and is not approved for use as a drug by the Food and Drug Administration. It is marketed as a food additive or supplement in Japan, England, Italy, and Portugal (Muzzarelli, 1999).

2.14. Summary

- Chitosan is a glucosamine polymer derived from shellfish that, among other properties, is capable of adsorbing lipophilic and acidic substances.
- Chitosan appears to be modestly effective in improving body composition, decreasing serum lipids, and decreasing blood pressure in mild-to-moderately overweight patients.
- Chitosan may be useful in treating anemia, pruritis, weakness, and fatigue associated with chronic renal failure in hemodialysis patients by removing nitrogenous wastes and uremic toxins.
- Chitosan is partially digested in the gastrointestinal tract and can be absorbed systemically.
- Gastrointestinal complaints are the most common adverse effects associated with chitosan.
- Theoretically, chitosan might interfere with the action of warfarin by increasing vitamin K levels, but no interactions have thus far been reported.

References

Au Natural Herbals. Chitosan history. Available from: URL: http://www.chitosan-weight-loss.net/history.html. Accessed November 22, 2001.

Candlish JK What you need to know: over the counter slimming products—their rationality and legality. Singapore Med J 1999;40:550–2.

Chang JJ, Hash JH. The use of an amino acid analyzer for the rapid identification and quantitative determination of chitosan oligosaccharides. Anal Biochem 1979;95:563–67.

Colombo P, Sciutto AM. Nutritional aspects of chitosan employment in hypocaloric diet. Acta Toxicol Ther 1996;17:278–302.

Ernst E, Pittler MH. Chitosan as a treatment for body weight reduction? A meta-analysis. Perfusion 1998;11:461–5.

Giustina A, Ventura P. Weight-reducing regimens in obese subjects: effects of a new dietary fiber integrator. Acta Toxicol Ther 1995;16:199–214.

Jing S, Li L, Ji D, Takiguchi Y, Yamaguchi T. Effect of chitosan on renal function in patients with chronic renal failure. J Pharm Pharmacol 1997;49:721–3.

Lopatin SA, Ilyin MM, Pustobaev VN, et al. Mass-spectrometric analysis of N-acetyl-chitooligosaccharides prepared through enzymatic hydrolysis of chitosan. Anal Biochem 1995;227:285–288.

Macchi G. A new approach to treatment of obesity: chitosan's effects on body weight reduction and plasma cholesterol's levels. Acta Toxicol Ther 1996;17:303–20.

Muzzarelli RAA. Clinical and biochemical evaluation of chitosan for hypercholesterolemia and overweight control. EXS 1999;87:293–304.

Pittler MH, Abbott NC Harkness, Ernst E. Randomized, double-blind trial of chitosan for body weight reduction. Eur J Clin Nutr 1999;53:379–81.

Sciutto AM, Colombo P. Lipid-lowering effect of chitosan dietary integrator and hypocaloric diet in obese subjects. Acta Toxicol Ther 1995;16:215–30.

Veneroni G, Veneroni F, Contos S, et al. Effect of a new chitosan dietary integrator and hypocaloric diet on hyperlipidemia and overweight in obese patients. Acta Toxicol Ther 1996;17:53–70.