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EVALUATION OF THE HEALTH ASPECTS OF LICORICE, GLYCYRRHIZA AND AMMONIATED GLYCYRRHIZIN AS FOOD INGREDIENTS

FEDERATION OF AMERICAN SOCIETIES FOR
EXPERIMENTAL BIOLOGY

1974

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NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) or prior sanctioned food substances being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 72-85 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

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Life Sciences Research Office
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I. INTRODUCTION

This report evaluates the health aspects of using licorice, glycyrrhiza and ammoniated glycyrrhizin as food ingredients. The evaluation has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1970.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally recognized as available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of December 19, 1974 (39 FR 43865 & 43866) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using licorice, glycyrrhiza and ammoniated glycyrrhizin as food ingredients. The Select Committee received no requests for such a hearing on licorice, glycyrrhiza and ammoniated glycyrrhizin.

As indicated in the Food, Drug and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in 21 CFR 121.1, revised April 1, 1974 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accordance with

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*The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconducted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

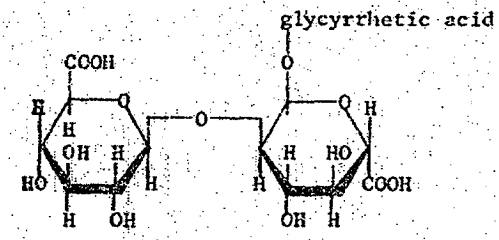
In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on licorice, glycyrrhiza and ammoniated glycyrrhizin and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug and Cosmetic Act.

II. BACKGROUND INFORMATION

Most commercially available licorice (glycyrrhiza) is an extract prepared from the roots and rhizomes of Glycyrrhiza glabra L., a leguminous shrub that grows wild or is cultivated in numerous temperate or semitropical regions of Europe and Asia. At least two varieties of G. glabra are recognized: var. typica (Spanish licorice) and var. glandulifera (Russian licorice). Licorice is known to be present in other species of Glycyrrhiza and other plants (1, 2, 4).

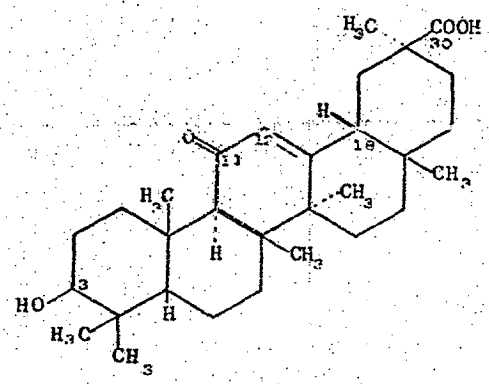
Licorice has long been valued as a remedy and is discussed in some of the earliest medical literature. Even today, licorice is still considered to have useful medicinal properties (3, 4).

An important biologically active constituent of licorice is glycyrshizin ($C_{42}H_{62}O_{16}$), a saponin in which an aglycone, glycyrrhetic acid, is linked to two molecules of glucuronic acid. Alternative names for glycyrrhizin are glycyrrhizic acid, glycyrrhizinic acid, glycyrrhetinic acid glycoside, and β, β'-glucuronido-glucuronide of glycyrrhetic acid (2, 4, 5). The structural formula is:



glycyrrhizin

Glycyrrhetic acid, the aglycone of glycyrrhizin, is a triterpenoid ($C_{30}H_{46}O_4$). Alternative names are glycyrrhetinic acid and 3-β-hydroxy-11-oxoolean-12-en-30-oic acid (2). The structural formula (2) is:



glycyrrhetic acid

A second glycoside, liquiretin, has been reported in licorice root (4). In this substance the aglycone, liquiretigenin (a flavanone) is linked to one glucose unit. The structure proposed is:

197006

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Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
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15. Supplementary Notes

16. Abstracts

This report, by a group of qualified scientists designated the Select Committee on GRAS Substances (SCOGS), provides an independent evaluation of the safety of licorice, glycyrrhiza and ammoniated glycyrrhizin, when used in food at present or projected levels of use.

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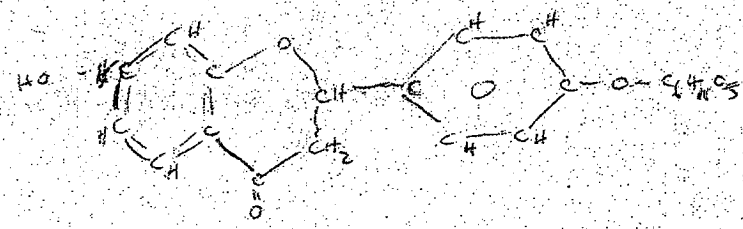

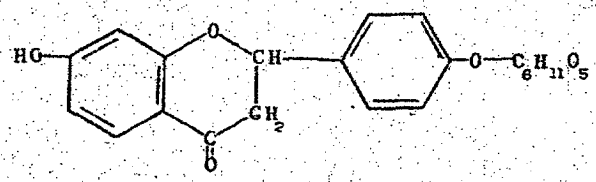

liquiretin

A number of substances are known to be present in licorice (1,4), some of which may be artifacts introduced in processing. Nieman (4) gives the following rough approximations for the content of various classes of constituents in licorice extract:

	Percent
Glycyrrhizin	20
Reducing sugars	5
Nonreducing sugars	5
Starch, dextrins, and gums	30
Ash	8
Moisture	17

As a tribasic acid, glycyrrhizin can form a variety of salts. The literature contains references to mono-, di-, and tri-ammonium glycyrrhizinates (5-8) and the corresponding potassium salts (8-13). Morris and Muller (5) and Mitchell (9) indicate that glycyrrhizin is naturally present in licorice root as calcium and potassium salts.

According to Nieman (4), licorice extract is commercially prepared by macerating the roots of the licorice plant, extracting with hot water and filtering, concentrating the extract to about 20 percent moisture, and pouring the product into molds. On cooling, the extract solidifies to a hard, brownish-black product, "block licorice". The yield of crude extract is 30 to 40 percent of the root. Recently licorice powder, which is macerated block licorice, has become an important commercial product.

The literature refers to "ammoniated glycyrrhizin" as a product of commercial importance. According to Larry et al. (14), this is prepared from a hot water extract of licorice root by sulfuric acid precipitation followed by neutralization with dilute ammonia. Morris and Muller (5)

indicate that the structural formula is the same as triammonium glycyrrhizinate; however, it appears more likely to be a mixture of the mono-, di-, and tri-ammonium salts. Mitchell (9) mentions the isolation of monoammonium glycyrrhizinate from commercial ammoniated glycyrrhizin.

The glycyrrhizin content of licorice varies with the place of origin. Oriental block licorice contains 20 percent or more of glycyrrhizin while Italian and Spanish products contain from 12 to 16 percent of the substance (4).

Ammoniated glycyrrhizin has been important to the food industry because it is 50 times sweeter than sucrose*, and for its foam stabilizing action. Morris and Muller (5) reported that ammoniated glycyrrhizin synergizes the sweetness of sucrose; the same workers found that the flavor of chocolate is potentiated about 35 percent when ammoniated glycyrrhizin is added to the food mix.

No specifications for licorice-related substances have been found in the Food Chemicals Codex. The U.S. Pharmacopoeia XVIII (15) describes glycyrrhiza (dried rhizome and roots of licorice) and a glycyrrhiza extract (a black pilular mass apparently similar to block licorice); glycyrrhiza fluid extract (extract diluted with water and ethanol); and glycyrrhiza syrup (fluid extract with added fennel oil, anise oil, and syrup).

The Food and Drug Administration's GRAS list (16) includes reference to licorice, glycyrrhiza, and ammoniated glycyrrhizin, as being derived from Glycyrrhiza glabra L. and other species of Glycyrrhiza, in the category of spices, seasonings, essential oils, oleoresins, and natural extractives.

The current percentage use of licorice-related substances in foods, according to a 1970 survey by a National Research Council subcommittee (17), is given in Table I.

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*Mitchell (9) and the Merck Index (2) indicate that it is glycyrrhizin itself, rather than the ammonium salt, which has the sweet taste. Klosa (11) states that potassium glycyrrhizinate has a sweet taste. Mitchell states that glycyrrhetic acid, the sapogenin of glycyrrhizin, is not sweet.

Table I

Use of Licorice-Related Substances in Foods

Food category	Licorice root		Licorice extract		Licorice extract powder		Ammoniated glycyrrhizin	
	Usual	Max	Usual	Max	Usual	Max	Usual	Max
	percent		percent		percent		percent	
Beverages Type II (alcoholic)	0.001	0.001	0.139	0.142	0.180	0.200	0.003	0.006
Baked goods, baking mixes	0.011	0.015	0.057	0.063	0.167	0.191	0.006	0.006
Chewing gum	---	---	2.880	2.880	0.582	0.582	0.072	0.228
Sugar confections	---	---	---	---	---	---	0.062	0.062
Frozen dairy desserts, mixes	0.052	0.064	0.047	0.055	0.020	0.040	0.005	0.009
Gelatins, puddings, fillings	---	---	0.019	0.021	---	---	0.005	0.008
Hard candy	24.400	24.400	0.446	8.020	0.401	0.535	0.040	0.068
Meat products	0.210	0.240	0.060	0.080	0.220	0.250	---	---
Beverages Type I (nonalcoholic)	0.018	0.020	0.017	0.020	0.002	0.004	0.002	0.005
Soft candy	0.186	0.204	1.214	2.509	1.223	3.279	0.076	0.151
Sweet sauces, toppings, syrups	---	---	0.001	0.002	---	---	---	---

The Select Committee has no specific information on the year that licorice-related substances were first used in foods in the United States; however, they have been used in foods as flavoring agents since the eighteenth century (4). The Census Bureau statistics (18) on U.S. imports of licorice root and licorice extract for all purposes (including tobacco and pharmaceuticals as well as foods) for the years 1965-1972 inclusive do not provide any information on licorice used only in foods. The Committee has no information on changes in use of licorice-related substances in foods, either by the categories shown in Table I, or in total usage during this period.

III. CONSUMER EXPOSURE DATA

The National Research Council subcommittee (17) has provided information on the possible daily human intake of licorice-related substances in the total diet, as shown in Table II for individuals in various age groups. The Select Committee has converted these figures to possible intakes per kilogram of body weight.

It is recognized that the figures calculated for the daily intake of licorice-related substances per kilogram of body weight in the age group 2 to 65+ years could be deceptively low, since most children from age 2 to maturity will weigh less than 60 kg. Accordingly, the daily load of licorice-related substances for children weighing about 20 kg could be three times higher than the figures indicated.

Certain other considerations have bearing on the reasonableness of the figures in Table II. The NRC subcommittee has pointed out that its calculations of intakes in most cases are overstated, often by considerable margins. That daily human intakes are overstated for licorice-related substances is borne out by the following calculations.

Other data supplied by the NRC subcommittee indicate the quantity of licorice-related substances used for food purposes in the United States in 1970 (17). These NRC figures, compiled from industry reports, are

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An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (17). The Select Committee finds this explanation reasonable and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey on the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."

Table II

Possible Daily Intake of Licorice-Related Substances

Total intake, mg									Intake, mg per kg body weight*							
0-5 mos.		6-11 mos.		12-23 mos.		2-65+ yrs.		AGE	0-5 mos.		6-11 mos.		12-23 mos.		2-65+ yrs.	
Av	Max	Av	Max	Av	Max	Av	Max	SUBSTANCE	Av	Max	Av	Max	Av	Max	Av	Max
4.01	13.09	43.80	236.36	166.38	402.37	369.44	826.00	Licorice root	0.80	2.62	10.48	29.54	15.12	36.58	6.16	13.77
6.29	31.64	67.72	182.98	114.53	258.12	283.06	644.92	Licorice extract	1.26	6.33	8.46	22.87	10.41	23.46	4.73	10.75
10.79	39.25	118.22	301.42	206.09	394.07	541.86	1039.41	Licorice extract powder	2.16	7.85	14.78	37.68	18.74	35.82	9.03	17.32
0.57	2.30	5.10	13.92	9.09	21.03	19.18	42.61	Ammoniated glycyrrhizin	0.11	0.46	0.64	1.74	0.83	1.91	0.32	0.71

*Calculations based on an average weight of 60 kg for an adult (19) and the following estimated weights of infants by age groups: 0-5 mos., 5 kg; 6-11 mos., 8 kg; and 12-23 mos., 11 kg (20).

assumed to comprise between 60 and 70 percent of the total quantity used annually in food. Assuming 60 percent recalculated to 100 percent and a U.S. population of 210 million, the per capita per day average intake of licorice-related substances can be estimated as shown in Table III and compared to the figures in Table II. Had sufficient data been available, it would have been preferable to adjust the figures in both Table II and Table III in terms of the glycyrrhizin content of the four licorice-related substances.

TABLE III

Consumption of Licorice-related Substances Based on Total Quantity Used Annually in the United States

Substance	Total 1970 usage in foods		Estimated individual usage[3]
	NRC data[1]	Adjusted data[2]	
	pounds	kg	mg per capita per day
Licorice root	9,871	7,478	0.098
Licorice extract	44,572	33,767	0.441
Licorice extract powder	180,735	136,920	1.786
Ammoniated glycyrrhizin	11,242	8,517	0.111

[1] From NRC Table 11C (17); figures assumed by NRC to be 60 percent of actual.

[2] NRC figures adjusted to 100 percent and converted to kg.

[3] Based on a U.S. population of 210 million.

An additional check on the validity of the consumption figures in Table II is provided by Census Bureau statistics (18), which show that for the years 1965 to 1972 inclusive, average annual imports of licorice root and licorice extract were 45,851,876 pounds (20,841,762 kg) and 1,028,533 pounds (467,515 kg), respectively. Nieman (4) estimates that in the United States, 90 percent of the total licorice production is used in tobacco products. Taking 10 percent of the foregoing figures, making no allowance for the use of licorice in pharmaceuticals and other nonfood products, and assuming a U.S. population of 210 million, the quantity of licorice root available for consumption is 27.19 mg per person per day, and 0.61 mg per person per day of licorice extract. This

figure for licorice root is substantially lower than the corresponding figure in Table II and the figure for licorice extract is also substantially lower than the combined figures for licorice extract and licorice extract powder in Table II. However, the 27.19 mg per day intake of licorice root is substantially higher than the corresponding value in Table III and the 0.61 figure for licorice extract is somewhat lower than the combined figures for licorice extract and licorice extract powder in Table III. Thus, it seems apparent that the data in Table II represent consumption levels that are not likely to be achieved by any of the age groups. The Select Committee believes the intake values calculated from the quantities of licorice imported (about 27 mg of licorice root and about 0.61 mg of licorice extract, per capita per day) represent the more realistic values.

The Joint FAO/WHO Expert Committee on Food Additives has not reported any consideration of an acceptable daily intake level for licorice-related substances.

IV. BIOLOGICAL STUDIES

Absorption, metabolism, and excretion

On oral administration of tritium-labelled monoammonium glycyrrhizinate to human subjects, Carlat et al. (6) determined that the substance was only slightly absorbed from the gastrointestinal tract, and was mainly hydrolyzed to form glycyrrhetic acid, which was excreted unchanged in the feces. Oral administration of labelled glycyrrhetic acid produced essentially the same results. However, when tritium-labelled β-glycyrrhetic acid was administered intraperitoneally (25 mg per kg of body weight) to male and female albino rats, an average of 100 percent of the label was absorbed and then excreted within 12 hours through the bile into the feces (21). The rate of excretion was slower when the substance was orally administered at a level of 60 mg per kg; an average of 83 percent of the label was excreted in the feces and one percent in the urine in one to three days. The bile contained three unidentified metabolites of glycyrrhetic acid. Parke et al. (21) suggested that Carlat et al. (6) might have made similar observations had they collected bile over a longer period than 4 hours.

Oral administration of ammoniated glycyrrhizin (about 7 g per kg), monoammonium glycyrrhizinate (about 2 g per kg), and glycyrrhetic acid (about 1.5 g per kg), to bilaterally adrenalectomized rats, significantly decreased sodium output and caused retention of urine (24). The first

two compounds had little or no effect on potassium output, but glycyrrhetic acid increased potassium retention. When given by any route to male albino rats, glycyrrhetic acid exhibits a strong antidiuretic effect and, when given orally (about 500 mg per kg), delays water absorption from the alimentary tract (23). Cats and rats administered glycyrrhetic acid intraperitoneally (200 mg and 125 mg per kg, respectively), exhibited a marked antidiuretic action; however, there was an increase in urinary potassium excretion (24). Following oral administration of as much as 1.5 g of glycyrrhetic acid per kg of body weight to male albino rats daily for 8 days, Linko and Vasama (25) noted an increase in excretion of potassium, while the body weight of the rats increased.

In vitro experiments by Whitehouse et al. (26) have shown that glycyrrhetic acid is a potent uncoupler of oxidative phosphorylation in rat liver mitochondria. Kraus (27) reported that when rats received 0.4 percent ammoniated glycyrrhizin in drinking water (about 500 mg per kg per day) for a week, their ability to mobilize glucose was decreased. The ability of mice, receiving drinking water containing 0.4 percent ammoniated glycyrrhizin (about 800 mg per kg per day), to withstand cold temperatures was decreased. These results led the investigator to suggest that glycyrrhizin decreases the output of ACTH. Evdokimova and Kamilov (10) found that potassium glycyrrhizinate (15 mg per kg daily), "injected internally" for two months, decreased experimental atherosclerosis in rabbits by decreasing the amount of cholesterol in the blood and reducing the cholesterol-lecithinic coefficient.

Gujral et al. (28) found that oral glycyrrhizin (100 to 160 mg per kg per day) exhibits antiarthritic and anti-inflammatory effects in adrenalectomised rats with Brownlee's formaldehyde-induced arthritis. Elmadjian et al. (29) found that monoammonium glycyrrhizinate and hydrocortisone have synergistic effects in the adrenalectomized patient. Sasano et al. (30) reported that simultaneous intravenous administration to rats of glycyrrhizin with dexamethasone inhibits the dexamethasone-induced atrophy of the adrenals, indicating adrenocortical stimulation by the glycyrrhizin. Asanuma (31) found that glycyrrhizin can either suppress or intensify the action of cortisone in adrenalectomized rats, depending on the immediate conditions, and can suppress the inhibitory action of dexamethasone on the pituitary.

Van Katwijk et al. (32) fed glycyrrhetic acid to two human subjects (one with Addison's disease and one with a jejunal ulcer) in amounts up to 2.5 g per day for unspecified periods. The urine of these patients showed no traces of glycyrrhetic acid. No data on fecal excretion were reported. However, the investigators isolated an apparent metabolite of the acid in the urine which was unidentified except for its red color and absorption maximum (555-560 mμ) when treated with sulfuric acid.

Acute and Short-term Toxicity Studies

The LD_{50} of various glycyrrhizin salts administered to mice has been determined by Fujimura (7,8), and Klosa (11), with results as shown in Table IV.

Table IV

Acute Toxicity of Glycyrrhizin Salts in Mice

Route	Glycyrrhizin salt	LD_{50}	Reference
		mg per kg	
Oral	ammonium (crude)	12,700	7
	diammonium	9,600	8
	potassium (crude)	12,400	7
	monopotassium	1,220	11
	dipotassium	8,100	8
Intravenous	monopotassium	412	11
Intraperitoneal	ammonium (crude)	1,050	7
	monoammonium	1,070	7
	diammonium	1,250	8
	potassium (crude)	1,260	7
	dipotassium	1,400	8
Intramuscular	monopotassium	695	11
Subcutaneous	monopotassium	697	11

Finney (24) using albino mice of both sexes, reported an intraperitoneal LD_{50} of 308 mg per kg for glycyrrhetic acid. Upon oral or subcutaneous administration, no deaths occurred with single doses as high as 610 mg per kg.

Tocco (33) observed that when pigeons received subcutaneous doses of glycyrrhizin of from 450 to 500 mg per kg of body weight, they became diarrhetic within an hour, and showed depression lasting about 24 hours. Guinea pigs receiving glycyrrhizin subcutaneously in doses of 1,000 mg per kg rapidly became depressed and diarrhetic, showed decreased urinary volume, and died within 24 hours. In dogs, intravenous doses of glycyrrhizin of about 500 mg per kg were fatal. The same dose given subcutaneously produced only a slight depression for up to 3 hours; by the oral route, this dose produced almost no adverse reaction.

Over a 50-day period, Girerd et al. (34) gave oral doses, to male Sprague-Dawley rats, of (a) 10 g of licorice per kg per day and (b) 1 g of ammoniated glycyrrhizin per kg per day. The experimental animals showed a progressive increase in blood pressure to about 190 mm as compared to 125 mm for a control group. They also showed a significant depression of growth, which was greater in the licorice-treated rats than in the animals fed ammoniated glycyrrhizin. Both absolute and relative weight increases were noted in kidneys, adrenals, and hearts of treated animals, and weight losses in hypophyses and testes. Severe renal and cardiovascular lesions were found in the licorice-treated rats; milder lesions were noted in the ammoniated glycyrrhizin group. The survival rate, after 50 days, was 36 percent for licorice-treated rats and 77 percent for those receiving ammoniated glycyrrhizin, as compared to 100 percent for controls.

Macabies et al. (35) administered glycyrrhizin orally to rats, at a level of 160 mg per rat per day, on the following schedule: 70 days of treatment; 50 days without treatment; another 35 days of treatment; and a final 20 days without treatment. There was no effect on weight, but a 25 percent increase in blood pressure during glycyrrhizin administration was observed; blood pressure returned to normal when the treatment was discontinued. In another study, the same workers (12) determined the hypertensive action of several licorice-related substances administered as shown below to male Wistar rats over a period of 10 to 25 days:

Route	Substance	Daily dose (mg per kg)
Intraperitoneal	Ammoniated glycyrrhizin	150 and 300
Intraperitoneal	Tripotassium glycyrrhizinate	150 and 300
Subcutaneous	Ammoniated glycyrrhizin	300
Oral	"Deglycyrrhizinated" licorice extract*	800
(Route unstated)	Glycyrrhetic acid (α and β isomers)	300

*Extract containing 3 to 4 percent glycyrrhizin, as compared to 20 to 25 percent in the original extract.

All of the glycyrrhizin salts increased the blood pressure which returned to normal when the treatment was ended. The glycyrrhetic acid isomers also had a strong hypertensive effect, but the duration of action was shorter; the beta isomer particularly appeared to be more effective in this respect than the salts. The "deglycyrrhizinated" licorice extract had only a very weak hypertensive action.

An extensive study of the effect of ammonium glycyrrhizinate on blood pressure, electrolytes, and corticosterone was conducted by Gordon (36). Dosing with technical ammonium glycerrhizinate at 1000 and 2000 mg per kg per day produced significant increases in the blood pressure of Sprague-Dawley rats within 2 to 3 weeks, but not in Osborne-Mendel rats over a 20 week period. There was a decrease in plasma corticosterone and increased kidney and heart weights at the 1000 mg per kg level. However, when the compound was fed at 4 percent of the diet (2000 mg per kg per day) for 5 weeks, plasma corticosterone, blood pressure, and organ weights all were increased.

Fujimura and Okamoto (8) fed diammonium and dipotassium glycyrrhizinates at dietary levels of 0.1 (approximately 100 mg per kg per day) and 0.5 percent to rats for 90 days. At the higher level the male animals showed a slower rate of weight gain than did the controls; at autopsy, no gross or histological abnormalities were noted in the organs. Klosa (11) observed no untoward effects when rats were given potassium glycyrrhizinate (route unstated) at a level of 60 mg per kg per day for 8 months.

Long-term Toxicity Studies

No reports of long-term studies on licorice-related substances have been found.

Special Studies

Tests of the teratogenicity of ammonium glycyrrhizinate have been conducted on laboratory animals that were given daily doses, by oral intubation, of up to 1000 mg per kg of the test substance, under the following schedule (37):

> 109 albino CD-1 outbred mice. Dosed for 10 days (6th through 15th day of gestation). Caesarian section performed on 17th day.
>
> 106 rats of Wistar-derived stock. Dosed for 10 days (6th through 15th day of gestation). Caesarian section performed on 20th day.

111 golden hamsters. Dosed for 5 days (6th through 10th day of gestation). Caesarian section performed on 15th day.

53 Dutch-belted rabbits. Dosed for 13 days (6th through 18th day of gestation). Caesarian section performed on 29th day.

It was concluded that the indicated dosages of ammonium glycyrrhizinate had no teratological effect and did not unfavorably influence maternal or fetal survival.

Mutagenicity screening studies have been conducted on ammoniated glycyrrhizin (38). It was found to be non-mutagenic in rats in the dominant lethal assay at oral doses up to 5000 mg per kg. It produced no detectable aberrations in rat bone marrow metaphase chromosomes when administered orally in doses up to 5000 mg per kg. It produced no significant aberrations in the anaphase chromosomes of human embryonic lung cells in tissue culture when tested at levels up to 1000 μg per ml. Results in the host-mediated assay in mice at oral levels of ammoniated glycyrrhizin up to 5000 mg per kg and using two Salmonella strains and one Saccharomyces strain were generally negative. Dose levels in all of these mutagenic studies greatly exceed estimated current dietary consumption levels.

The Select Committee is not aware of any studies on the possible carcinogenic properties of the glycyrrhizinates.

Consumption of large amounts of candy or beverages containing licorice has caused untoward effects in human subjects. An adult male developed shortness of breath, ankle edema, headache, weakness, elevated blood pressure and "apparent hypokalemia" attributed to eating 700 g of licorice candy within 9 days (39). The symptoms disappeared when he stopped eating the candy. Another adult male consumed a 35 g licorice bar every day for about 6 months and developed a hypertension with "unpleasant cardiac sensations" (40). After two weeks on a salt-free diet, bed rest, mild sedation and discontinuance of the candy he returned to normal. A 19-year-old girl developed a chronic edema of the legs and ankles and an elevated blood pressure after prolonged eating of large amounts of licorice candy (41). Abstinence from licorice caused the symptoms to disappear.

Five persons (age unstated) experienced intoxication after drinking an unknown amount of "antesite," an alcoholic drink flavored with licorice extract (42). The chief symptoms were hypertension and polydipsia. One subject was found to have hypokalemia. Recovery followed when the antesite was no longer consumed. Two men over 50 years old who were chronic users of licorice were treated by Potton et al. (43) because they developed arterial hypertension and severe neuromuscular symptoms with

episodes of hypokalemia and hypernatremia. In both cases there was a total regression of symptoms within 20 days after abstinence from licorice was instituted.

Three planned studies on feeding licorice-related substances to human subjects have been reported. Molhuysen (44) administered daily, to ten persons for periods up to 3 weeks, 20 to 45 g of licorice extract. Hemoglobin and total serum protein decreased, and venous pressure, blood pressure, and pulse pressure rose considerably. Louis and Conn (45) fed ammonium glycyrrhizinate to 10 persons, up to 6 g per day for 3 days or 4 g per day for 5 to 10 days. They noted a significant decrease in 17-ketosteroids, indicating inhibition of the pituitary-adrenal system, and a decrease in release of MSH (melanocyte-stimulating hormone) from the pituitary. Card et al. (46) fed block juice (dried licorice extract) to two adult males for two periods of four days each, at levels of 20 and 36 g daily. They noted a gain in body weight and a slight rise of systolic and diastolic blood pressures.

Nishiyama (47) has reported on the use of licorice substances in the treatment of ulcers. He found that favorable results were evident in 44 patients with peptic ulcers. The administration of licorice extract to rats rendered ulcer-prone by ligation of the pylorus (Shay rats) (48) was found to check the growth of ulcers, while crude glycyrrhizin was without effect.

Revers (49) investigated the effectiveness of licorice extract in treating gastric ulcers in 45 patients. Three times daily, patients were treated with one teaspoon per person of a preparation consisting of 100 g of powdered extract and 50 g of water; progress was checked with x-rays every two or three weeks. In nearly two-thirds of the cases, the ulcers disappeared. This treatment of duodenal ulcers with licorice extract was not as effective as it was in the treatment of gastric ulcers. Edema occurred in some patients but ceased to occur when dosage was lowered or discontinued.

V. OPINION

Orally administered licorice and licorice derivatives are absorbed to some extent and the principal metabolic products are excreted through the bile, but most of an ingested dose is hydrolyzed in the digestive tract and the products excreted through the feces. Acute and short-term animal studies on licorice and licorice derivatives reveal that they are substances of a very low order of toxicity, capable of eliciting a variety of pharmacological effects but only at levels considerably higher than

are likely to be achieved in usual diets. None of these effects suggests cause for concern at current or foreseeable dietary levels of consumption. However, the capacity of licorice and licorice derivatives to elicit transitory hypertensive effects, at higher dosage levels in animals and man, requires more definitive clarification as far as its practical implications are concerned. This would be particularly important for the unknown number but probably few individuals who may indulge themselves with excessive intakes of licorice-containing candies and/or beverages. The Select Committee has found no long-term toxicological data on licorice-related products administered to animals or man. Until the long-term as well as the acute dose relationships of the hypertensive effect are clarified, it appears inappropriate to conclude that unrestricted use of licorice and licorice derivatives in food would be without hazard to consumers in general.

In the light of the foregoing and the information elsewhere in this report, the Select Committee concludes that:

> There is no evidence in the available information on licorice, glycyrrhiza, and ammoniated glycyrrhizin that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

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