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(54) BILE ACID BINDING COMPOSITION

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, of 345 Park Avenue, New York, State of New York, 10022, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:-

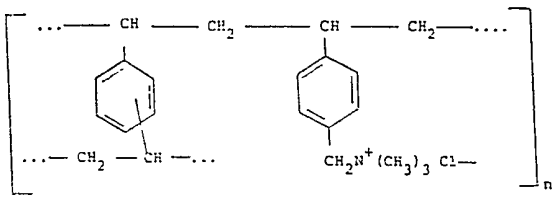
The present invention relates to a composition for binding bile acids in the digestive tract of a human having need thereof into an unabsorbable, excretable form.

The present invention provides a bile acid binding composition in unit dosage form effective upon oral administration for the purpose of increasing bile acid excretion which comprises from 1.2 to 6 g. of calcium carbonate and from 6 to 20 g. of a polymeric, water-insoluble, non-toxic styrene-divinylbenzene quaternary ammonium anion exchange resin having a molecular weight in excess of 3,000, a polymer skeleton inert to digestive enzymes, and a water content greater than 65 wt% after equilibration with air at 100% relative humidity.

The composition of the invention comprises an orally administrable mixture comprising a polymeric, water-insoluble, non-toxic, styrene-divinylbenzene quaternary ammonium anion exchange resin and calcium carbonate. Resins suitable for the bile acid binding composition of the present invention are preferably those styrene-divinylbenzene polymers containing from 1-4% but not more than 5% divinylbenzene described in U.S. Patent No. 3,383,281. These resins, when used in combination with calcium carbonate, produce entirely unexpected increases in bile acid excretion compared to that achieved by the administration of either ingredient separately.

Particularly preferred compositions of the present invention comprise a mixture of from 6 to 20 g. of cholestyramine and from 1.2 to 6 g. of calcium carbonate. Another preferred composition of the present invention comprises 12 g. of cholestyramine and 5.6 g. of calcium carbonate. A still further preferred composition of the present invention comprises 8 g. of cholestyramine beadlets and 2 g. of calcium carbonate.

Cholestyramine, a member of the aforementioned class of water-insoluble, non-toxic, strongly basic anion exchange resins containing quaternary ammonium functional groups attached to a styrene-divinylbenzene copolymer, is a product of commerce available from the Dow Chemical Company, Midland, Michigan, as Dowex (Registered Trade Mark) 1 x 2 and from Rohm and Haas, Philadelphia, Pennsylvania, as Amberlite (Registered Trade Mark) XE-268. The main polymer groups of cholestyramine are represented schematically by the following formula:



In the above formula, the degree of polymerization is represented by the index "n". Because of three-dimensional cross-linking, a precise numerical value cannot be given. However, it is to be understood that the index "n" is of sufficient value to provide a molecular

weight greater than 3,000. These quaternary ammonium resins are useful in the form of non-toxic salts, such as chlorides, sulfates, acetates, or phosphates, or in the hydroxyl form. Preferred resins of the composition of the present invention are the "Dowex 1" series, as for example "Dowex 1x1, Dowex 1x2, and Dowex 1x4" which contain 1%, 2% and 4% divinylbenzene, respectively.

Two discrete forms of cholestyramine may be employed in the cholestyramine-calcium carbonate compositions of the instant invention. Those forms are pharmaceutical grade cholestyramine and cholestyramine beadlets. As used herein, the term pharmaceutical grade cholestyramine refers to the water-insoluble styrene-divinylbenzene quaternary ammonium anion exchange resin employed in Questran. This anionic resin is a relatively fine powder having a particle size characterized according to G. R. Jansen, et al., J. Pharm. Sci.*54* 833-837 (1965) in that not less than 100% of the particles pass through No. 100 mesh screen and not less than 80% of the particles pass through a No. 200 mesh screen. The foregoing specifications establish maximum possible particle size of pharmaceutical grade cholestyramine but are indefinite with respect to a minimum particle size. As determined by the Coulter Automatic Particle Size Counter (Coulter Counter is a Registered Trade Mark), particle size distribution of commercially available cholestyramine found in Questran ranges from 15-75 microns with 96% of the particles having a diameter ranging from 20 to 60 microns. It is to be understood that as used herein, the term "cholestyramine beadlets" refer to small spherical beads in contrast to "pharmaceutical grade cholestyramine" which is characterized by irregular shaped jagged particles. For the present composition, cholestyramine beadlets having a diameter of from 150 to 900 microns are suitable, with a diameter of 300 to 850 microns preferred.

The effective non-toxic resin/calcium carbonate compositions of the present invention can be orally administered with or without pharmaceutical carriers. If desired, the compositions can be orally administered in conventional pharmaceutical formulations such as tablets, elixirs, syrups or suspensions prepared in accordance with standard pharmaceutical methods. The resins are also suitable for incorporation in orally ingested carriers such as fruit juice, apple sauce, puddings, cereals, desserts and conventional foodstuffs.

In man, the daily dosage usually is preferably given in divided form prior to meal time but also may be administered once or twice a day if desired. The dosage may be varied by the physician depending upon the weight of the subject, the nature of the patient's condition, and the intensity of the effect desired. For instance, a suitable regimen for aggressive therapy comprises the administration of a composition comprised of 20 g. of cholestyramine and 6 g. of calcium carbonate.

The admixture of from 6 to 20 g. of the aforementioned non-toxic anionic exchange resins with from 1.2 to 6 g. of calcium carbonate has a pronounced synergistic effect with respect to sequestering bile acid in the digestive tract thereby increasing fecal bile acid excretion substantially above the additive effect of the individual components. Standard *in vivo* feeding tests with laboratory animals such as rats or human subjects may be employed in demonstrating the effectiveness of the resin-calcium carbonate compositions of the present invention. One suitable laboratory test involved feeding rats cholestyramine at a constant 0.5% of the diet with varied levels of calcium carbonate based on recommended daily allowances established by the National Research Council. Feces are collected and fecal exretion patterns relative to bile acid excretion determined by standard fecal bile acid assay.

The figure is a dose-response graph of one such study wherein a mixture of calcium carbonate with cholestyramine beadlets or pharmaceutical grade cholestyramine was employed.

Values for bile acid excretion are given in micro moles per 100 g. body weight per day. Calcium levels refer to the percent of the National Research Council recommended daily allowance (NRC-RDA). Calcium carbonate alone is represented by circles (-O-), cholestyramine beadlets alone and in combination with various amounts of calcium carbonate is represented by triangles (-△-) and pharmaceutical grade cholestyramine alone and in combination with various amounts of calcium carbonate is represented by the letter "X". The results are given in the accompanying Figure of the drawings.

It is evident from the Figure that without resin in the diet, added levels of calcium did not significantly alter bile acid excretion whereas, on an essentially calcium-free diet (0% NRC-RDA), addition of resin increased bile acid excretion 3- to 4-fold over controls and 4- to 5-fold at the normal calcium level (100% NRC-RDA). It is further evident that additional calcium above the 100% NRC-RDA level significantly increases bile acid excretion with the cholestyramine beadlet and that beadlets of cholestyramine-calcium carbonate at the 200 and 400% NRC-RDA calcium level increases fecal bile acid excretion by some 40 and 60% respectively over that obtained with the cholestyramine pharmaceutical grade, indicating that the calcium carbonate-cholestyramine beadlet composition is more effective as a bile acid sequestrant than the pharmaceutical grade cholestyramine-calcium carbonate composition.

The following preferred specific embodiments are to be construed as merely illustrative and not limiting the invention in any way whatsoever.

EXAMPLE 1

Fecal Bile Acid Excretion - Human

The effectiveness of the non-toxic anionic resin/calcium carbonate compositions of the invention may be demonstrated by standard clinical pharmacology studies in which the subject is used as the control. In one such study following a 12-day base line control determination, pharmaceutical grade cholestyramine and/or calcium carbonate was administered to a male subject prior to mealtime in three equal portions of the daily dose as set forth in Table I.

TABLE I

Test Protocol

Test Agent[a]	Days Administered	Days Feces Collected
Cholestyramine	1-8	4-9
Cholestyramine plus $CaCO_3$	9-16	10-17
$CaCO_3$	17-25	20-25

[a]cholestyramine daily dose = 12 g. ($CaCO_3$ daily dose equivalent to 2.25g calcium.)

Feces were collected according to the above schedule, dried at 50°C. for 16 hours and then ground to pass through a No. 20 mesh screen. One-Half gram aliquots of the dry, ground feces were placed into 16 x 150 mm. screw-cap test tubes and 10 ml. of 0.5 N HCl in 75% *tert.*-butyl alcohol was added to each sample and the sample tube vigorously agitated on a vortex mixer for 10 sec. at 5 min. intervals for 1 hour at room temperature. The extract was then clarified by centrifugation at 100 G for 10 min. to provide 0.2 ml. aliquots of supernatent fluid anlayzed for bile acids according to the following methodology. The 0.2 ml. aliquots were transferred to test tubes labeled T and E. Appropriate standard and blank tubes were also labeled T and E. To each tube was added 4.0 ml. of a reagent mixture consisting of equal parts of 0.1 M sodium pyrophosphate buffer adjusted to pH 9.5 with 1.0 N HCl and 1.0 M hydrazine hydrate adjusted to pH 9.5 with 2.0 N H_2SO_4. The contents of each tube were mixed with 0.5 ml. of 5.0 mM nicotinamide-adeninedinucleotide (NAD) adjusted to pH 7.0 with saturated $NaHCO_3$ and contents again mixed. One-half ml. of 0.03 M Tris-(hydroxymethyl)amino-methane buffer, pH 7.2 in 1.0 mM (ethylenedinitrilo)tetraacetic acid disodium salt (EDTA), was added to the filtrate tubes labeled T, aqueous cholic acid standards in 0.5 N HCl in 75% *tert.*-butyl alcohol ranging from 0.625 to 5.0 mM and the reagent blanks also labeled T. To the filtrate tubes labeled E (standards and blanks) was added 0.5 ml. Tris-(hydroxymethyl)aminomethane buffer containing 1.0 mg. 3-α-hydroxysteroid: NAD phosphate oxidoreductase (EC 1.1.1.51) cell-free powder (minimum 0.5 units per mg.) per ml. After mixing, the tubes remained at room temperature for 60 min. at which time the optical density (OD) was determined at 340 nm with a Hitachi (Registered Trade Mark) Perkin-Elmer 139 spectrophotometer equipped with a flow cell of 0.5 cm. light path. The quantity of bile salt in the filtrate was calculated using the differences in optical density (ΔOD) between the pairs of unknowns, standards, and blanks. The blank ΔOD was subtracted from the ΔOD of the filtrate and from the ΔOD of the standard.

Results are summarized in Table II.

TABLE II

Fecal Bile Acid Excretion (FBA) - Man

	Mean FBA μ mole/day	*FBA Increase* over control
Control	451	
$CaCO_3$	522	71[a]
Cholestyramine	2754	2302[a]
Cholestyramine plus $CaCO_3$	3626	3175[b]

[a]The sum of these values (2374) is the additive effect of the components.
[b]This observed value less the additive effect (2374) is the synergistic effect (801).

The foregoing results clearly establish that a mixture of cholestyramine and calcium carbonate induces a synergistic fecal bile acid excretion value of some 34% (801/2374) greater than the expected additive effect of 2374 μ mole/day. This increase of 34% in fecal bile acid excretion constitutes a synergistic effect which is entirely unexpected.

EXAMPLE 2

Fecal Bile Acid Excretion - Rat

Male weanling rats were individually housed and fed a standard basal diet containing calcium at 100% of the National Research Council-recommended daily allowance (NRC-RDA) for a 10-day pre-test period. Groups of 10 rats were then selected on the basis of body weight and weight gain and fed test diets for 14 days containing graded levels of calcium carbonate and/or cholestyramine (pharmaceutical grade) or cholestyramine beadlet at 0.5% of diet as shown in Table III. Feces were collected during the last 4 days of the test period and analyzed for bile acids by hydroxysteroid dehydrogenase methodology of Example 1 with the results shown in Table III.

TABLE III

Fecal Bile Acid Excretion - Rat

Treatment	*Calcium Level % of NRC-RDA*	*Bile Acid, micro moles /100 g. body weight/day*
Control		
Group 1	0	3.5 ± 0.9
Group 2	100	5.2 ± 1.7
Group 3	200	6.3 ± 2.8
Group 4	400	5.9 ± 2.9
Cholestyramine (0.5% of diet) (pharmaceutical grade)		
Group 5	0	11.5 ± 4.6
Group 6	100	18.1 ± 3.2
Group 7	200	17.1 ± 5.1
Group 8	400	18.2 ± 5.0
Cholestyramine Beadlet (0.5% of diet)		
Group 9	0	13.0 ± 4.7
Group 10	100	20.3 ± 4.2
Group 11	200	25.0 ± 3.4
Group 12	400	29.3 ± 5.3

It will be seen from Table III that increasing calcium levels 2-4 times that of NRC-recommended daily allowance (i.e., 100% NRC-RDA) did not appreciably increase bile acid excretion whereas calcium carbonate administered in combination with cholestyramine beadlets (as 0.5% of diet) substantially increased fecal bile acid excretion. It is also clearly evident that the increase in fecal bile acid excretion provided by the admixture of cholestyramine beadlet and calcium carbonate is not merely the result of additive effects of each component but rather constitutes true synergistic activity. For instance, the cholestyramine beadlet in combination with calcium carbonate at 400% of the NRC-RDA level provided fecal bile acid excretion of 29.3 micro mole/100 g. body weight per day. This value represents an increase of about 40% above the calculated additive effects (20.3 + 0.7) of cholestyramine with calcium carbonate at 100% of the NCR-RDA level and further illustrates the synergistic advantage of the instant process.

EXAMPLE 3

Tablet

Ingredient	*Amount*
Cholestyramine (pharmaceutical grade)	819.0 mg.[a]
Calcium carbonate	250.0 mg.
Povidone	12.5 mg.
Carbopol (Registered Trade Mark) 934	30.0 mg.
Talc	22.0 mg.

EXAMPLE 3 (Cont'd)

Magnesium stearate	10.5 mg.
Hydroxypropyl methylcellulose	10.0 mg.
Ethylcellulose	4.0 mg.
Opaspoxy yellow	1.0 mg.
Carnauba wax[b]	
White wax[b]	

[a]To give 750 mg. anhydrous basis.

[b]Amount retained during polishing is negligible.

Weigh and transfer proportional amounts of the cholestyramine, calcium carbonate, and Carbopol 934 into a suitable mixer and blend until homogeneous. Granulate using distilled water. Dry the wet granulation.

Pass the granulation through a comminutor and blend this in a suitable mixer with the talc and magnesium stearate. Compress the granulation into tablets to provide 750 mg. cholestyramine/250 mg. calcium carbonate. Coat and polish the tablets using hydroxypropyl methylcellulose, ethylcellulose, color, white wax and carnauba wax.

EXAMPLE 4

Powder Composition

Ingredient	*Amount*
Cholestyramine (Pharmaceutical grade)	819.0 mg.
Calcium carbonate	250.0 mg.
Acacia powder	140.0 mg.
Kelcoloid (Registered Trade Mark) (low viscosity)	42.0 mg.
Flavor	69.0 mg.

Blend proportional amounts of the above ingredients and seal in moisture proof packaging, e.g., sealed metal can. Add the required dose by volumetric measure (calibrated scoop) to a glass (6 oz.) of water or fruit juice, stir to disperse and administer.

WHAT WE CLAIM IS:-

1. A bile acid binding composition in unit dosage form effective upon oral administration for the purpose of increasing bile acid excretion which comprises from 1.2 to 6 g. of calcium carbonate and from 6 to 20 g. of a polymeric, water-insoluble, non-toxic styrene-divinylbenzene quaternary ammonium anion exchange resin having a molecular weight in excess of 3,000, a polymer skeleton inert to digestive enzymes, and a water content greater than 65 wt% after equilibration with air at 100% relative humidity.
2. A composition as claimed in claim 1 wherein the polymer is a quaternary ammonium anion exchange resin with less than 5% cross-linkage.
3. A composition as claimed in claim 2 wherein the polymer is a quaternary ammonium anion exchange resin with 2% cross-linkage.
4. A composition as claimed in any one of the preceding claims wherein the polymer is cholestyramine.
5. A composition as claimed in claim 4 wherein said polymer is pharmaceutical grade cholestyramine.
6. A composition as claimed in claim 4 wherein said polymer is cholestyramine in beadlet form.
7. A composition as claimed in claim 6 wherein the beadlets have a diameter in the range of from 150 to 900 microns.
8. A composition as claimed in claim 7 wherein the beadlets have a diameter in the range of from 300 to 850 microns.
9. A composition as claimed in any one of the preceding claims which comprises 12 g. of cholestyramine and 5.6 g. of calcium carbonate.
10. A composition as claimed in any one of claims 1 to 4 or 5 to 8 which comprises 8 g. of cholestyramine in beadlet form and 2 g. of calcium carbonate.
11. A composition as claimed in any one of the preceding claims which includes therein a pharmaceutically acceptable carrier.
12. A composition as claimed in any one of the preceding claims which is in the form of tablets, an elixir, a syrup or a suspension.
13. A composition as claimed in claim 1 substantially as hereinbefore described with reference to the foregoing Examples.

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