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CONTROLLING THE BLOOD CHOLESTEROL LEVEL BY ADMINISTRATION OF DIHODO-TYROSINE POLYPEPTIDE

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This invention relates to a method and means for reducing and controlling the blood cholesterol level, and it more particularly relates to a method and means for stimulating the reticulo-endothelial system.

Hypercholesteremia has come to be generally associated with certain diseases, particularly with atherosclerosis and the aging effects in the body.

It has been known for some time that the liver and thyroid gland are, in some manner, associated with the cholesterol metabolism of the body. It has now been discovered that the liver removes the excess of cholesterol from the circulation and the thyroid gland controls the excretory function of the liver. The excess of cholesterol in the blood stream, after being removed by the liver, is excreted with the bile into the intestinal canal.

Further investigation has shown that not even the entire liver is operative in excretion of the excess cholesterol but that it is only a certain group of cells in the liver, the so-called reticulo-endothelial cells, otherwise referred to as the Kupffer cells, which are engaged in this function. These Kupffer cells comprise about one-third of the cellular components of the liver.

The Kupffer cells line the blood channels of the liver and come into direct contact with the circulating blood. Any foreign particles in the circulating blood, including excess cholesterol, are engulfed by the Kupffer cells which then release these particles into the bile to be eliminated with the feces.

When the Kupffer cells reach their phagocytic limits, that is, when they cannot absorb any more particles, the excess particles remain in the circulating blood for prolonged periods depending on their number and molecular size. However, when the Kupffer cells release the engulfed particles into the bile, their phagocytic activity is reestablished and they can again pick up the foreign elements from the circulation. This process repeats itself until all foreign substances are eliminated from the blood stream.

The Kupffer cells respond to an excess of free cholesterol in the circulation in a manner similar to foreign bodies since this excess cholesterol has a similar irritating effect.

In addition to their phagocytic and excretory activities, the reticulo-endothelial or Kupffer cells also act as transporters of fat. The fat particles in the blood serum passing through the sinusoids of the liver are taken up by the Kupffer cells and transferred to the parenchymal cells of the liver.

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The various fat components, usually found in the blood after the intake of a fatty meal, are of large molecular size and cannot enter the epithelial cells of the liver unless they are first engulfed by the hepatic reticulo-endothelial cells and broken down into smaller particles. They are then transferred to the parenchymal cells of the liver where they undergo further metabolic changes. If, however, the Kupffer cells are obstructed by blocking agents or damaged by disease, this function is impaired.

Obstruction of the Kupffer cells may be effected by the accumulation of lipid particles in the cytoplasm of the cells; these lipid particles consisting primarily of neutral fat, fatty acids, cholesterol, cholesterol esters and phospholipids. Such accumulation may, for example, be caused by conditions associated with persistent and sustained hyperlipemia such as result from starvation, diabetes, etc.

In normal activity, the thyroid gland acts on the Kupffer cells in such a manner that the cells are stimulated to release the stored lipids from their cytoplasm, thereby permitting other fat particles to be taken up from the circulation. However, in those cases where the thyroid gland cannot, for some reason, so act upon the Kupffer cells, the cells cannot rid themselves of these accumulated lipids and consequently become blocked and cease taking up the lipids or fatty substances from the circulation.

Since practically all the cholesterol of the blood is carried by the lipids or, more precisely, by the alpha and beta globulin lipoprotein portions of the lipids, and since these lipids tend to accumulate in the blood when the Kupffer cells are blocked, this results in hyperlipemia and hypercholesteremia. It should be noted that in patients with atherosclerosis, there is an increase in the beta and a decrease in the alpha lipoproteins.

It has been discovered that thyroid therapy consisting of the administration of the total thyroid secretion (thyroglobulin) acts on the Kupffer cells to release the stored lipid particles and take up new lipid particles from the blood stream, thereby reducing the concentration of the total lipids in the blood stream, as well as decreasing the cholesterol level of the circulation. However, thyroglobulin also produces toxic effects on the body and therefore its use as a lipotropic agent is limited.

Thyroglobulin is a protein of very high molecular weight, ranging from 350,000 to 400,000, and it is made up of fifteen different amino acids. Thyroglobulin may be broken down by various methods of hydrolysis into two organic iodine containing fractions: Thyroxine fraction and diiodotyrosine fraction. The latter is free of thyroxine. The end product of such hydrolysis may be in the form diiodotyrosine peptides, diiodotyrosine peptones and diiodotyrosine in amino acid form. The molecular size of diiodotyrosine peptide is the largest, whereas the diiodotyrosine amino acid molecules are the smallest.

Because of the difference in molecular size of these thyroid iodine compounds, there is a difference in their respective physiological activities. The diiodotyrosine molecules in the amino acid form are very small and are

rapidly eliminated from the circulation by the kidneys, thus producing an insignificant effect on the Kupffer cells. The peptones of diiodotyrosine, which are somewhat larger in their molecular size, remain in the circulation for somewhat longer periods but even these periods are insufficient to produce the desired effect upon the excretory function of the Kupffer cells. The diiodotyrosine peptides, which are of much larger molecular size, cannot be eliminated by the kidneys and therefore remain in the circulation for prolonged periods because they are only slowly eliminated from the blood stream by the liver. Thus they produce a continuous and prolonged stimulating effect on the Kupffer cells of the liver, resulting in greater excretion of the excess cholesterol from the circulation.

Although both of the thyroid iodine components (the thyroxine and the non-thyroxine fractions) produce a reduction in the cholesterol level of the blood, the thyroxine fraction causes a toxic effect on the body, whereas the diiodotyrosine fraction does not indicate any toxic effect. Therefore this investigation is concentrated on the non-thyroxine fraction of the thyroid as a lipotropic agent; an agent which reduces the level of cholesterol, as well as other lipids of the blood, in individuals with hyper-

cholesteremia and/or hyperlipemia in order to prevent or delay the development of atherosclerosis.

The present invention overcomes the above difficulties by providing a diiodotyrosine compound, namely diiodotyrosine polypeptide in colloidal suspension. This colloidal suspension cannot be excreted by the kidneys because of the large size of its molecules. The only organ of the body which is capable of removing it from the blood stream is the liver. However, the excretion of the colloidal diiodotyrosine polypeptide by the liver is far slower than the excretion of diiodotyrosine in amino acid form by the kidneys. As a result, the diiodotyrosine polypeptide particles remain in the circulation for sufficient periods of time to produce a prolonged and sustained physiological effect on the Kupffer cells. In this manner the polypeptide produces a continuous and repeated stimulating effect on the Kupffer cells, thereby increasing the rate of elimination of the excess cholesterol particles from the blood stream and reducing the cholesterol level thereof.

It has been found that even small dosages of diiodotyrosine polypeptide are extremely effective in reducing the blood cholesterol level, as is evidenced by the clinical data embodied in the following table:

EFFECT OF ORAL ADMINISTRATION OF COLLOIDAL DIIODOTYROSINE POLYPEPTIDE ON SERUM CHOLESTEROL AND LIPOPROTEINS

Case	Age, Sex	Diagnosis	Daily dose, mg.	Weeks Treated	Total Serum Cholesterol	Lipoproteins Percent		
						Alpha	Beta	Neutral
1	45 M	Coronary sclerosis	0	0	319	16.43	76.72	6.85
			1.0	12	306	21.98	70.33	7.69
			0.66	12	306	24.00	68.70	7.30
2	57 M	Coronary Insufficiency	1.33	13	284	31.78	61.37	6.85
			0	0	306	28.10	68.54	3.86
			1.0	24	252	32.34	61.43	6.23
3	48 M	do.	0	0	267	33.33	60.00	6.67
			1.0	4	189	35.89	55.06	9.05
			0	0	366	10.18	85.29	4.53
4	49 M	Coronary sclerosis	1.0	5	290	17.39	70.19	12.42
			0.5	10	297	17.76	76.56	5.68
			0	0	346	26.68	69.74	3.58
5	64 M	do.	1.0	10	327	26.28	64.66	9.06
			0	0	310	17.68	77.79	4.53
			1.0	21	271	20.00	70.84	9.16
6	67 M	do.	0	0	497	11.89	84.80	3.31
			1.33	10	425	11.29	79.88	8.83
			0	0	450	9.25	86.55	4.20
7	68 M	do.	1.33	8	306	22.88	70.79	6.33
			0	0	432	9.34	86.72	3.94
			1.0	25	375	17.35	77.93	4.72
8	52 F	Coronary sclerosis and diabetes	0	0	347	15.49	78.21	6.30
			1.0	0	299	24.00	60.00	7.00
			1.33	4	436	5.85	91.29	2.86
9	55 F	do.	1.0	7	320	12.34	77.52	10.42
			0	18	427	17.46	78.52	4.02
			0	0	283	34.20	62.44	3.36
10	57 M	Diabetes	0.66	25	267	34.12	60.09	5.79
			0	0	428	11.18	85.17	3.65
			1.33	12	328	10.10	81.39	8.59
11	52 F	do.	0.66	18	351	15.20	81.19	3.61
			0	0	405	10.16	84.36	5.48
			1.0	9	312	17.74	74.66	7.70
12	60 F	do.	0	0	330	9.57	86.79	3.64
			1.0	4	299	14.03	81.45	4.52
			1.0	7	340	15.62	78.32	6.06
13	52 F	do.	0	0	415	14.37	80.36	5.27
			1.0	12	338	20.29	73.97	5.74
			0	0	419	10.67	84.71	4.62
14	64 F	do.	1.0	16	315	16.33	74.13	9.54
			0	0	445	15.85	81.42	2.73
			1.0	6	387	16.15	78.47	5.38
15	55 F	do.	0	0	395	19.18	74.63	6.19
			1.33	4	306	21.37	70.46	8.17
			0	0	371	14.30	77.57	8.13
16	48 M	Idiopathic hypercholesteremia	1.0	5	295	17.62	71.61	10.77
			1.33	11	291	25.98	68.04	5.98
			0.5	5	324	17.03	77.46	5.51
17	44 M	do.	1.0	14	286	22.84	69.87	7.29
			0	0	370	17.17	75.24	7.59
			1.33	9	327	22.11	66.40	11.49
18	64 F	do.	0	0	320	13.85	82.18	3.97
			1.0	24	303	18.84	73.07	8.09
			0	0	652	4.16	89.89	5.95
19	50 M	do.	1.33	25	355	9.92	81.43	8.59
			0	0	435	18.76	74.13	6.51
			1.0	3	407	14.72	74.06	11.22
20	48 M	Familial hypercholesteremia	1.33	5	370	17.03	71.97	11.00
			1.0	4	390	12.39	73.20	14.41
			1.33	13	387	20.36	71.68	7.96

In order to create a product to produce the most effective physiological action as a lipotropic agent, an enzymatic method of hydrolysis is used which does not denature the protein molecules and therefore prevents the breaking down of the peptides into peptones and amino acid of diiodotyrosine. Furthermore, when the diiodotyrosine fraction is in liquid form it loses its potency within two to three months even when kept under constant refrigeration. This is overcome in the process of this invention by converting the liquid into solid form by vacuum and freeze method. The methods of preparing the solid diiodotyrosine polypeptide are illustrated by the following example:

EXAMPLE

A quantity of fresh hog thyroid glands are frozen and then rapidly minced in a grinder. The minced glands are then suspended in a 0.1 M aqueous sodium acetate solution by adding 1 cc. of the sodium acetate solution to each gram of the minced gland. The suspended gland is then agitated for three hours and allowed to stand for about 12 hours. The mixture is then strained through butter muslin and the solid residue is submitted to a second extraction by repeating the aforementioned procedure.

The two filtrates are then mixed and the mixture is treated with dilute acetic acid at the isoelectric point. In this treatment, 8 cc. of water and 1 cc. of 0.1 N acetic acid are added to each cc. of the extract mixture. The resultant mixture is allowed to stand for between 1 and 2 hours, after which the supernatant fluid is removed. The residue is then re-precipitated in the same manner at least twice more in order to obtain greater purity. The resulting precipitate is thyroglobulin.

The thyroglobulin obtained in the above manner is suspended in a 1% aqueous solution of sodium bicarbonate. To this solution is added sufficient pancreatin to obtain a weight concentration of 0.2% pancreatin relative to the total solution. A few drops of alcoholic thymol solution is added as a preservative.

The above mixture is then adjusted to a pH of 8.0 by the addition of sodium hydroxide and incubated for approximately 72 hours at a temperature of between 37° and 39° C. During this incubation period, the pH is adjusted daily by the addition of sodium hydroxide to maintain its value at 8.0. After the first 48 hours of this incubation period, the pancreatin is renewed. After the 72 hour period, the mixture is removed from the incubator and the pH is adjusted to 5.0 by the addition of hydrochloric acid.

This incubation period is important because it is necessary to have a slow hydrolytic action in order to prevent denaturing of the proteins, while, at the same time, the hydrolytic action cannot be permitted to extend too long since it will subject the thyroglobulin to

the danger of being broken down into amino acids. Upon adjustment of the pH to 5.0, precipitation of the insoluble fraction takes place. The precipitate is removed by filtering; the resulting filtrate being the acid-soluble fraction of the thyroglobulin.

The filtrate obtained in the above manner is admixed with 0.5% phenol or benzyl alcohol 1 to 2% as a preservative and this filtrate is then dehydrated by lyophilization or freeze drying. This freeze drying, which stabilizes the product and is a highly important step in the process, is accomplished by treating the filtrate in a substantial vacuum (2-5 mm. mercury) at a temperature of between about -40° to -60° C. until the filtrate is converted into solid form. The resultant solid is then ground to a powder and the powder is placed in sealed capsules to prevent decomposition thereof.

The colloidal diiodotyrosine polypeptide obtained in the above manner is administered orally in a preferable dosage of about 1.0 to 1.5 mg. of iodine content daily.

The invention claimed is:

1. A method of controlling the cholesterol content of the human blood stream which comprises decreasing the concentration of beta-lipoproteins and increasing the alpha:beta lipoprotein ratio by the oral administration of sufficient solid diiodotyrosine polypeptide to contain about 1 to 1.5 mg. of iodine.

2. The method of claim 1 wherein said diiodotyrosine polypeptide is administered in successive interludes of about 24 hours.

3. The method of claim 1 wherein said diiodotyrosine polypeptide is administered in an air-tight, water-soluble capsule.

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