EFFECT OF CHOLESTEROL RICH DIET ON SOME ARTERIES OF RABBIT AND THE PROTECTIVE EFFECT OF BEZAFIBRATE

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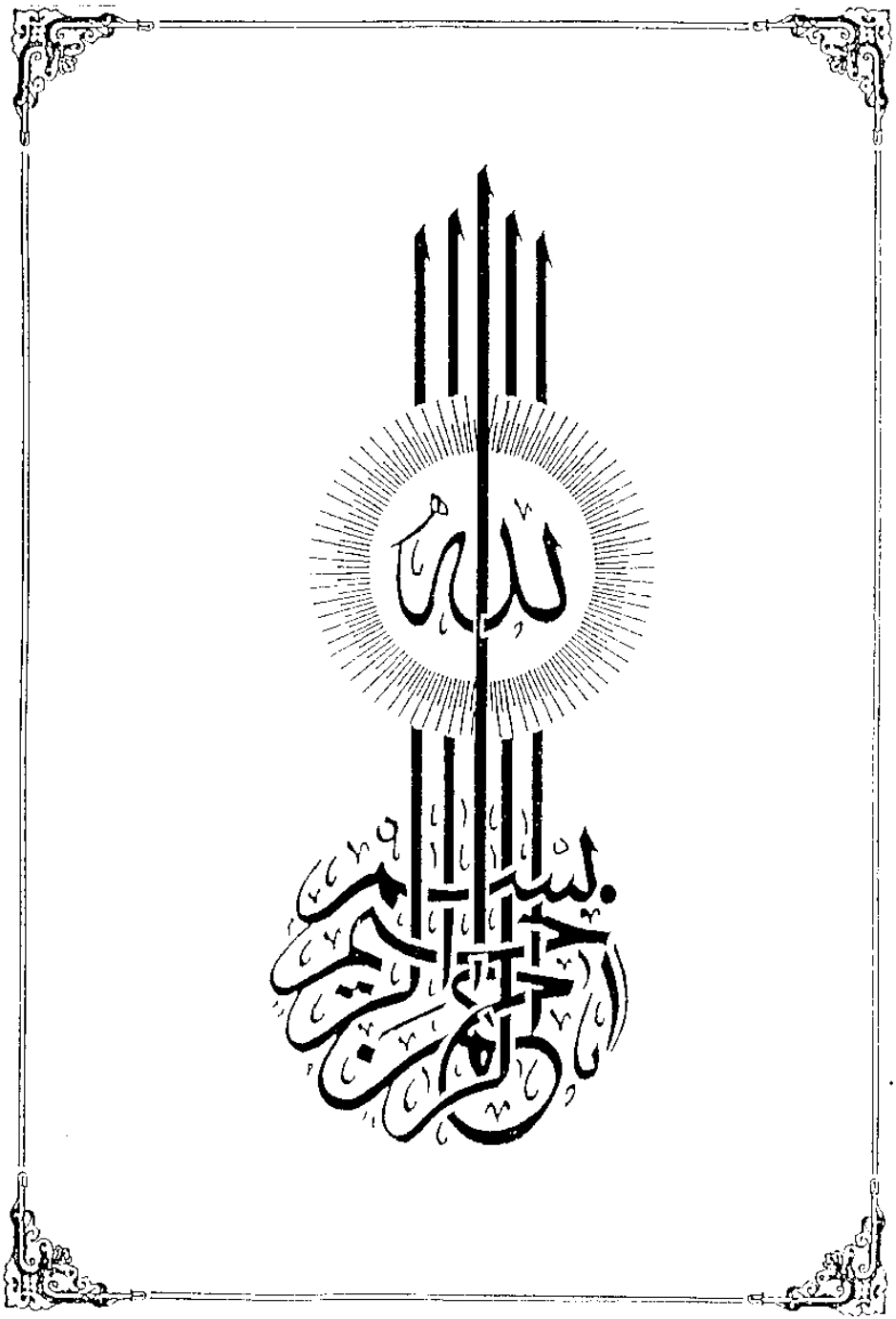

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INTRODUCTION AND AIM OF WORK

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Atherosclerosis is a well established disease. The role of primary and secondary factors in the pathogenesis of atherosclerosis is a highly controversial subject. The role of dietary factors in the development of atherosclerosis has arisen the interest of many investigators ***(Ignatowski, 1908; Stuckey, 1910; Dominguez, 1927; Mann and Andrus, 1956; Taylor, Cox, Counts and Yogi, 1959; Mattson, Erickson and Kligman, 1972; Yasui, Yano, Yoshida, Yoshimasu, Ota and Oshima, 1989; and Wang, Li, Hao, He, Li, Zhang, Tang, Wu, Chen and Wang, 1990).***

Many investigators have demonstrated a correlation between the raised serum lipid levels, atherosclerosis and the incidence of coronary heart disease ***(Sabiston, Smith, Talbert, Gutelius and Vasko, 1961; Taylor, Patton and Cox, 1963; Malinow, Maruffo and Perley, 1966; Bullock, Clarkson, Lehner, Lofland and Clair, 1969; Armstrong, Warner and Connor, 1970; Olsson, Ostlund, Bondjers, Wiklund and Olofsson, 1988; and O'Keefe, Lavie and O'Keefe, 1989).***

Of serum lipids, cholesterol has been the one most often singled out as being chiefly concerned in this relationship ***(Wesselkin, 1913; Lowenthal, 1925; Turner, 1933; Connor, Hodges and Bleiler, 1961; Strong and Mc Gill, 1967; Connor and Jagannathan, 1973; Tipton, Leung, Johnson, Brooks and Beitz, 1987; Booth, Honey, Martin, Lindon, Farrant, Carpenter and Hall, 1990; Robert, Peter, Daryl and Victor, 1991; and Guyton and Klemp, 1992).***

Cholesterol is typically a product of animal metabolism and occurs, therefore, in foods of animal origin such as meat, liver, brain and egg yolk which is a particularly rich source ***(Robert et al., 1991).***

The evidence is likewise clear-cut populations consuming a low cholesterol, low fat diet have little coronary heart disease, whereas in populations of the western world, where the diet is concentrated in animal foods rich in cholesterol and saturated fat, the incidence of coronary heart disease is very high ***(Connor and Connor, 1990).***

The aim of this study is to examine the nature and distribution of the vascular changes produced by a high cholesterol diet (egg yolk) and to find the relation between the changes produced and the duration of the diet regimen. Bezafibrate (a drug which lowers the elevated blood lipids) will be used to demonstrate its protective effect when used immediately after the development of hypercholesterolaemia and its curative effect when used after a sustained period of hypercholesterolaemia with histopathological changes.

REVIEW OF LITERATURE

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I- EFFECT OF DIET ON BLOOD CHOLESTEROL LEVEL AND ON THE STRUCTURE OF BLOOD VESSELS

Ignatowski (1908), studied the effects of high protein diets on rabbits. He noticed the occurrence of fatty plaques on the intimal surface of the aorta in some of his animals, as well as fatty accumulations in the liver and kidneys and marked enlargement of the suprarenal glands. The feedings consisted of meat, eggs and milk. He attributed the pathological changes to the effect of a diet rich in animal proteins on herbivorous animals.

Similarly, ***Starokadomsky and Ssobolew (1909),*** had given rabbits a diet of eggs and milk. They believed that the arterial lesions were due to the toxic effect of proteins in the diet.

Fraser and Gardner (1910), reported that, when cholesterol was given with the food to rabbits, the free cholesterol and cholesterol esters increased in the blood.

Stuckey (1910), fed rabbits with milk, egg-white, egg yolk and meat juice in various combinations and found that the diets containing egg yolk produced marked fatty changes in the aorta and some of its large branches, while milk, egg-white and meat juice alone had no damaging effect on the arteries.

Later on, ***Stuckey (1912),*** succeeded in producing identical lesions by feeding brain tissue. These findings led the author to the conclusion that the lesions in the arteries were not due to the protein fraction of the diet, but rather to some lipoid constituent common in egg yolk and brain tissue.

Anitschkow and Chalatow (1913), demonstrated that the feeding of pure cholesterol dissolved in oil could produce arterial lesions in rabbits. They observed the earliest deposits of fat as fine droplets or granules prior to the appearance of any fat-containing cells in the thin layer of ground substance beneath the intimal endothelium.

Steinbiss (1913), fed rabbits a diet made of dried powdered horse liver (freed of fat) mixed with moistened bread. The aorta of rabbits presented small raised plaques as early as two weeks from the beginning of the experiment. Microscopic examination showed these lesions to be medial. Even in the most advanced cases, the intima was normal. In some animals, the aorta was normal but the media of the peripheral vessels was extensively involved.

Wacker and Hueck (1913), reported that pure cholesterol in solid form, added to the ordinary food of rabbits, could produce arterial lesions and a marked increase in the blood cholesterol. They observed that the first appearance of lipoids was within cells of the lining endothelium or of the subendothelial layer.

Wesselkin (1913), fed rabbits on egg and milk and others on lecithin and milk. In the former group, he obtained abundant deposits of cholesterol esters in the liver, aorta and spleen. In the lecithin-fed rabbits, he also found phosphatides deposits in the same organs. However, because no pathological changes followed the deposition of phosphatides in the organs, while marked changes were produced by cholesterol esters, he concluded that the injury could be ascribed to the cholesterol content of the egg yolk and not to its lecithin content.

Adler (1914), found atherosclerotic lesions in dogs fed cotton seed oil and cholesterin. The lesions were primarily and principally localized in the intima. At the beginning, there was proliferation of rather large flat cells and elastic fibres began to split off from the internal elastic lamina. Simultaneously, minute sudanophil droplets were seen in the intima.

Saltykow (1914), was convinced that the cholesterol added to the animal diets had caused the vascular disease. He pointed out that, the aortic lesions found in the rabbits that had been injected with staphylococci might have been in part or wholly due to the diet, since his animals had received a considerable amount of milk ***(Saltykow, 1908)***. In order to prove his hypothesis, he injected various strains of staphylococci into a group of rabbits whose diet contained 100 c.c. of milk daily. A second group of rabbits received no treatment other than the addition to the diet of 400 c.c. of milk daily. He found that milk feeding caused as great changes in the aorta as the combination of milk feeding and the injection of bacteria.

Bailey (1915), studied the possibility of the production of atheroma of the aorta in guinea pigs by cholesterol feeding. Four guinea pigs were fed on daily doses of 0.1-0.5 gm of cholesterol dissolved in cotton seed oil for periods of 18-72 days. These animals showed enlargement of the adrenals and abundant deposits of anisotropic fat in the liver and spleen. The aortae showed no gross lesions. Microscopically, there were small patches of fatty infiltration in the intima and media. One guinea pig, which received 20 gm of cholesterol in 72 days, showed pronounced atheroma. He concluded that longer periods and larger doses were necessary for the production of an atheroma in the guinea pig.

Knack (1915), fed one group of rabbits an entirely normal diet to which he added very large amounts of cholesterol (4.5 gm daily). The second group was fed milk, one egg and greens daily, resulting in an intake of only 0.3 gm cholesterol. The milk-egg feeding, in spite of containing much less cholesterol and not being continued so long, produced a well marked atherosclerosis in every rabbit of this group. On the other hand, most of the animals that ate large amounts of pure cholesterol showed no vascular injury, a few of them exhibited small intimal lesions. He concluded that pure cholesterol when fed with a normal diet did not cause atherosclerosis and that the milk-egg diet produced atherosclerosis because such a diet was accompanied by abnormal metabolic products which caused the primary vascular injury.

Bailey (1916), found that feeding rabbits with egg yolk or pure cholesterol dissolved in cotton seed oil produced an abundant deposits of fat in

various organs. Prominent among these lesions was an atheroma of the aorta very similar in the gross appearance and histologically to the human lesion. Microscopically, these lesions were mainly intimal which showed pronounced thickening due to collection of fat filled cells. With further development of the lesion, the thickness of the intima was even greater than that of the underlying media.

Dewey (1916), injected rabbits with cholesterol emulsions by intra-peritoneal or intravenous route. Very small quantities of cholesterol administered by intravenous injections and relatively small amounts injected intraperitoneally were sufficient to produce pathological changes in some organs of rabbit. The pathological changes were marked in the kidney and liver. However, the involvement of the aorta was slight and was in the form of the presence of large intima cells. He also observed a uniform and marked infiltration with fat of the entire intima and some adjoining layers of the media.

Adler (1917), injected cholesterol dissolved in sesame oil into the jugular vein of four young dogs for 7 to 8 months. This caused nodules protruding to some degree into the lumen of the pulmonary artery as well as some diffuse thickening. Microscopically, the lesions were seen to be primary in the media especially in the inner third. They consisted of disorganization of the elastic elements and displacement of the muscular tissue. There was no evidence of fatty degeneration. Typical sclerotic hyperplasia of the intima was found over some of the prominent median nodulations while over others, the intima was normal.

Anitschkow (1922), studied the effect of high cholesterol diet on seven guinea pigs which he fed from 1/3 to 1 egg yolk daily. The longest experiment lasted 183 days. Lesions were seen in the first part of the arch of aorta in the form of diffuse infiltration of lipoid material in the subendothelial layer of the intima and in the inner layers of the media. The fatty deposits in the media were located in areas from which the muscle cells had disappeared. The more advanced lesions showed cellular accumulations in the intima consisting of cells like small blood lymphocytes and of large foam cells. The internal elastic lamina was sometimes split and in some cases it showed breaks near which cells were found in the media similar to those seen in the intima.

Newburgh and Clarkson (1923), reported that the term atherosclerosis should be applied only to primary disease of the intima characterized by fatty degeneration and hyperplasia of elastic tissue. They believed that the high cholesterol content of the abnormal diets was not the sole source of experimentally produced atherosclerosis. To prove that, the authors fed rabbits diets containing two different concentrations of protein (dried powdered beef). They observed that prolonged ingestion of excessive amounts of protein by rabbits resulted in extensive atherosclerosis of the aorta. They concluded that the occurrence and extent of atherosclerosis were determined by the amount of protein in the diet and by the duration of feeding.

Anitschkow (1925), studied the effect of cholesterol feeding on rabbits. He found the earliest microscopic changes in the aortae of his rabbits in 30 to

45 days when a total of 11 to 18 gm of cholesterol in oil had been given by mouth.

Lowenthal (1925), fed white mice 0.015 gm of cholesterol in oil daily for 4½ months. In addition to being fed cholesterol, some of the mice were given egg-white and others were castrated. 75% of mice which received cholesterol only presented fatty deposits in the arteries while only about 50% of mice fed cholesterol and egg-white and in the castrated group showed fatty deposits in the aorta.

Clarkson and Newburgh (1926), fed a group of rabbits different doses of cholesterol for different periods. They found that the small doses of cholesterol did not affect the blood cholesterol nor caused arterial disease. Rabbits receiving large amounts of cholesterol developed hypercholestero-laemia and atherosclerosis but there was no parallelism between the two. High blood readings were found in rabbits with normal aortae and atherosclerotic rabbits sometimes had a normal blood cholesterol level. The involvement of the aortae was primarily intimal.

Dominguez (1927), fed five rabbits egg yolk. The duration of the experiment varied from 81 to 391 days. The number of eggs consumed varied from 40 to 531. All the animals developed varying degrees of atherosclerosis of the aortae.