The Hypercholesteremic and Atherogenic Properties of Various Purines and Pyrimidines

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THERE IS still no agreement on the relative importance of various dietary factors in the development of atherosclerosis. Until recently the nitrogenous components of the diet had received little attention in regard to this disease. However, studies emanating from this[1-4] and other[5-8] laboratories indicate that dietary protein is an important consideration toward the understanding of the role of diet on the regulation of cholesterol metabolism. Furthermore, the quality[1] as well as the level[4] of the protein is believed to influence the development of experimentally induced atherosclerosis.

Another group of nitrogenous compounds, the purines and pyrimidines, is considered in the present report. It was shown in a preliminary communication that several of these compounds favor hypercholesteremia as well as an increased amount of incipient atherosclerosis in the rat.[9] Uracil, in particular, was found to be a potent hypercholesteremic agent. These studies were all carried out in the rat, since atherosclerosis, including coronary artery lesions, can be readily induced in this animal. One of the procedures for inducing this disease is to feed diets containing cholesterol and cholic acid. When dietary thiouracil, a sulfur-containing pyrimidine, is included, the pathogenic process is, as expected, accelerated.[3] The basal atherogenic regimen used in the following experiments contains cholesterol and cholic acid but no thiouracil.

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EXPERIMENTAL

The animals used were 12-week-old male albino rats of the Charles River strain which had been maintained on a diet consisting only of Purina Laboratory Chow prior to the introduction of the experimental regimens. All animals were then fed ad libitum a mild atherogenic regimen containing 0.5 per cent cholic acid, 1.5 per cent cholesterol, 20 per cent fat in the form of a hydrogenated cottonseed oil, 20 per cent casein, 54 per cent sucrose, 4 per cent salts, 0.2 per cent choline chloride, and a vitamin mixture as previously described.[2] To this regimen each of the various nucleic acids, purines, or pyrimidines to be studied was added at the levels indicated below. Control animals were fed this diet for the same period of time. These trials were not all executed concurrently, and for this reason it should be noted that the values for control animals are not the same for Figs. 1–3. Detailed comparisons of some of these compounds will be the subject of discussions elsewhere.[10,11]

A total of 78 rats are considered in the present report. The animals were bled at the end of 2, 3, 5, and 10 weeks of dietary treatment for the analyses of the various serum lipid components, including the total cholesterol.[12] At the end of this 10-week period each group was sacrificed, and the hearts,

aortas, kidneys, thyroids, and the other major organs were removed for histologic examination. The heart and aorta were opened in situ, fixed in 10 per cent formalin, and then stained with saturated Sudan IV in 70 per cent ethanol. The degree of cardiovascular sudanophilia was measured in ocular grid units with the aid of a dissecting microscope equipped with an ocular grid.[13] One hundred of these units is equivalent to 3.41 sq mm of surface.

RESULTS

Dietary Ribonucleic Acid (RNA) and Desoxyribonucleic Acid (DNA)

Either 1.5 per cent RNA or 1.5 per cent DNA was added to the atherogenic regimen; a third group received both 0.75 per cent RNA and 0.75 per cent DNA.

It was found that RNA was a slightly more potent agent than DNA for enhancing hypercholesteremia during the first five weeks of the study. However, at the end of the 10-week trial both dietary nucleic acids showed terminal responses of cardiovascular sudanophilia and hypercholesteremia that were not significantly different (Fig. 1). Among the rats which received the same atherogenic diet but with both 0.75 per cent RNA and 0.75 per cent DNA, a less marked over-all hypercholesteremic response was noted during the first 5 weeks. Although these animals finally attained the same magnitude of terminal cholesterolemia, the amount of cardiovascular sudanophilia was significantly less than seen among those groups receiving either RNA or DNA alone ($p < 0.01$). This finding suggests that when RNA and DNA are combined in the diet a protective synergism against arterial lipid deposition may occur.

Another group of seven animals previously had been fed higher levels of RNA, initially at a 3.3 per cent level, and as this diet was poorly tolerated the level was reduced eventually to 1.65 per cent.[9] In these animals hypercholesteremia was further aggravated. For example, at the end of a similar 10-week period, although endocardial sudanophilia was within the same range as above (e.g., 11.0),

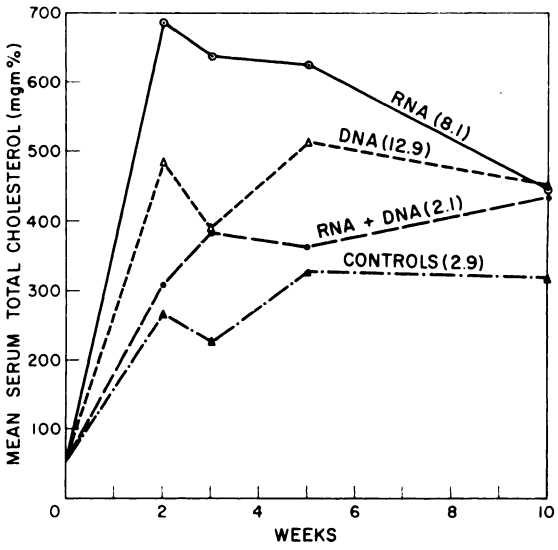

Fig. 1. Effect of dietary nucleic acids on hypercholesteremia and cardiovascular lipid deposition. The values in parentheses indicate the terminal amount of gross sudanophilia in the endocardium expressed in ocular grid units. Each point on the curves represents at least four and not more than twelve animals.

the mean serum cholesterol response was 840 mg per 100 ml. However, these higher levels of RNA resulted in striking degenerative changes in the aorta and renal lesions similar to, but less severe than, those observed in the rats described below receiving adenine supplementation.

Dietary Purines

In this trial four purines are considered (Fig. 2). Uric acid, xanthine, and guanine were tolerated at a 3.3 per cent dietary level. These supplements were studied for 10 weeks. On the other hand, adenine was poorly tolerated and the dietary level was reduced to 1.65 per cent at the end of 3 days to 0.75 per cent at the end of one week, to 0.5 per cent at the end of two weeks, and finally to 0.33 per cent for the remaining 5 weeks of the study. This "adenine" group was finally sacrificed at the end of only 8 weeks because the survival of these animals was in doubt.

In each instance the addition of the purine to the basic atherogenic diet resulted in significant increments in the hypercholesteremia as well as moderate increases in endocardial

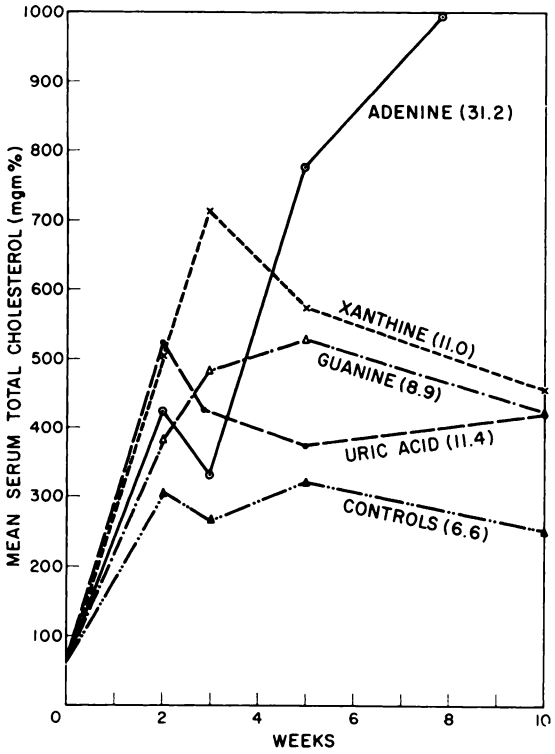

Fig. 2. Effect of various purines on hypercholesteremia and cardiovascular lipid deposition. The values in parentheses represent the amount of endocardial sudanophilia expressed in ocular grid units. Each point on the curve represents at least four and not more than eight animals, except among the rats treated with adenine where only three animals were available for terminal examination.

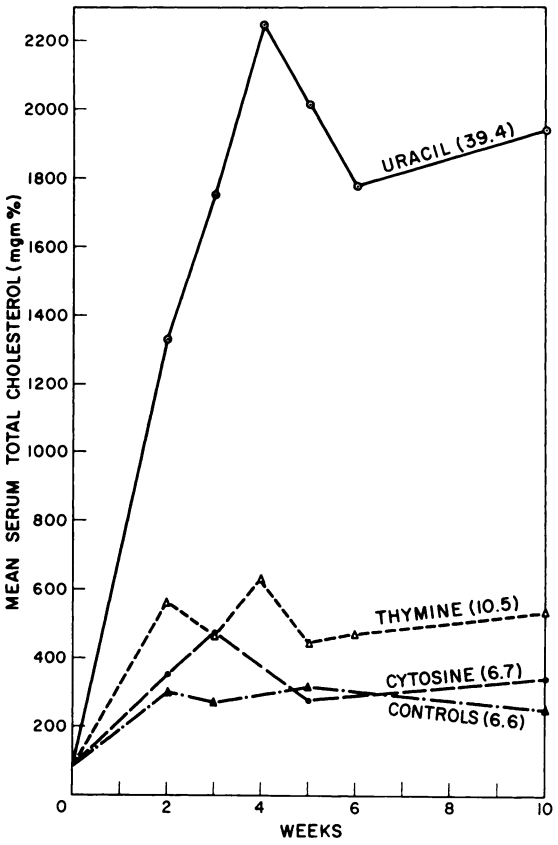

Fig. 3. Effect of various dietary pyrimidines on hypercholesteremia and cardiovascular lipid deposition. Each value in parentheses indicates the mean endocardial sudanophilia expressed in ocular grid units. Each point on the curve represents at least four and not more than eight animals.

sudanophilia. These changes were distinctly most pronounced among the group supplemented with adenine despite the lower dietary level (of purine) and the shorter duration of feeding. In addition, this group showed the same aortic and renal lesions previously seen in those animals receiving the massive levels of RNA,[9] though distinctly more advanced than in the latter. Similarly at a microscopic level both vascular and renal lesions from the two groups of animals were identical, the only differences being those of degree.

The aortic lesions consisted of patchy foci of degeneration in the media with loss of smooth muscle, collapse of elastic lamellae, and calcification. Overlying the larger lesions there was an apparently secondary proliferation of fibrous intimal plaques. Both the medial and intimal lesions were frequently though not invariably associated with moderate amounts of grossly visible sudanophilic lipid. These lesions were seen at all levels of the aorta, being most pronounced in the abdominal portion. Each of the major branches of the aorta was involved on occasion and microscopic involvement of coronary arteries, while not prominent, was present. The marked thinning of the media frequently resulted in aneurysmal dilatation, most pronounced in the ascending aorta. It should be stated that the aortic sudanophilia associated with these lesions was not included in the grading of endocardial sudanophilia. The renal lesions were characterized by the deposition within the cortical tubules of large amounts of

birefringent crystalline material. Acute inflammation associated with foreign-body giant-cell reaction and tubular dilatation were marked. To date these aortic and renal lesions have been seen only in those experimental groups receiving adenine or massive dietary doses of RNA. A more detailed report of the histologic changes will be reported elsewhere.[11]

Dietary Pyrimidines

Three pyrimidines were studied (Fig. 3). These pyrimidines were initially offered at a 3.3 per cent dietary level, but since this dose was poorly tolerated, as evidenced by mild anorexia, levels of 1.5 per cent were offered after the first week and continued during the remainder of the 10-week study. Uracil was the most potent of this group of compounds. The uracil-treated animals displayed an exceptional hypercholesteremia and extensive endocardial as well as aortic sudanophilia. In addition, the thyroid glands of these animals showed a histologic picture of hyperplasia similar to, though less marked than, that induced by thiouracil. No degenerative aortic changes or renal lesions such as were seen in the rats receiving dietary adenine were found in any of the groups supplemented with pyrimidines.

Animals treated with thymine had significantly less sudanophilia as well as less hypercholesteremia than those receiving uracil. Cytosine appeared to be the least reactive of this group.

DISCUSSION

It is interesting to note that both adenine, a purine, and uracil, a pyrimidine, potentiate vascular lipid deposition in the rat. It is not known at the present time exactly how each acts in this process. Although each is associated with an increased degree of hypercholesteremia, these effects seem to be mediated via different pathways. Uracil's action seems to be mediated via the thyroid gland, while adenine's action may be related to renal damage.

In another report a comparison of the hypercholesteremic, atherogenic, and thyroidal effects of uracil and thiouracil at various dietary doses will be presented.[10] To our knowledge, this uracil thyroid hyperplasia has not been heretofore appreciated, suggesting that thiouracil's hypothyroid action may not be entirely related to the presence of a sulfur moiety. With uracil, dietary levels of 0.25 per cent or higher are necessary to induce a thyroidal hyperplasia in acute studies such as described herein. Thus, it would appear that the efficacy of uracil in potentiating dietary hypercholesteremia may, in part at least, be mediated through the thyroid gland.

Of the various recent reports of experimental vascular lesions associated with induced renal damage, the studies of Lehr and coworkers would appear most pertinent to the present material.[14–16] These workers fed a highly insoluble sulfonamide to rats and rabbits which resulted in severe obstructive renal lesions. The latter lesions were associated with hyperfunction of the parathyroid glands and a resultant extensive medionecrosis of the aorta and its branches. Although certain differences were noted, there are marked similarities between the lesions observed by Lehr *et al.* and those herein reported. It is assumed that the lipid associated with the present mediodegenerative changes (and absent in Lehr's material) was induced by the dietary lipids and the resultant serum lipid abnormalities. In the animals described in our study renal damage was induced by feeding adenine with the deposition within the renal tubules of an insoluble adenine derivative, presumably 6,8-dioxyadenine. This lesion has been well defined.[17–19] It is of note that massive amounts of RNA (which contains adenine) produced renal lesions less severe than, but otherwise identical to, those produced by adenine.

At present, it is not clear to what extent the renal lesions may themselves play a role in the hypercholesteremic effect of adenine. There is little resemblance between the renal lesion induced with adenine and the nephrosis produced in rats by administration of amino nucleoside,[20–22] despite the presence in the configuration of the latter compound of a methylated adenine-like moiety. The amino nucleoside-induced lesion is accompanied by

a classical nephrotic syndrome including hypercholesteremia.

It is also interesting that when dietary RNA and DNA are added in equal amounts to an atherogenic diet, less early cardiovascular lipid deposition appears to take place than is seen with RNA or DNA alone, despite the fact that the animals receiving both RNA and DNA demonstrate a distinctly higher degree of hypercholesteremia than seen among the controls. This implies that, although the level of hypercholesteremia is related to the quantity of lipid deposit in vessels, processes may exist which can delay the deposition of excess lipid material in the intracellular and extracellular components of the tissue. This suggests either a more efficient lipid transport system or a more favorable metabolic system of catabolism and synthesis in the tissues to combat an exaggerated serum lipid load. Furthermore, these data substantiate the conclusion previously noted[4] that endocardial sudanophilia reflects serum cholesterol level to a reliable degree only within well-defined experimental parameters.

Thus, it appears that the atherogenic process can, perhaps, be severely affected by both the purines and pyrimidines. These present findings do not diminish the important position the lipids enjoy in the study of this disease, but serve to emphasize that in order to learn why arterial lesions develop one must be aware of all the metabolic disorders that accompany atherosclerosis. It is also interesting to note that certain patients with coronary artery disease also displayed abnormal serum uric acid levels.[23] Although the chief end product of purine metabolism in the rat is allantoin, as compared to uric acid in man, this species difference does not invalidate such comparisons; yet it certainly would be premature to relate the present experimental work directly to the human disease at this time.

SUMMARY

The hypercholesteremic and atherogenic properties of RNA, DNA, four purines, and three pyrimidines were assayed in rats fed moderately atherogenic diets for 10 weeks. It was found that either RNA or DNA supplementation alone favors a mild increase in hypercholesteremic response and cardiovascular sudanophilia. When RNA and DNA were combined in such diets, the level of cardiovascular sudanophilia was reduced to levels seen among the control rats.

Among the four purines studied, adenine was found to be the most atherogenic and hypercholesteremic. In addition, these animals displayed severe obstructive renal lesions and medionecrosis of the aorta and its branches, including the coronary arteries. The aortic lesions were associated with aneurysmal dilatation, fibrous intimal plaque formation, and lipid deposition. On the other hand, the rats fed either guanine, uric acid, or xanthine all demonstrated significant elevations in the serum cholesterol response and only mild increases in the amount of cardiovascular sudanophilia without the above renal damage or vascular necrosis.

Among the rats fed pyrimidines, uracil treatment resulted in a marked hypercholesteremia and cardiovascular sudanophilia. The nature of these changes as supported by thyroidal hyperplasia is reminiscent of the changes seen among rats treated with dietary thiouracil. This uracil effect on the thyroid therefore may account for the singular properties noted in this pyrimidine. The rats fed thymine demonstrated a mild increase in hypercholesteremia and cardiovascular sudanophilia, while the cytosine group's response was not significantly higher than the control rats. Significant thyroidal changes were not noted among these latter two pyrimidine groups.

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