zyme with 0.1 mM (+)-catechin for 1 h and 3 h at 4 °C resulted in 15 and 24% inhibition, respectively.

Figure 3, A, shows the gel formed by 0.4 mg of guinea-pig skin soluble collagen dissolved in 0.5 ml of 0.05 M Tris-HCl buffer pH 7.5 containing 0.1 mM calcium chloride; B shows the disappearance of the gel upon addition of 40 µg of crude collagenase and incubation for 3 h at room temperature; C shows the persistence of a coarse gel when the same amount of enzyme is incubated with the same amount of collagen pretreated with 0.1 mM (+)-catechin.

Discussion. Although (+)-catechin has been proposed as a stabilizer of collagen[2] and, under experimental conditions has been proven to reduce collagen solubility[3] and the severity of lesions secondary to defective formation of collagen crosslinks[4,5], evidence for its binding to collagen has not been provided.

In this study we have reasoned that a possible binding of (+)-catechin to collagen might change its conformation and alter its sensitivity to the action of specific collagenases. The results of our experiments demonstrate that incubation of 2 different types of soluble collagen with increasing amounts of (+)-catechin affords increasing resistance to degradation by mammalian collagenase but not to degradation by bacterial collagenase. Considering the different mechanism of action of the 2 types of enzymes, it is reasonable to assume that the conformation changes caused by (+)-catechin must be moderate, to the extent that they cannot prevent the activity of bacterial collagenase.

It has been postulated that the interaction between soluble collagen and (+)-catechin occurs through hydrogen bonds. From our results, it is evident that exhaustive dialysis of the complex does not remove the bound flavonoid, nor eliminates the protective effect against the activity of mammalian collagenase. Our experiments also exclude the possibility that the resistance to the action of this enzyme might be ascribed to a direct inhibition of the enzyme rather than to stabilization of the substrate. These results reinforce the rationale that (+)-catechin be tried as a therapeutic agent in human diseases considered to involve either structural abnormalities of collagen fibres or their excessive enzymic degradation. Although the safety and lack of toxicity of (+)-catechin in humans have been abundantly proven[9], the questions related to its intestinal absorption and solubility suggest that the development of suitable derivatives might help to achieve effective concentrations at the level of the peripheral tissues.

1 Acknowledgments. This work was supported by grants from the Easter Seal Research Foundation of the National Easter Seal Society for Crippled Children and Adults (R-7821), and the National Institutes of Health (HL-20447). (+)-Catechin was a generous gift from Zyma SA, CH-1260 Nyon, Switzerland.

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An enzymatic spectrophotometric assay for inosinic acid

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Summary. An enzymatic, spectrophotometric assay for inosinic acid is described. Inosinic acid is reacted with excess pyrophosphate in the presence of magnesium ions and hypoxanthine guanine phosphoribosyltransferase to form hypoxanthine which is oxidized to uric acid in the presence of xanthine oxidase. Inosinic acid concentration is measured by the increase in absorption at 293 nm at the end of the reaction.

Inosinic acid (IMP) is the first product in de novo purine biosynthesis to possess a complete purine ring system, and is an intermediate for the biosynthesis of both adenylic acid (AMP) and guanylic acid (GMP)[1]. It appears to be of widespread occurrence in nature and has been detected in microorganisms and in various animal tissues[2]. The determination of this nucleotide is of particular interest not only in metabolic studies[3] but also in the analysis of products in the foodstuff industry[4]. IMP is generally determined after purification by column or thin layer chromatography by means of the characteristic UV absorption[5,6]. In the present paper an enzymatic spectrophotometric method for the determination of IMP is described. IMP is pyrophosphorylated to hypoxanthine by hypoxanthine guanine phosphoribosyltransferase (HGPRT) in the presence of excess pyrophosphate (PP_i) and of magnesium ions. Hypoxanthine is oxidized to uric acid by xanthine oxidase (XOD).

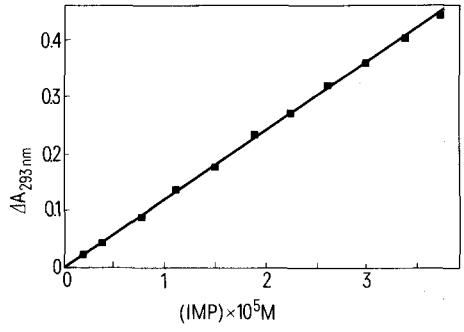
Materials. HGPRT was purified to apparent electrophoretic homogeneity from human erythrocytes as previously described[7]. XOD and IMP were purchased from Boehringer A.G. Paper chromatography according to Gerlach et al.[8] showed no significant impurity in the commercial sample of the nucleotide. All other reagents were high-purity commercial samples from Boehringer A.G. and Merck A.G.

Methods. IMP was assayed by the following procedure: IMP was reacted with excess PP_i in the presence of magnesium ions, HGPRT, and XOD[7] to form uric acid. At the end of the reaction, uric acid concentration was measured by the increase in absorption at 293 nm.

The reaction mixture contained 0.1 M Tris HCl, pH 7.4, 0.01 M $MgCl_2$, 0.001 M PP_i, 0.04 IU/ml XOD, and IMP ranging from 0 to 3.7×10^{-5} M. The 1st spectrophotometric reading was made at 293 nm to determine the initial

absorbance. HGPRT (1.5×10^{-4} IU) was then added to 1 ml of the mixture and the reaction was followed to its completion at 293 nm. The reaction went to completion after 2 h at 37 °C. The reaction mixture without IMP was used as the blank.

Results and discussion. The calibration curve for the IMP assay obtained by applying the procedure described above on IMP solutions of known concentration is reported in the figure. The relationship between IMP concentration and the optical density variation at 293 nm is linear in the range studied. From the same series of experiments a SD of ± 0.005 OD was obtained for the IMP assay.



Calibration curve for the enzymatic assay of IMP. The experimental conditions were as described in the text.

It is well established that HGPRT catalyzes IMP pyrophosphorolysis[7,9] and that the reaction can be followed spectrophotometrically at 293 nm in the presence of XOD activity[7]. When, at the end of the reaction, 0.04 IU of uricase were added to 1 ml of the incubation mixture, a decrease in optical density at 293 nm to the initial absorbance value was observed confirming that uric acid was the chief end product absorbing at this wavelength.

Human HGPRT has been studied extensively with regard to affinity for substrates[10]. The enzyme binds 6-oxo and 6-thiopurines but not 6-amino compounds. HGPRT, in the presence of magnesium ions, also catalyzes the reaction between GMP and PP_i to form guanine and phosphoribosylpyrophosphate. Since guanine is not a substrate for XOD, uric acid is not formed in the presence of GMP. AMP is not a substrate for HGPRT. In the presence of xanthine and/or hypoxanthine, the IMP assay can be carried out by adding HGPRT only after the purine bases have been completely oxidized to uric acid by XOD.

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Sequential changes in serum glucose, triglycerides and cholesterol in aging of normal and alloxan-diabetic rats[1]

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Summary. 16-week-old Wistar, alloxan diabetic rats exhibited progressive elevations in levels of serum glucose, total triglycerides, cholesterol and creatinine over a period of 8 weeks; hyperglycemia preceeded hyperlipidemia and hypercreatininemia and hypertriglyceridemia preceeded hypercholesteremia. Age-matched control rats failed to develop any signs of hyperglycemia or hypercreatininemia, but did develop both hypercholesterolemia and hypertriglyceridemia at 24 weeks of age. This suggests that the progressive cardiovascular derangements (e.g., atherosclerosis, hypertension) noted in experimental diabetes mellitus and in the normal aging (and maturation) process may be brought about by distinctly different biochemical processes.

There is considerable evidence, both experimental and clinical, that the high incidence of cardiovascular complications of aging and diabetes mellitus are related to derangements in carbohydrate and lipid metabolism[2-8]. It is not certain whether progressive alterations in the serum content of glucose, cholesterol and total triglycerides are causally interrelated to the progression of the cardiovascular derangements noted in aging and diabetes mellitus. The mechanism(s) responsible for the increased incidence of hypertension and atherosclerosis seen in animals and human subjects in diabetes mellitus, and on aging, are incompletely understood. There is clinical evidence to suggest that the former may be related to a progressive hyperlipidemia preceded by a hyperglycemia[8,9].

With these points in mind, the present study was undertaken to determine if, under controlled experimental conditions, progressive changes in these serum parameters could be observed in 16-week-old rats made diabetic with alloxan as well as in age-matched (17, 20 and 24 weeks old) rats. A restricted time interval of 8 weeks was chosen in the hope that subtle biochemical alterations would be observed in early aging (i.e., maturing adult) versus diabetic animals. In addition, recent in vivo and in vitro experiments reveal that such normal aging and diabetic rats exhibit progressive alterations in blood pressure, and arterial and arteriolar reactivity, at these time intervals[6,10,11].

Methods. Male Wistar strain rats, initially 16 weeks of age (i.e., 300-335 g), bred and housed in our laboratory were