sion[16,17]. In view of the above observations, and the higher concentration of progesterone recorded during luteal phase there is the possibility of its role in the implantation of the blastocyst during early pregnancy in buffaloes also.

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2 To whom reprint requests should be addressed.
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In vitro esterification of plant sterols by the esterifying enzyme of the small intestine of rat[1]

A.K. Bhattacharyya

Departments of Pathology and Physiology, Louisiana State University Medical Center, New Orleans (Louisiana 70112, USA), 23 February 1979

Summary. The in vitro esterification of plant sterols, β-sitosterol, campesterol and stigmasterol, by the esterifying enzyme of the small intestine of rat was studied in the presence of saturated and unsaturated fatty acids. Campesterol esterification was highest, followed by sitosterol and stigmasterol irrespective of the type of fatty acid. Both campesterol and sitosterol esterification was greater with unsaturated fatty acids than with saturated fatty acids.

The intestinal absorption of plant sterols is considered practically negligible[2-5]. Thus, only minute amounts of plant sterols are detectable in blood and tissues[2-6], although the usual diet may contain appreciable amounts of these sterols[7]. 5 years ago, a new inherited lipid storage disease involving plant sterols was reported in humans[8]. In the disease called 'β-sitosterolemia and xanthomatosis', considerable amounts of plant sterols, β-sitosterol in particular, were found in the blood and tissues of the patients[8,9].

One cause of the disease was found to be greatly increased intestinal absorption of sitosterol[8,10]. After reviewing the events involved in sterol absorption in the small intestine, Bhattacharyya and Connor[8] suggested that enhanced absorption of plant sterols in the new disease probably involved the esterification and incorporation of the plant sterols into chylomicrons. This implied that the enzyme responsible for esterification of cholesterol (cholesterol esterase) within the intestinal mucosa may have lost its specificity for structural requirement of the sterol substrate and acts upon plant sterols thereby facilitating abnormally the absorption of the latter. Since no information is available regarding the esterification of plant sterols by the intestinal esterifying enzyme, the present study was undertaken to study the esterification of plant sterol, β-sitosterol, campesterol and stigmasterol, using acetone-dried powder preparation of the enzyme from the rat intestine.

Materials and methods. Plant sterols, cholesterol and fatty acids (Applied Science Labs., State College, Pa.) used in the study were between 97 and 99+% pure and used without further purification.

Preparation of acetone-dried powder of the intestinal esterifying enzyme. 5 normal Sprague-Dawley rats maintained on regular diet (Purina rat chow, Purina Ralston Co., St. Louis, Mo.) were killed and the small intestine was removed quickly, stripped off of any mesentary and collected in ice-cold saline. The intestine was washed thoroughly with ice-cold saline, blotted on filter paper and placed in 10 volumes of acetone pre-cooled to −15 °C. The tissue was homogenized in a Virtis homogenizer and acetone-dried powder was prepared[11]. The powder was suspended in cold distilled water in the proportion of 1 g powder per 10 ml water for 1 h and centrifuged at 4 °C at 10,000×g for 10 min. The clear supernatant was suitably diluted and was used as the enzyme source.

Assay of the esterifying enzyme activity. The incubation mixture for the assay of the esterifying enzyme activity contained in a total volume of 5 ml, 5 μmoles of the free sterol and 10 μmoles of the given fatty acid in 0.5 ml ethanol, 3 ml of potassium phosphate buffer, 0.1 M, pH 6.1, 25 mg of sodium taurocholate and the enzyme extract representing about 25 mg of acetone-dried intestinal powder containing about 8 mg protein. The incubation was carried out in a metabolic shaker incubator at 37 °C for 1 h. Reactions were terminated by adding 5 ml ethanol to the system and total and free cholesterol determined before

Plant sterol and cholesterol esterification by the esterifying enzyme of the small intestine of rat

	Fatty acids			
	Palmitic 16:0*	Stearic 18:0	Oleic 18:1	Linoleic 18:2
Plant sterols				
β-Sitosterol	1.04±0.6	1.10±0.6	4.71±1.1	4.71±1.3
Campesterol	3.74±1.0	3.49±1.1	8.53±1.7	8.16±1.9
Stigmasterol	0.47±0.3	0.55±0.2	0.94±0.3	0.59±0.3
Cholesterol	6.7 ±0.8	6.5 ±0.9	41.7 ±2.6	88.6 ±6.7

Values are mean±SD of 4 determinations and expressed as nmoles esterified/mg protein/h. The assay system contained in a total volume of 5 ml: approximately 25 mg intestinal powder containing about 8 mg protein; potassium phosphate buffer 0.1 M, pH 6.1, 3 ml; sodium taurocholate 25 mg; sterol, 5 μmoles and fatty acid, 10 μmoles. * No. of carbon atoms: no. of double bond.

and after the incubation period by GLC[8]. Protein was determined by the method of Lowry et al.[12] with bovine serum albumin as standard.

Results and discussion. Of the 3 common plant sterols studied, campesterol was esterified the most, β-sitosterol was next and stigmasterol was esterified very little irrespective of the type of fatty acid present in the incubation medium (table). The esterification of both campesterol and sitosterol was higher with unsaturated fatty acids (oleic and linoleic acids) than with saturated fatty acids. Cholesterol esterification also showed a similar trend. For example, with palmitic acid, cholesterol esterification was only 6.7 nmoles/mg protein/h as compared to about 80 nmoles/mg protein/h when linoleic acid was present in the medium. Since cholesterol, campesterol and sitosterol showed increased esterification with unsaturated fatty acids as compared with saturated fatty acids, the present work is in support of the view that unsaturated fatty acids probably enhance sterol absorption.

In rats, the absorption of sitosterol, measured by radioactive sitosterol feeding, has been reported between 5 and 32%[5]. No information is available regarding the absorption of campesterol and stigmasterol in this species. Based on the present study, it is suggested that in the rat, the absorption of campesterol would be higher than either sitosterol or stigmasterol. Perhaps stigmasterol would not be absorbed. In rabbits fed 2% plant sterols for 10 weeks, it was concluded based on the plasma sterol concentrations that campesterol absorption was greater than sitosterol and probably stigmasterol was not absorbed[13]. Greater absorption of campesterol than sitosterol has also been reported in other species[14–20].

In the newly described lipid storage disease 'β-sitosterolemia and xanthomatosis' involving plant sterols, the absorption of β-sitosterol was very high (about 30% of the radioactive dose fed)[10]. However, campesterol was also found, although in less amount than sitosterol, in the blood and tissues of the patients thereby suggesting absorption of this sterol in amounts less than sitosterol. Both rat and rabbit absorb campesterol in much greater amount than sitosterol. Thus, both these species of animals could not be used as a model for studying the etiology of the newly described lipid storage disease 'β-sitosterolemia and xanthomatosis'.

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Isometric relaxation in rat myocardium: Load dependence and influence of caffeine

C. Poggesi, L. Ricciardi, C. Reggiani and R. Minelli

Institute of Human Physiology, University of Pavia, via Forlanini 6, I-27100 Pavia (Italy), 5 April 1979

Summary. When caffeine plus calcium is added to the perfusing medium, isometric relaxation of rat myocardium is no longer affected by length changes occurring during the twitch. The dependence of isometric relaxation on the initial muscle length is still present and more pronounced after caffeine addition.

In mammalian myocardium[1–3] and skeletal muscle[4,5], isometric relaxation is influenced by displacements or load changes, occurring during the twitch. On the contrary, relaxation in smooth muscle[6] and frog heart[1] is not dependent on the load. The observed difference between these tissues may be related[1] to their different rate of Ca^{2+} removal during relaxation. The high sequestering activity of the sarcoplasmic reticulum (SR) of mammalian heart and skeletal muscle determines an early decline of intrasarcomeric [Ca^{2+}]; cross-bridges cannot further recycle and, if a change in length or load occurs, tension decay depends on the ratio between the number of attached cross-bridges and the load applied.

Because of the feeble action of SR in smooth muscle and frog heart, Ca^{2+} decline is slow: cross-bridges can be reformed and load changes cannot affect the tension fall: in these tissues relaxation depends only on the activation level.

The aim of the present study is to investigate whether, in rat myocardium, caffeine, which has been shown to impair Ca^{2+} uptake by SR[7,8] modifies the dependence of relaxation on load or length changes. In order to describe quantitatively the course of isometric relaxation, it should be useful to fit the curve of tension fall with a mathematical function. The fact that, in rat myocardium, as in skeletal muscle[4,5], tension decay becomes exponential, offers such