PHYSIOLOGY

Effect of β-Sitosterol on Cholesterol and Lipid Metabolism in the Rat

IT is well established that β-sitosterol lowers serum cholesterol-levels in animals and humans[1–3]. In addition it has been found that in fowls[4] β-sitosterol feeding reduces cholesterol- and lipid-levels in the livers and aortas as well as in the serum, at the same time reducing the incidence of atherosclerosis. In a previous experiment[5] doubt was cast on the hypothesis[3] that β-sitosterol lowers serum cholesterol-levels by inhibiting intestinal absorption of cholesterol. We now report new evidence which further elucidates the nature of the action of β-sitosterol on cholesterol- and lipid-levels.

Twelve-month-old male Wistar rats, fed over a period of three months a low (1·4 per cent) fat diet, were used. Five experimental groups of 3 animals were given daily injections of 5 mg emulsified β-sitosterol per rat intraperitoneally on the last 25 days, while an equal number of rats in the control groups were given similar injections without β-sitosterol.

During the 25-day experimental period it was found that food consumption decreased from 13 g to 7 g/rat/day in the first few days in the experimental animals but reverted to the original value after six days. The average body-weight of the experimental animals showed a decline from 390 g to approximately 355 g and levelled off after approximately 1 week, remaining unchanged during the remainder of the experiment. All animals appeared active and healthy during the experimental period. Each animal finally received an intraperitoneal injection of 40 microcuries $CH_3.^{14}COONa$ and thereafter the exhaled carbon dioxide was collected and precipitated as calcium carbonate. One hour after the injection of $CH_3.^{14}COONa$ the animals were killed by bleeding.

The skins, adipose tissue, muscle (rectus femoris), intestines, livers, hearts, aortas, plasma, testes, adrenals and the remainder of the carcasses were examined for lipid- and cholesterol-levels as well as for specific activities, and the relative amounts of carbon-14 incorporated into the expired carbon dioxide were determined. Fæcal sterol excretion and incorporation of carbon-14 into fæcal sterols were measured. A summary of the findings is given in Table 1.

Table 1. RELATIVE LEVELS AND SPECIFIC ACTIVITIES OF THE TOTAL LIPIDS AND TOTAL CHOLESTEROL IN THE TISSUES OF RATS FOLLOWING β-SITOSTEROL INJECTIONS. (CONTROLS = 100)

Organ or tissue	Total lipid-level	Specific activity (^{14}C) in lipids	Total cholesterol-level	Specific aetivity (^{14}C) in cholesterol
Carcasses	76	—*	87	231
Pelts	65	—	110	106
Adipose tissue	105	69	116	173
Muscle	85	171	80	187
Livers	109	108	94	233
Intestines	72	112	101	163
Hearts	75	136	84	168
Aortas	32	136	55	125
Plasma	113	123	89	94
Testes	108	—	103	100
Adrenals	—	—	51	189

* Not determined.

Comparing β-sitosterol-injected animals with the controls, the expiration of carbon dioxide was increased by 7 per cent and the carbon-14 content of the carbon dioxide by 14 per cent, but the amount of fæcal sterol excreted was unchanged.

Although the level of cholesterol in the adipose tissue of the experimental animals had been raised, the total amount of this substance, after allowing for the reduction in weight of this tissue, is less than in the control animals.

An increase in the rate of biosynthesis of lipids (except in adipose tissue) and of cholesterol in the tissues is shown by the specific activity data in Table 1. At the same time β-sitosterol appears to increase the rate of degradation of these constituents as shown by their reduced levels in most tissues. The increased expiration of carbon-14 is also consistent with this view.

As already mentioned β-sitosterol has been assumed to inhibit cholesterol absorption by blocking its passage through the intestinal wall. This mechanism was based on the then prevalent view that phytosterols, including β-sitosterol, do not pass through the intestinal mucosa[3]. However, according to Gould[6], β-sitosterol is absorbed to some extent. It is, therefore, unnecessary to invoke the blocking action of β-sitosterol as the only mechanism for its lowering cholesterol-levels in the animal. In the present work there was no exogenous cholesterol and the question of intestinal absorption was not involved. Nevertheless the cholesterol-levels were lowered considerably, showing that β-sitosterol has a substantial effect in the tissue themselves. These findings may be of significance in connexion with atherosclerosis problems.

T. GERSON
F. B. SHORLAND

Fats Research Laboratory,
Department of Scientific and
Industrial Research,
Wellington, New Zealand.

G. G. DUNCKLEY

Nutrition Research Department,
Medical Research Council,
Dunedin, New Zealand.

[1] Peterson, D. W., *Proc. Soc. Exp. Biol.*, **78**, 143 (1951).
[2] Beveridge, J. M. R., Connell, W. F., Mayer, G. A., and Haust, H. L., *Canad. J. Biochem. Physiol.*, **36**, 895 (1958).
[3] Glover, J., and Green, C., *Proc. Second Intern. Conf. Biochemical Problems of Lipids*, edit. by Popjak, G., and Le Breton, E., 359 (Butterworths Sci. Pub., London, 1955).
[4] Betzien, G., Bracharz, H., Diezel, P. B., Franke, H., Kuhn, R. B., and Seidl, T., *Arzneimittel-Forsch.*, **11**, 751 (1961).
[5] Gerson, T., Shorland, F. B., and Adams, Y., *Biochem. J.*, **81**, 584 (1961).
[6] Gould, R. G., *N.Y. Nat. Acad. Sci.*, **18** (2), 129 (1955).

Excitation of Cerebellar Neurones by Acetylcholine

ALTHOUGH the overall content of acetylcholine and choline acetylase within the mammalian cerebellum is relatively low[1], acetylcholinesterase is associated with the cell bodies and axones of granule cells[2]. Since the synaptic endings of the parallel fibres (granule cell axones) on the dendrites of Purkinje cells are excitatory in nature, in contrast to the inhibitory endings of the basket cells which are confined to the bodies of Purkinje cells[3], it would not be unreasonable to consider that acetylcholine could be an excitant of Purkinje cells. Topically applied acetylcholine, in the presence of physostigmine, does indeed excite elements in the cerebellar cortex[4]; but hitherto the actual cells involved have not been identified.

In the series of experiments reported here extracellular spike potentials have been recorded from individual neurones in the vermis cerebelli of cats which were anæsthetized with pentobarbital sodium (35–40 mg/kg, intraperitoneally). A small pressure plate, which was occasionally surrounded by agar jelly[3], was used to stabilize the cortex. The spike potentials were recorded by means of the central barrel of 5-barrel micropipettes[5] of overall tip diameter 4–8μ, the outer barrels of which contained aqueous solutions of compounds to be tested. These substances were ejected electrophoretically by appropriately directed electrical currents. Most cells were located by periodic ejection of DL-homocysteic acid. The neurones were identified as Purkinje cells by the following criteria: depth beneath the cortical surface, antidromic response to stimulation near the fastigial nucleus by means of a stereotaxically placed concentric electrode[6], and the response to local stimulation of parallel fibres at a point 0·5–1 mm along the same folium from the site of recording[3].