fore and 1 and 2 h following 3 h of vibration (120 Hz, 0.3 mm amplitude). The frequency response curves before and after vibration were similar in the rings exposed to vibration and controls (fig. 2). Following 16 h of vibration similar contractions to electrical stimulation (4–16 Hz, 9 V, 2 msec pulse duration) were observed before and 2 h after vibration (120 Hz, 0.2 mm amplitude) (fig. 3).

Contractions to exogenous norepinephrine (3×10^{-6} M) were studied in 6 rings before and 2 h following 3 h of vibration (120 Hz, 0.2 mm amplitude). After correction for time dependent changes in the control rings contractions to norepinephrine were on the average 42.6 ± 20.7% greater than before vibration. In individual rings, however, contractions were either augmented (n = 4), unchanged (n = 1) or depressed (n = 1) and the difference induced by vibration was not significant.

Neuronal uptake of tritium labeled norepinephrine was not significantly different in six control rings (9989 ± 1585 dpm/mg) and in 6 rings vibrated (120 Hz, 0.2 mm amplitude) for 16 h (7980 ± 1085 dpm/mg).

Azuma et al.[5] reported that prolonged vibration (3 h, 50 Hz, 500 µm amplitude) of helical strips (length 15 mm) from canine femoral arteries were followed by augmented contractions to exogenous norepinephrine 2–5 h after vibration. This suggested a possible pathogenetic mechanism for 'white fingers' induced by the prolonged use of vibrating tools. However, with the procedures outlined in this study, 3–16 h of vibration was not sufficient to uncover any significant persistent abnormality in a canine cutaneous artery following cessation of vibration.

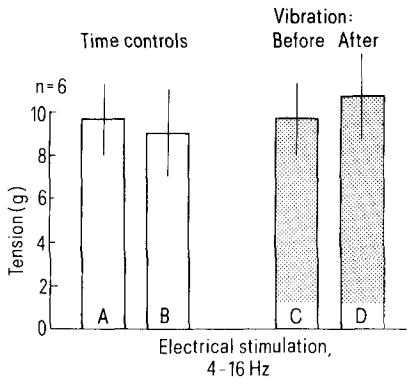

Figure 3. Absence of effect of 16 h vibration (120 Hz, 0.2 mm amplitude) of rings of canine saphenous arteries on contractions to electrical stimulation. Studies were made before (C) and 2 h after Vibration (D) (hatched rectangles). Control rings (clear rectangles) were studied at similar times (A and B, respectively).

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Cholesterol content and cholesterol esterifying activity of various organs in guinea pigs[1]

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Summary. In various organs of the guinea pig, the total cholesterol content of an organ was significantly correlated with the percentage of esterified cholesterol present in this organ. Cholesterol esterifying capacity was shown in most organs, with highest activities in the adrenals, the spleen and the liver. The significant correlation found between the cholesteryl ester content of an organ and its acyl cholesterol acyltransferase activity suggests a possible role of this enzyme in determining the level of the total and esterified cholesterol in a tissue.
Key words. Guinea pig; cholesterol content; cholesterol esterifying capacity; cholesterol, total; cholesterol, esterified; acyl cholesterol acyltransferase.

Esterification of cholesterol constitutes a major step in cellular metabolism[3]; nevertheless, little is known about the cholesterol esterifying activity of different tissues and the possible role of this esterification capacity in regulating cholesterol metabolism in normal and in cholesterol-fed animals. Thus, we decided to study the degree of cholesterol esterification and the cholesterol esterifying capacity (acyl Co-A cholesterol acyltransferase; ACAT, EC 2.3.1.26) in various organs. As experimental animals, guinea pigs were chosen because when they are fed a cholesterol rich diet, a marked plasma and tissue cholesterol accumulation (mainly in the esterified form[4–7] ensues.

Material and methods. Thirty-five male guinea pigs were used; they were on a standard diet for periods extending from 1 to 24 months and weighed between 225 and 1100 g when sacrificed. Twenty-nine guinea pigs weighed between 225 and 595 g; five animals weighed between 965 and 1100 g.

The diet was prepared by Hope Farms BV, Woerden (The Netherlands). The animals had free access to food and water containing ascorbic acid (1 g/l). Animals were sacrificed between 08.00 and 10.00 h and were not fasted. The tissues were quickly excised, placed in ice-cold 0.9% saline and weighed. The following organs were studied; stomach, small intestine (divided into three equal portions named proximal, middle and distal parts), colon, liver, kidneys, adrenals, lungs, spleen and aorta. For free and esterified cholesterol determinations[8], tissue samples were homogenized in 0.9% saline and extracted with petroleum ether. For measurement of the tissue cholesterol esterifying capacity, tissues samples were homogenized in

0.25 M sucrose; the homogenate was centrifuged at 4 °C (4500 × g for 10 min) and the supernatant was kept frozen or immediately used for enzyme activity measurement.

CoA-dependent cholesterol esterifying activity of the liver was estimated by using the method of Goodman et al.[9] as modified by Beck and Drevon[10]. The incubation mixture contained in a volume of 0.75 ml, cellular extract from 5 mg liver (0.3–0.6 mg protein), bovine serum albumin 8 mg/l, ATP 26.7 mM, CoA 1.07 mM and about 0.05 µCi of [4-^{14}C]cholesterol. [4-^{14}C]cholesterol (the Radiochemical Center, Amersham, England) was added in the incubation tube, as [4-^{14}C]cholesterol-albumin solution which was prepared by slowly adding the [4-^{14}C]cholesterol dissolved in acetone to a solution of 100 mg bovine serum albumin (fraction V, Sigma Chemical Co) in 1 ml of 0.1 M phosphate buffer pH 7.4; acetone was removed by evaporation. The tubes were incubated in a shaking water bath maintained at 37 °C; the reaction was stopped by the addition of 10 ml of chloroform/methanol (2:1; v/v) and lipids were extracted by the method of Folch, Lees and Sloane-Stanley[11].

Free cholesterol and cholesteryl esters were dissolved in a small volume of chloroform (500 µl) and separated by thin layer chromatography on silica gel H using light petroleum/diethylether/glacial acetic acid (85:15:3; v/v/v). The spots containing the radiolabeled free cholesterol and cholesteryl esters were made visible with iodine vapor, and scraped into vials containing 4 ml of a mixture containing naphthalene 320 g, POPOP 2 g, PPO 20 g, toluene 1540 ml, dioxane 1540 ml, absolute ethanol 920 ml.

Cholesterol content and ACAT activity of various organs of normal guinea pigs

Organ studied	Total cholesterol (mg · g organ^{-1})	Cholesteryl esters (%)	ACAT (% · h^{-1})
Liver	2.5 (16)*	22.6 (16)	13.8 (20)
	2.5 (1.6–3.6)	22.0 (6.0–46.4)	14.6 (7.1–18.6)
Spleen**	3.6	11.8	14.3
	3.6 (3.0–4.4)	8.9 (0–27.0)	11.6 (1.7–36.4)
Adrenals**	5.9	60.0	30.7
	3.8 (2.4–12.0)	63.9 (20.0–84.4)	34.5 (15.9–48.6)
Kidneys	3.6 (14)	11.5 (17)	8.8 (11)
	3.4 (2.4–5.3)	8.9 (3.7–31.5)	8.8 (3.1–14.9)
Lungs	4.3 (16)	12.4 (16)	1.2 (9)
	3.8 (3.2–6.9)	12.7 (0.3–27.0)	0.7 (0–4.6)
Aorta**	1.6	10.3	1.5
	1.5 (0.4–3.3)	12.3 (0–16.7)	2.0 (0.1–2.7)
Stomach	1.9 (9)	14.1 (17)	3.0 (11)
	1.9 (1.2–2.6)	6.4 (0–55.6)	2.5 (0–13.0)
Small intestine			
proximal	1.4 (17)	11.2 (24)	11.5 (20)
	1.4 (1.1–1.9)	7.2 (0–57.3)	8.6 (1.5–31.5)
middle	1.5 (17)	11.5 (24)	3.3 (18)
	1.4 (1.2–2.0)	8.9 (0–54.5)	2.4 (1.0–15.2)
distal	1.5 (17)	8.9 (24)	4.4 (18)
	1.5 (1.2–1.8)	3.7 (0–31.7)	3.5 (0.7–10.1)
Colon	2.0 (18)	12.2 (18)	9.0 (13)
	1.6 (1.3–3.7)	11.0 (0.7–44.3)	10.1 (0.6–14.0)

* First line, mean values and, in brackets, the number of animals studied. Second line, median values and, in brackets, the extreme values.
** Due to their small size, the spleens , the adrenals and the aortas had to be pooled on several occasions. For the spleen, the values represent the means of six determinations corresponding in four cases to a pool of two spleens and in two cases to a pool of three spleens. For the adrenals, the values represent the means of four determinations corresponding in one case to a pool of adrenals from eight animals, in one case to a pool of adrenals from five animals, and in two cases to a pool of adrenals from four animals. For the aortas, the values represent the means of seven determinations corresponding in four cases to one aorta, in one case to a pool of four aortas, in one case to a pool of five aortas and one case to a pool of eight aortas.

For the whole procedure, recoveries of radioactive free cholesterol and radioactive cholesteryl oleate were respectively 85.2 and 75.9%. The radioactivity was quantitated in a liquid scintillation counter (Intertechnique SL 31). Protein content was analyzed by the method of Lowry et al.[12].

The ACAT activity in the other organs was assayed by the technique used for the liver. In the liver, as in most organs, the rate of radioactive cholesteryl ester formation is linear for 90 minutes (fig. 1). Therefore, a 90-min incubation time was used in subsequent experiments; for adrenals, spleen and aorta, the relationship between ACAT activity and time could not be assessed, due to the lack of sufficient tissue material. Figure 2 shows that in the liver, as in the adrenals and in the small intestine, there is a linear relationship between ACAT activity and content of protein per incubation vial up to 1000 µg. Esterification rate was calculated as fractional esterification rate (FER) (percentage of cholesteryl ester formed per h and per g of tissue). For analyzing the relationship between the total cholesterol content, the cholesteryl ester content and the ACAT activity, in the different tissues, a linear correlation coefficient was calculated by using the mean values of these parameters. Indeed, because several organs (spleen, adrenals, aorta) had to be pooled (table), individual values could not be obtained in these cases; moreover, the coefficient of variation of the mean values is approximately of the same magnitude for

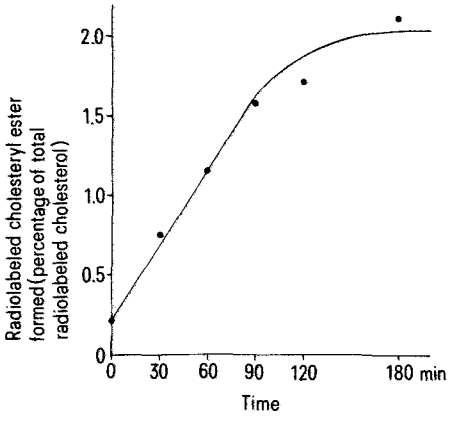

Figure 1. Cholesteryl ester formation in relation to time. Enzyme assays were performed as described in the material and methods section. The reaction was stopped after 30, 60, 80, 120 and 150 min by addition of chloroform/methanol. The results shown in the graph are those obtained from assays performed with liver extract.

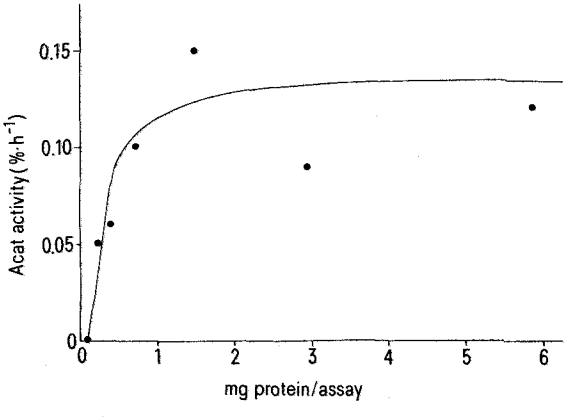

Figure 2. Cholesteryl ester formation in relation to protein content of the tissue extract (in this case, liver). Enzyme assays were performed as described under material and methods.

the different parameters in the different organs. Thus the method used for the statistical analysis of the data seemed to be acceptable.

Results and discussion. Except for cholesterol content (mg/g organ) in the liver which is significantly correlated with body weight ($r = +0.71$; $p < 0.01$), no significant correlation was found in the different organs studied between the different parameters measured and the body weights.

The accompanying table shows that the cholesterol content of the adrenals, the liver, the lungs, the spleen and the kidneys is higher than in the other tissues. The cholesteryl esters represent 9–14% of total cholesterol in most organs, being higher, however, in the liver and overall in the adrenals. There is a significant correlation between the cholesterol content of each organ and the percentage of esterified cholesterol in the same organ ($r = +0.73$; $p < 0.02$). Cholesteryl ester is considered as a form of storage from which it can be mobilized for several structural and metabolic functions[3]. The high cholesteryl ester content of the liver and the adrenals can be related to their high metabolic rate directed mainly to lipoprotein synthesis and steroidogenesis.

Norum and coworkers have demonstrated the presence of a coenzyme A-dependant esterification mechanism for cholesterol in intestinal mucosa from guinea pigs[13]; likewise, ACAT activity was shown to be present in the liver[10], the kidneys, the spleen and the adrenal glands of guinea pigs[14].

As it is shown in the table, the present work demonstrates that cholesterol esterifying activity is present in most organs studied.

The FER is found to be highest in the adrenals; the spleen, the liver, the proximal part of the small intestine, the kidneys and the colon have lower but still substantial cholesterol esterifying activity. On the other hand, the middle and the distal parts of the small intestine, the stomach, the aorta and the lungs have only slight ACAT activity. For each organ, the values of FER are significantly correlated with the values of the percentage of esterified cholesterol of the same organ ($r = +0.86$; $p < 0.001$). This latter finding suggests that ACAT could play a major role in determining the level of tissue ester cholesterol content. However, care must be taken before firm conclusions can be drawn. Indeed, more precise information ought to be obtained by taking into account the possible specific requirements of each organ when measuring its ACAT activity[15], and the possible role of a recently-described sterol carrier protein (SCP_2) necessary for effective esterification of cholesterol by the hepatic microsomes. Moreover, it must also be noted that differences in ACAT activity between tissues can mean either differences in the quantity of enzyme or differences in the cholesterol content of the microsomes. Finally, a cholesterol esterase was demonstrated by Drevon in several organs of the guinea pigs[14]; its activity has not been evaluated in our study.

Nevertheless, in support of an important role of ACAT in cholesterol metabolism and cholesterol disposition is the demonstration that in cholesterol-fed guinea pigs the increase in the cholesteryl ester content of various organs is relatively well correlated with the increase of the ACAT activity in these organs[17].

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Acute ^{60}Co-gamma irradiation of rats decreases the inhibitory effect of succinate on the lipid peroxidation of liver mitochondria

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Summary. Succinate inhibits NADPH-dependent lipid peroxidation of liver mitochondria. This effect of succinate decreased 12 h after whole-body ^{60}Co-gamma irradiation, depending on the dose of irradiation.

Key words. Rat liver; liver, rat; mitochondria; irradiation, ^{60}Co-; lipid peroxidation; succinate.

Lipid peroxidation is a consequence of free radical production in biological systems, which may cause irreversible damage. Protection against free radicals is generally attributed to the superoxide dismutase-catalase system and to glutathione. Lipid peroxidation is known to occur in mitochondria in the presence of NADPH and physiological quantities of ferric ions[1]. The increase of lipid peroxidation is considered to be an indirect, deleterious effect of ionizing radiation[2,3,4] which can be demonstrated in mitochondria 2–3 days after irradiation in vivo[5,6,7].

Mészáros and co-workers[8] have observed that succinate inhibits NADPH-dependent (ADP-Fe stimulated) lipid peroxida-