Materials and methods. 17 males of Wistar strain, 15 homozygous (DI) and 15 heterozygous (non-DI) male rats of Brattleboro strain aged 90 days and reared from birth under standard laboratory conditions were used. 8 animals in each group were deprived of water 24 h before the onset of the experiment. Tissue ^{86}Rb-uptake was used as an indicator of local tissue blood flow[8] as modified for unanaesthetized animals[9]. The ^{86}Rb-distribution in the course of the first min after i.v. injection corresponds to cardiac output distribution. 20 μCi ^{86}Rb in the form of RbCl (Isocommertz, GDR) were injected into the tail vein and 40 sec later the rat was decapitated. The activity of samples and of ^{86}Rb standard was measured by Autowell II (Picker, USA). According to Sapirstein[8], tissue ^{86}Rb-content was expressed in percent of total administered dose (organ flow fraction in percent of cardiac output) and in percent of total ^{86}Rb-dose per g tissue (corrected for a 'standard rat' weighing 200 g). ^{86}Rb-uptake was measured in anterior and posterior pituitary separately and for comparison also in heart, kidneys and in samples of liver, skin, intestine and muscle. Data were statistically processed by means of Student's t-test.

Results and discussion. In accordance with earlier observations[5], no changes were observed after water deprivation either in DI or in non-DI rats in anterior pituitary weight, flow fraction and ^{86}Rb/g uptake. On the other hand, there was a significant increase in flow fraction of cardiac output and in ^{86}Rb/g uptake in neurohypophysis of DI and Wistar rats. The increase of corresponding values was not significant in non-DI rats. In agreement with data reported by Sokol and Valtin[10], heavier neurohypophysis were found in DI than in non-DI and Wistar rats, and this weight further increased after water deprivation only in DI rats. Dehydration did not influence ^{86}Rb/g-uptake in other organs studied in non-DI rats, while in DI rats ^{86}Rb/g-uptake was decreased in intestine (0.95 ± 0.109 vs. 0.41 ± 0.049) and in skin (0.17 ± 0.016 vs. 0.12 ± 0.010). After water deprivation, the weight of myocardium increased in DI rats (316.3 ± 5.7 vs. 333.0 ± 4.6 mg/100 g b.wt).

An increase of the neurohypophyseal flow fraction of cardiac output and of ^{86}Rb/g-uptake was observed after osmotic load produced by 24 h of water deprivation of Wistar males. But the increase of both parameters was much more expressed in homozygous Brattleboro rats although they do not synthetize VP. This increase need not be in relation to the synthesis or release of VP, and it might be related to the oxytocin release[6], the turnover of which seems to be enhanced in DI rats[11].

8 L. A. Sapirstein, Am. J. Physiol. *193*, 161 (1958).
9 J. Kapitola, O. Schreiberová and I. Jahoda, Čslká Fysiol. *16*, 462 (1967).
10 H. W. Sokol and H. Valtin, Endocrinology *77*, 692 (1965).
11 R. J. Balment, I. W. Henderson, J. A. Oliver, Gen. comp. Endocr. *26*, 468 (1975).

Vitamin C and gallstone formation: A preliminary report

S. A. Jenkins[1]

Pye Research Centre, Walnut Tree Manor, Haughley Green (Suffolk IP14 3RS, England), 9 June 1977

Summary. Gallstone formation in hypovitaminotic C guinea-pigs fed a high cholesterol diet was associated with qualitative changes in the gallbladder bile, namely, a high cholesterol concentration, a lowered bile acid content and diminished phospholipid-to-cholesterol and bile acid-to-cholesterol ratio.

The formation of cholesterol gallstones has been induced experimentally in several species of animal by modifications to their diet[2–6]. Although experimentally induced vitamin A- and to a lesser extent vitamin D-deficiency have been shown to promote gallstone formation[7], no evidence for a relationship between vitamin C status and gallstone formation has appeared. This is perhaps surprizing in view of the considerable amount of experimental evidence linking vitamin C with cholesterol metabolism[8–12]. During the course of a wide-ranging study of the effects of atherogenic diets in guinea-pigs, a high incidence of gallstone formation was observed in animals with latent vitamin C-deficiency. We report here on an initial experiment designed to examine the relationships between vitamin C and gallstone formation.

Methods. 36 male guinea-pigs (Dunkin Hartley) weighing approximately 200 g were given access to water and a standard pelleted laboratory diet ad libitum for 2 weeks, and then transferred to a pelleted high cholesterol (0.5%) scorbutic diet (Cooper Nutrition). Chronic hypovitaminosis C was induced in 18 of the animals by the daily p.o. administration of 0.5 mg. L-ascorbic acid (Sigma Ltd) in 0.2 ml of 20% sucrose solution. The remaining animals were similarly dosed with 5.0 mg of the vitamin in the same volume of vehicle. This dietary regimen lasted for 5 weeks after which time the animals were weighed, anaesthetized and the bilary tree examined for concrements. Following an overdose of anaesthetic, the cystic duct was ligated prior to cholecystectomy. The gallbladder contents were centrifuged at 5000 rpm for 20 min to separate the bile from gallstones and associated debris,

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2 R. F. Borgman, Am. J. vet. Res. *26*, 1167 (1965).
3 F. J. Caldwell, Jr, K. Levitsky and B. Rosenberg, Am. J. Physiol. *209*, 473 (1965).
4 F. Christensen and H. Dam, Acta physiol. scand. *211*, 1069 (1966).
5 L. G. Schoenfield and K. J. Sjövall, Am. J. Physiol. *211*, 1069 (1966).
6 T. Osuga and O. Portman, Proc. Soc. exp. Biol. Med. *136*, 722 (1971).
7 J. G. Fortner, Surgery *36*, 932 (1954).
8 G. C. Chatterjee, in: The Vitamins, vol. 1, p. 399. Academic Press, New York and London 1967.
9 E. Ginter, Science *179*, 702 (1973).
10 E. Ginter, J. Babala and J. Cerven, J. Atheroscler. Res. *10*, 341 (1967).
11 E. Ginter, P. Bobek and M. Ovecka, Int. Z. VitamForsch. *38*, 104 (1967).
12 R. E. Hughes, J. hum. Nutr. *30*, 315 (1976).

Table 1. Weight changes, mortality and incidence of gallstones in guinea-pigs receiving either 0.5 or 5 mg of vitamin C daily

Group	Daily dosage of vitamin C	No. of animals Started	Survived	Average wt change (g)	No. of survivors with gallstones
A	0.5	18	14	+ 2.89	14
B	5	18	17	+ 25.67	0

Table 2. Bile acid, cholesterol and phospholipid content of the gallbladder bile of guinea-pigs receiving either 0.5 or 5 mg of vitamin C daily

Group	Daily dosage of vitamin C	Bile acids (BA)	Cholesterol (C)	Phospholipids (P)	Ratio BA: C	Ratio P: C
A	0.5	8.99 ± 0.81*	2.11 ± 0.12*	7.19 ± 0.62	4.4*	3.7*
B	5	14.54 ± 0.97	1.22 ± 0.17	8.41 ± 0.82	16.7	9.3

*Significantly different from group B values at $p < 0.01$ (t-test). Values are mmoles/l ± SEM.

and the bile analyzed for phospholipid[13], cholesterol[14] and bile acids[15]. The gallstones were washed in distilled water, dried under vacuum, extracted with alcohol, and the cholesterol content determined[14].

Results and discussion. Table 1 summarizes the result of the experiment in terms of weight gain, mortality and incidence of gallstones. During the experimental period the increase in weight of vitamin C replete animals was considerably greater than that of animals subjected to the low vitamin C regimen, but inanition was not present in any of the guinea-pigs. Gallstones were present in the gallbladder and occasionally in the lumen of both the cystic and common bile duct of all survivors that had received 0.5 mg vitamin C per day. The dried pooled gallstones weighed 18.96 mg and 52.8% of this weight was accounted for by the presence of cholesterol.

The gallbladder bile of hypovitaminotic C guinea-pigs had a higher concentration of cholesterol and a lower concentration of bile acids than that of control animals, and consequently a lower bile acid : cholesterol ratio (BA:C). Although there was no significant difference between the bile phospholipid concentrations in the 2 groups of animals, the phospholipid : cholesterol (P:C) ratio in the hypovitaminotic C guinea-pigs was significantly lower than that of the controls as a result of the higher bile cholesterol concentration in these animals. Similarly, lower BA:C and P:C ratios concomitant with gallstone formation has been reported by other investigators[3, 5, 16]. Cholesterol is held in micellar solution in bile in combination with phospholipids and bile acids and it has been postulated that the BA:C and P:C ratios determine its solubility rather than absolute values[17]. Consequently, a lowering of the BA:C and P:C ratios favours cholesterol precipitation and provides a satisfactory explanation for gallstone formation in the hypovitaminotic guinea-pigs.

13 D. B. Zilversmit and A. K. Davis, J. Lab. clin. Med. *35*, 155 (1950).
14 D. Watson, Clin. chim. Acta *5*, 637 (1960).
15 S. J. Levin, J. L. Irvin and C. G. Johnson, Analyt. Chem. *33*, 856 (1961).
16 H. Dam, Proc. Int. Cong. Nutr., Edinburgh *6* (1964).
17 B. Isaksson, Acta. Soc. Med. upsal. *59*, 296 (1954).

Protein bound calcium in piscine red and white muscles

B. Jamila Begum and R. V. Krishnamoorthy[1]

Department of Zoology, University of Agril, Sciences GKVK, Bangalore 562142 (India), 23 February 1977

Summary. The levels of Ca^{++} and calcium binding properties of sarcoplasmic proteins in red and white muscles of cat fish were compared. The white muscle is characterized by greater water content, ionic calcium and Ca-binding capacity, whereas the red muscle is characterized by a higher calcium sensitive protein content.

Calcium levels and Ca-binding properties of sarcoplasmic proteins of vertebrate red and white muscles are not known. Sreter[2] compared the uptake of Ca^{++} in red and white muscles of rabbit. In view of the importance of Ca^{++} in muscle contraction, the rate of which differs in red and white muscles[3, 4], the present investigation was undertaken, to examine whether the muscles are characterized by Ca-binding capacity and Ca-bound proteins. Cat fish, *Clarias batrachus* (Linn), were caught from Hebbal (fresh water) tank, stored in laboratory aquaria and fed daily on earthworms. Red and white muscles from the anaesthetized (with chloroform) fish were excised and transferred to aluminium pans kept on ice piles. The water content of the muscles was assessed by

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2 F. A. Sreter, Fedn Proc. *23*, 930 (1964).
3 M. Barany, K. Barany, T. Richard and A. Volpe, Archs Biochem. Biophys. *109*, 185 (1965).
4 M. Barany, J. gen. Physiol. *50*, 197 (1976).