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.

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Protein bound calcium in piscine red and white muscles

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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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Properties of the sarcoplasmic proteins of red and white muscles of cat fish, *Clarias batrachus*

	Red muscle $\bar{x} \pm SD$	White muscle $\bar{x} \pm SD$	t	p
Calcium binding capacity (μmole Ca/mg protein)	0.92 ± 0.06	1.70 ± 0.06	23.73	0.01
Percentage water content	78.48 ± 2.43	82.09 ± 1.86	2.885	0.02
Calcium precipitated proteins (mg Ca bound protein/mg protein)	0.56 ± 0.09	0.34 ± 0.05	5.348	0.01
Ca-insensitive proteins (mg protein/mg total protein)	0.48 ± 0.09	0.61 ± 0.097	2.380	0.05
Ionic calcium (μg Ca/mg protein)	0.15 ± 0.03	0.32 ± 0.09	7.291	0.01
Ash content (percent wet weight)	1.66 ± 0.05	1.27 ± 0.05	1.879	0.2
Total calcium (μmole Ca/g dry weight)	10.80 ± 4.96	8.80 ± 2.59	0.875	0.2

Values are $\bar{x} \pm SD$ of 6; t = Student's t-test.

estimating the water loss when the wet tissue (2 g) was dried to constant weight at 105 °C. The dried muscle slices were ashed in porcelain crucibles in a muffle furnace at 650 °C and weighed to compute the ash content. A single pan electric balance (Mechaniki, made in Poland) was employed for these studies. 2 g of fresh muscle was homogenized in 10 ml redistilled water using a Potter-Elvinjin-homogenizer. A clear homogenate was obtained after centrifugation at 6000 × g for 20 min, and it dialized using a cellophane tube 15 cm long × 0.5 cm diameter against 20 ml water for 24 h at 5 °C. The Ca content of the dialysate was estimated by complexiметric method[5].

Sarcoplasmic proteins were extracted into 0.47 M KCl pH 7.0 according to Barany et al.[3]. Calcium binding proteins of them were estimated according to Weller[6]. Ca^{++} levels in the homogenate was estimated flame photometrically after analyzing the dialysate obtained after equilibrium dialysis[6]. The proteins of the homogenate was precipitated by addition of equal volume of 0.1 M Calcium acetate. The precipitate was collected by centrifugation at 600 × g for 20 min and the protein content of it was estimated colorimetrically[7]. The sarcoplasmic proteins in a 5 ml of the homogenate were precipitated with 10% trichloroacetic acid (TCA) and the precipitate was ashed in the muffle furnace at 650 °C. The calcium content of the ash was estimated by complexiметric method[5]. The TCA precipitated proteins in the same volume were estimated colorimetrically[7] to express the amount of bound calcium to unit weight of protein. The results were presented in the table.

The white muscle is characterized by greater water content and ionic calcium than the red muscle. The latter contains more Ca-precipitated proteins. Calcium insensitive (for charge neutra-lization at pH 7.0) proteins occur in greater concentration in white muscles. The muscles do not differ in total calcium and ash contents. Ca-binding capacity is more in white muscle, whereas calcium sensitive proteins are more in red muscle. The occurrence of high Ca binding capacity and rich Ca^{++} and a low Ca bound protein content of the white muscles corroborate the findings of Sreter[2] in the *Vastus lateralis* muscle of rabbit. Our results on Ca^{++} content in red and white muscles are not in agreement with those of Beecher et al.[8] who reported that the muscles do not differ in Ca levels. Another interesting observation is that the total Ca in the muscles do not vary but the availability of Ca in ionic or bound form varies with reference to the muscle.

The occurrence of more Ca-precipitated proteins (table) in the red muscle suggests the latter's low affinity to Ca^{++}. This affinity is mainly an electrostatic attraction between the protein and Ca^{++}[9]. These attractions have immense significance in the contractility of the muscle fibre[10].

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Proposed effects of brain noradrenaline on neuronal activity and cerebral blood flow during REM sleep

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Summary. We propose that the observed increases of both neuronal activity and cerebral blood flow seen throughout the brain during REM sleep may be effects of decreased central noradrenaline release.

Several groups of neuronal cell bodies located in the mammalian brainstem have been shown to contain[1] and to synthesize[2] noradrenaline (NA); the most well-known of these cell groups is the locus coeruleus. The axons of some of these NA-containing cells ramify widely throughout the spinal cord, brainstem, cerebellum and forebrain[3–10]. Pharmacological studies suggest that increased release of NA from axon terminals is coupled to increased discharge rates of the parent brainstem cells[11–15]. Central NA's precise functional role in central nervous system (CNS) is uncertain. Recent evidence, described below, suggests that central NA may affect both neuronal firing rates and cerebral blood flow (CBF) in regions innervated by NA-containing axons. We wish to propose here that the increases of both neuronal firing rates and CBF observed throughout the brain during rapid-eye-movement (REM) sleep are the result of decreased central NA release.

Recent experiments suggest that central NA may influence both neuronal firing rates and CBF. Stimulation of