all specimens. However by the histochemical technique employed in the present study no haemosiderin could be detected in any of the organs even after such a prolonged period of starvation.

Discussion. There are many plausible explanations that could account for this absence of haemosiderin from the melano-macrophages of starved dogfish. The phagocytic system may be less well-developed than that of teleosts such that dogfish macrophages may be unable to process effete erythrocytes and to store haemosiderin for possible future recycling. It is also possible that in spite of the long period of starvation the fish was still utilising other body tissues as energy sources thus sparing those tissues (presumably including the blood cells) that are more crucial for survival. Moreover the catabolic pathways of haemoglobin breakdown in dogfish could be different from those in higher fish. Thus for instance it is possible that in dogfish excessive ferric iron is stored in forms other than haemosiderin such as ferritin; this latter substance is water-soluble and thus not detectable by the techniques employed here. Whatever may be the answer the present findings clearly indicate that the melano-macrophages of elasmobranchs differ from those of teleosts not only on morphological but also on functional grounds.

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Serum calcium and inorganic phosphorus level of *Rana tigrina* in response to glucagon administration

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Summary. In *Rana tigrina*, i.p. injection of glucagon (1 mg/kg/day) evokes a progressive hypocalcemia up to day 3 which declines after day 5. It also induces hypophosphatemia which continues throughout the experiment.

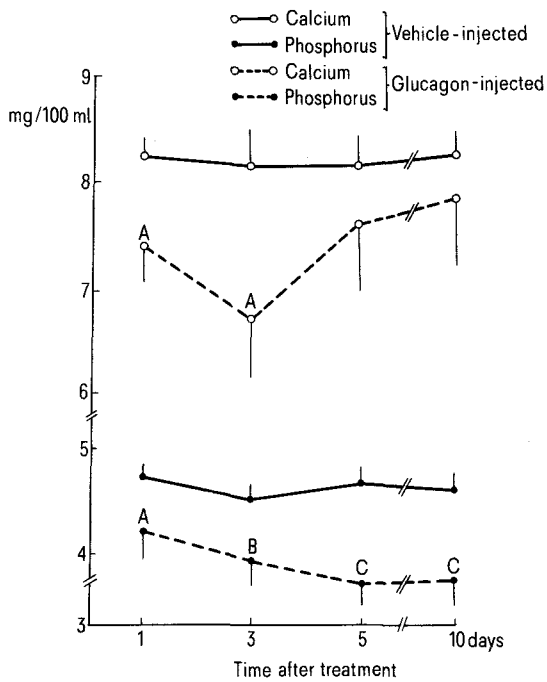
The phosphocalcic response to glucagon administration has been well demonstrated in mammals[1-13] but not to the best of our knowledge in amphibians. The present investigation was therefore undertaken to determine the effect of glucagon administration on serum calcium and inorganic phosphorus levels in the frog, *Rana tigrina.*

Material and methods. 48 male adult frogs of the species *Rana tigrina*, weighing from 130 g to 180 g were collected during the end of March (spring season) and maintained under laboratory conditions for 2 weeks prior to use. They were then divided into 2 numerically equal groups, a) vehicle-injected (control); and b) glucagon-injected (experimental).

The experimental frogs were injected i.p. with 1 mg/kg b.wt of glucagon[14] daily for 10 days. The hormone was dissolved in 0.005 N HCl (pH 2.6) and diluted with 0.6% sodium chloride solution containing 0.1% gelatin (vehicle). The control frogs were injected i.p. with 1 ml/kg b.wt daily with vehicle. Blood samples from both the groups were collected by cardiac puncture on the 1st, 3rd, 5th and 10th day of the treatment. In all cases, the last injection was given 2 h before the frogs were sacrificed. The sera were analyzed for calcium and inorganic phosphorus levels according to Trinder's[15] and Fiske and Subbarow's[16] methods, respectively.

The frogs were not fed during the experiment. To avoid the effects of circadian rhythm, the injections were administered at the same time and the blood samples were collected at approximately the same h of the day throughout the experiment. The differences in the serum calcium and inorganic phosphorus levels of vehicle- and glucagon-injected specimens were evaluated using Student's t-test.

Results. In experimental animals, the hormone (glucagon) induces hypocalcemia on day 1 which reaches its maximum



Changes in the serum calcium and inorganic phosphorus level of *R. tigrina* after daily administration of vehicle and glucagon for 10 days. The blood samples were collected 2 h after the last injection on the 1st, 3rd, 5th and 10th days of the treatment. Each point indicates mean ± SD of 6 determinations. The significant differences in the serum calcium and inorganic phosphorus levels of vehicle- and glucagon-injected specimens are indicated by A, B and C which represent $p < 0.01$, < 0.002 and < 0.001, respectively.

on day 3 ($p < 0.01$). Thereafter, the serum calcium concentration rises again toward control levels (fig.). Glucagon administration evokes a progressive decrease in the serum inorganic phosphorus level (fig.). This fall outlives the hypocalcemic response of glucagon.

Discussion. The glucagon-induced hypocalcemia in *Rana tigrina* is in consonance with the earlier reports using other vertebrates[1-13,17]. This also derives support from the enhancement of urinary excretion of calcium and other electrolytes in dog[18-21] and man[22] after glucagon administration.

In mammals, glucagon has been reported to stimulate calcitonin release[1,2,7,17]. The hypocalcemia observed in the present study may be attributed to the release of the hypocalcemic factor from the ultimobranchial cells (known to be the source of calcitonin in non-mammals[23,24]) as is evident from the activity of the gland, occurrence of hyperplasia up to day 3 and thereafter, indications of the degeneration of the gland (own, unpublished results).

Glucagon also induces hypophosphatemia in *R. tigrina*. This is in agreement with earlier reports[1,2,17,25-27]. This response can be attributed to the enhanced release of calcitonin caused by glucagon treatment. Talmage et al.[28] have reported that calcitonin lowers the serum calcium by preventing release of calcium from bone whereas it (calcitonin) lowers the serum phosphate by increasing its exit from the circulation rather than by inhibiting its release from bone. Thus, the hypocalcemic and hypophosphatemic effects of calcitonin are independent.

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Ca-antagonistic substance from soft coral of the genus *Sarcophyton*[1]

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Summary. A 14-membered ring diterpenoid named cembrane, possessing Ca-antagonistic action on the isolated rabbit aorta, has been isolated from a soft coral of the genus *Sarcophyton*, and its structure established by X-ray and spectroscopic data.

As part of a program to search for biologically active substances from marine organisms[2-7], methanol extracts of numerous soft corals[8] collected in Okinawa water were screened on the isolated rabbit aorta. The screening revealed that the extract of a soft coral of the genus *Sarcophyton*, like Ca-antagonists[9-11], inhibited markedly the contraction of the aorta induced by KCl but did not affect that induced by norepinephrine (NE). In this paper, the isolation and structure of the Ca-antagonistic substance **I** from a soft coral of the genus *Sarcophyton* are described.

Male albino rabbits (2-3 kg) were used. The procedure for preparing the rabbit isolated aorta and the technique of measurement of contractions were carried out as previously described[12]. Soft corals (wet wt 450 g), collected in October 1981 and stored at −20 °C until used, were homogenized in methanol and extracted with the same solvent. The com-

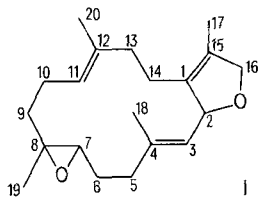

Figure 1. Chemical structure of compound **I**.