with those of malondialdehyde standard preparations in aqueous solution (fig. 2). The molar extinction coefficient for malondialdehyde was calculated from a standard curve at pH 11.3 ($\varepsilon = 6.68 \times 10^7$ cm/mole).

Malondialdehyde could be detected in normal, untreated murine cardiac and hepatic tissues in very small amounts using the method described above. 48 h after i.p. injection of 15 mg/kg doxorubicin malondialdehyde concentrations reached a peak level in the liver and at 72 h in the heart (table 1). There was distinct evidence for higher malodialdehyde concentrations in heart than in liver-tissues of treated animals. Untreated C 57 BL mice of different ages showed different malondialdehyde concentrations in heart tissue, indicating an increased lipid peroxidation in untreated older animals (table 2).

The process of doxorubicin induced malondialdehyde production in heart tissue could unequivocally be reduced by pretreatment with antioxidative substances (table 3).

Discussion. Up to now, only Myers et al.[3] have produced evidence of lipid peroxidation taking place in vivo; they found an increase in the malondialdehyde content of the hearts of doxorubicin-treated mice with respect to untreated controls. Their published method is difficult to perform and not easily reproducible because of great losses of malondialdehyde by the lyophilization process of the homogenized tissue extract (unpublished observation in our own laboratory). To investigate the effect of antioxidative substances on the potency of doxorubicin as a lipid peroxidation inducer we adapted a distillation method used in food research[11] to our requirements. With this micro-Kjeldahl-distillation apparatus we were able to detect malondialdehyde even in normal mice in very low concentrations and were able to investigate easily the protective activity of some SH-containing substances as possible inhibitors of doxorubicin-induced lipid peroxidation. Our findings that the malondialdehyde content of cardiac tissues is dependent on the age of untreated animals gives support to the observation that age-dependent accumulation of lipid peroxides follows as a consequence of increased radical formation in mitochondria[12]. Doxorubicin treatment can induce malondialdehyde production mainly in cardiac tissues of mice. This effect can be inhibited to a large extend by i.p. injection of tocopherol, glutathione, cysteamine and cysteine 24 h before doxorubicin-treatment. The smaller malondialdehyde content in hepatic tissues after doxorubicin-treatment could be the consequence of the existing higher content and turnover of reduced glutathione in the liver compared with the heart[13], which could be a protecting factor against this treatment. We conclude from our results that there is good evidence to justify clinical trials of the application of antioxidative substances in doxorubicin treatment in order to diminish drug-induced cardiac toxicity.

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On the failure to detect haemosiderin in the melano-macrophages of dogfish *Scyliorhinus canicula* (L.) after prolonged starvation

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Summary. When teleost fish are starved, the number of melano-macrophages increases markedly within the spleen and kidney. This increased pigment deposition is almost certainly a consequence of catabolic tissue breakdown. One of the pigments, haemosiderin, resulting from the breakdown of haemoglobin of red blood cells, accumulates almost exclusively in the melano-macrophages of the spleen but not within the kidney melano-macrophages. In contrast when elasmobranchs, as exemplified by the dogfish *Scyliorhinus canicula* are starved, melano-macrophages accumulate predominantly in the liver and to a lesser extent in the spleen. However no haemosiderin deposits could be detected in the melano-macrophages of either of these two organs. This is suggestive of functional differences between the melanomacrophages of elasmobranchs and teleosts.

Cells with a macrophage-like morphology and containing abundant amounts of pigments are a common feature of certain fish tissues. These pigments can be of the melanin, haemosiderin, lipofuscin or ceroid series and all 4 pigment types can occur in one and the same cell. While in cartilaginous fish and in primitive bony fishes these cells are found mainly in the liver, in the advanced bony fishes they are more abundant within the 2 main haemopoietic organs, the spleen and kidney. Also, whereas in all bony fishes (except the salmonids) these cells accumulate in large numbers to form discrete centres within which are also found leucocytes, in salmonids and cartilaginous fish they are randomly distributed throughout the tissues[2,3].

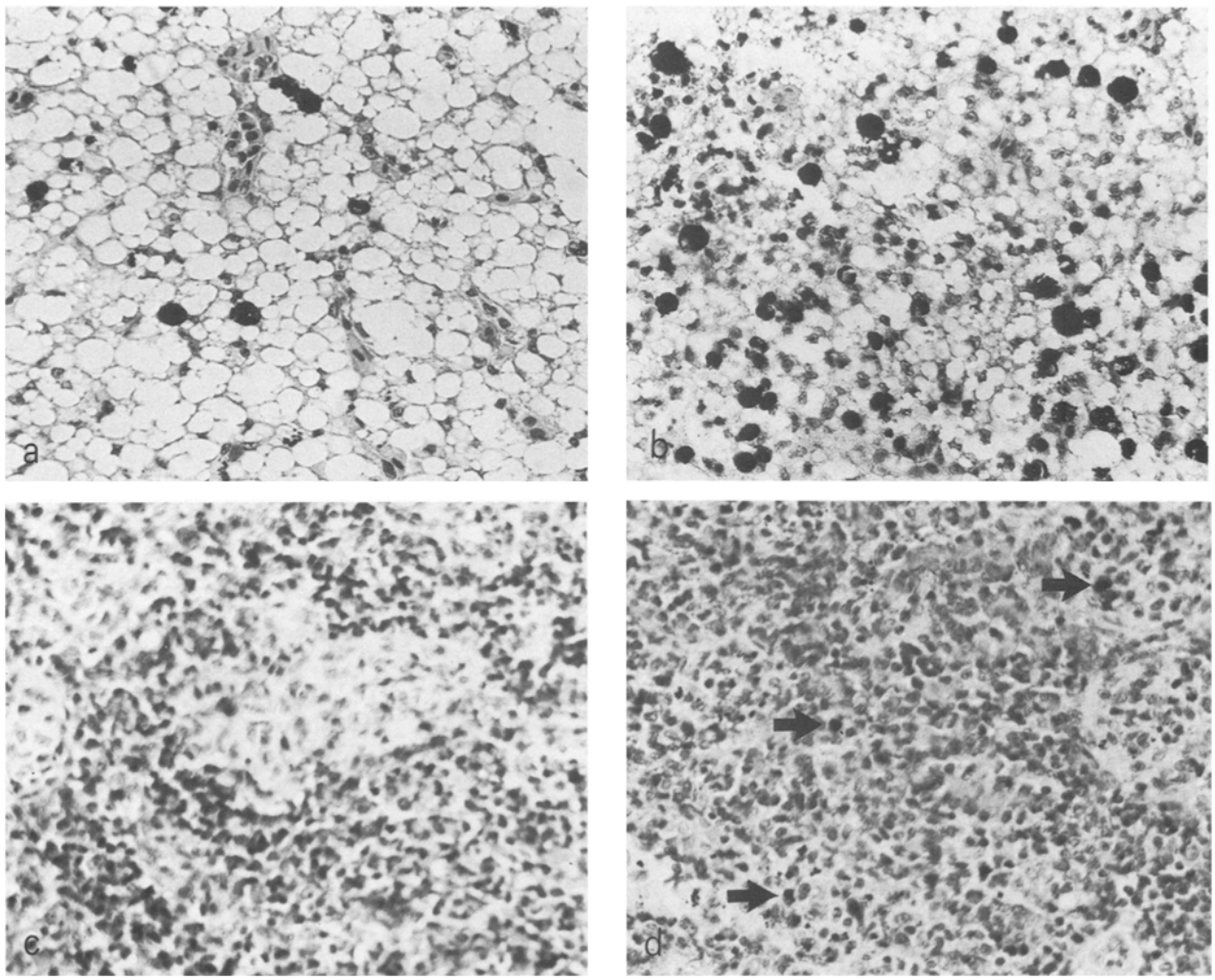
Starvation experiments with brown trout *Salmo trutta,* rainbow trout *Salmo gairdneri,* European eels *Anguilla anguilla,* plaice *Pleuronectes platessa,* swordtails *Xiphophorus helleri* and many *Tilapia* and *Sarotherodon* species have shown that prolonged periods of starvation result in considerably increased deposition of melano-macrophages within the spleen and kidney[4,5]. Concomitantly there is noted

an increased deposition of haemosiderin in the melano-macrophages of the spleen but by comparison, the content of haemosiderin in the kidney and liver melano-macrophages generally remains very low[6]. When rainbow trout are splenectomized and subsequently starved, accumulation of haemosiderin is diverted to the kidney melano-macrophages[7].

In contrast, when the dogfish *Scyliorhinus canicula,* an elasmobranch, is subjected to prolonged starvation, melano-macrophages accumulate predominantly in the liver and to a lesser extent in the spleen[4]. The objective of the present study was to investigate haemosiderin deposition in starving dogfish.

Materials and methods. Specimens of dogfish *Scyliorhinus canicula* varying in body length from 40 to 45 cm were caught off the West Coast of Scotland, transported to the laboratory and split into 4 circular tanks (20 fish were placed in each tank) with recirculated sea-water of salinity 3%. Each tank had a diameter of 2 m and a depth of water of 0.5 m. The temperature was maintained constant at 12 °C throughout the experiment. The fish were weaned onto chopped rainbow trout and left to acclimatize for 4 weeks. The fish in 2 of the tanks were then completely deprived of food whereas the fish in the remaining 2 control tanks continued to be fed normally. The experiments lasted 8 months during which time no mortalities occurred. Two fish were sampled from each tank every month. After killing by pithing, portions of the spleen, kidney and liver were dissected out and placed in 10% formal saline. After routine processing, 5-µm-thick paraffin sections were stained with haematoxylin and eosin (H and E) and with Perls' Prussian blue method for the demonstration of ferric iron bound as haemosiderin[8].

Results. After 8 months of complete starvation the number of melano-macrophages in the liver had increased from 40 to 700 per mm^2 of section while in the spleen there was observed an increase from 20 to 140 per mm^2 of section (fig.). Melano-macrophages were absent from the kidney of



a Section of the liver from a clinically normal dogfish containing a small number of randomly distributed melano-macrophages (H and E, × 200). *b* Markedly increased deposition of melano-macrophages in the liver of a dogfish that had been starved for 8 months (H and E, × 200). *c* Section of the spleen from a clinically normal dogfish. In elasmobranchs the spleen is much less heavily pigmented than the liver and in fact no melano-macrophages can be seen in this field (Schmorl's ferricyanide stain, × 200). *d* Section of the spleen from a starved dogfish. A small number of melano-macrophages (arrowed) resulting from prolonged starvation can be seen in this field (Schmorl's ferricyanide stain, × 200).

When stained by Perls' Prussian blue method no traces of haemosiderin could be seen in any of the sections from dogfish even after prolonged starvation. The pigments in the spleen are of a very light yellow color in H and E stain, therefore Schmorl's ferricyanide method for lipofuscins (which show up blue) was employed for micrographs *c* and *d*.

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.

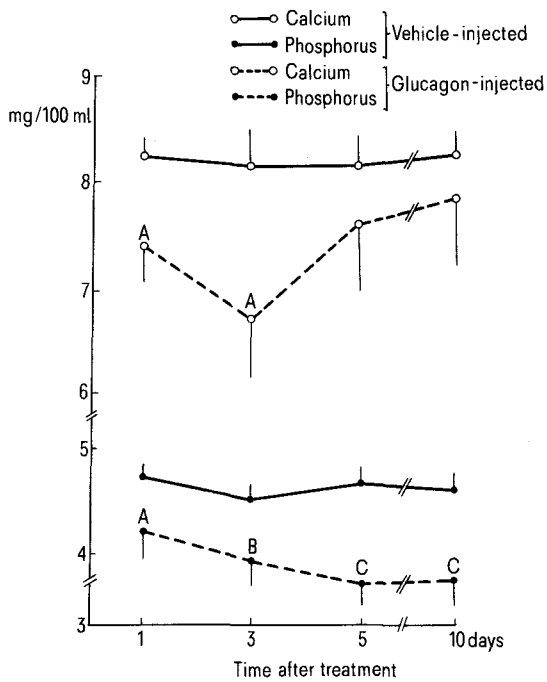
The phosphocalcic response to glucagon administration has been well demonstrated in mammals[1-13] but not to the best of our knowledge in amphibians. The present investigation was therefore undertaken to determine the effect of glucagon administration on serum calcium and inorganic phosphorus levels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