cuticles and other invertebrate structures. Under undefined conditions, however, chitin has been reported to show a reluctance to display itself in the tanned exocuticle of certain myriapods[3]. Similarly, the hyaline exocuticle of the scorpion *Pandinus imperator*, though presumably containing chitin, was found to give a negative iodine–chitosan colour test[4].

The reason for this peculiarity has been examined during the course of an investigation into the cuticle of the scorpion *Buthus quinquestriatus*. Sheets of cuticles of this scorpion were subjected, after chitosan transformation, to different treatments: they were either stored in alcohol or washed in several changes of distilled water. Sections of cuticles after the first treatment showed the hyaline exocuticle as an intact layer which, as found in *Pandinus*[4], neither dissolved in dilute acetic acid nor gave a positive iodine–chitosan reaction. The distinctive violaceous colour with iodine was, however, readily obtained in cuticles treated in the second way. In these cuticles, the hyaline exocuticle was split into separate laminæ which yet resisted solution in dilute acetic acid.

The application of histochemical tests revealed a sterol–protein complex impregnating the hyaline exocuticle of *Buthus* in a similar manner to the impregnation of the tanned exocuticle of insects in preparation for hardening[5]. During transformation of chitosan, the sterol, being unsaponifiable, resists alkali treatment, and appears to protect the impregnated chitosan laminæ against solution during subsequent treatment with dilute acetic acid. The protein moiety, in contrast, is readily destroyed under the same conditions, but its hydrolytic products are not completely dispersed until after thorough washing in water. In alcohol, on the other hand, they remain as an insoluble residue which so tightly holds the laminæ together as to allow reaction with iodine. It is suggested that a similar lipoprotein complex, impregnating the hyaline exocuticle of *Pandinus* as in *Buthus*, is responsible for having prevented a positive iodine–chitosan reaction occurring in that scorpion[4].

In the full account of this work to be given elsewhere it will be shown that the epicuticle of *Buthus*, as in all arthropods, is free of chitin. This is important, for Kirshnan *et al.*[6] claimed, on the basis of X-ray diffraction and chromatographic studies, that the epicuticle of the scorpion *Palamneus swammerdami* contains chitin. It appears, as has already been suggested[4], that misinterpretation of the hyaline exocuticle of this scorpion for the cuticulin layer of the epicuticle has led to that conclusion.

S. R. A. Malek

Department of Zoology,
University of Alexandria,
Egypt.

[1] Rouget, C., *C.R. Acad. Sci., Paris*, **48**, 792 (1859).
[2] Campbell, F. L., *Ann. Entomol. Soc. Amer.*, **22**, 401 (1929).
[3] Blower, G., *Quart. J. Micro. Sci.*, **92**, 141 (1951).
[4] Kennaugh, J., *Quart. J. Micro. Sci.*, **100**, 41 (1959).
[5] Dennell, R., and Malek, S. R. A., *Proc. Roy. Soc.*, B, **143**, 414 (1955).
[6] Krishnan, G., Ramachandran, G. N., and Santanam, M. S., *Nature*, **176** 557 (1955).

Effect of Ethyl *m*-Aminobenzoate (MS 222) on the Elasmobranch Electrocardiograph

Randall[1] noted that the perfusion of water containing the teleost *Tinca tinca* with ethyl *m*-aminobenzoate (MS 222) caused an increase in heart and respiration rates. Although these increases did not correlate significantly, he concluded that there was probably some connexion between the cardiac and the respiratory centres in the central nervous system.

We have recently carried out a series of experiments on the electrocardiography of 10 elasmobranch fishes (3 stingrays, *Dasyatis* spp.; 6 grey sharks, *Carcharinus obscurus*; and 1 hound shark, *Mustelus manazo*). The length-range of these fishes was 50–100 cm. In these investigations, an exploring electrode was inserted into the body cavity next to the heart, by direct dissection in the stingrays, and by the coracoid bar-drilling method of Perry Gilbert[2] in the sharks. The anæsthetic used was MS 222; but it was found that the optimum concentration for inducing these species was 1·7 parts in 1,000 of water as opposed to that recommended by Gilbert[3] of 1 part in 1,000. 80–100 ml. of this solution induced anæsthesia in a grey shark 70 cm in length in 45 sec, and a single dose allowed of 15–20 min of anæsthesia. One of the grey sharks survived 5 such doses in the course of a week.

The pulse rate taken when the fish had quietened down in the tank after the operation showed that MS 222 apparently caused a bradycardia. This pulse slowing was confirmed by taking resting E.C.G.'s in *C. obscurus*, anæsthetizing the fish with MS 222, and taking further E.C.G.'s. A similar effect was noted in the stingray (*Dasyatis uarnak*) in which direct epicardial leads were taken from the exteriorized heart of a partly conditioned animal out of the water, in a supine position, before and after the pharyngeal administration of MS 222. After large doses, a bradycardia of 30/min was found, as opposed to the measured resting pulse rate of 50/min. This fall in pulse rate was also accompanied by a fall in respiration rate of 11–8/min.

In the rays, we were unable to record respiration rates accurately, because of the difficulty of the operating procedure, and the violent action during and after recovery from anæsthesia. Our findings are of interest in the light of Randall's observations that the direct effect of MS 222 on the isolated teleost heart was to decrease the frequency of the beat, and that the drug caused bradycardia in the tench with bilateral vagal section[1]. Furthermore, the coincident fall in pulse and respiration rate that we noted with large doses of MS 222 in the stingray supports Randall's contention that there may be a neurological relationship between the cardiac and respiratory centres in the brain.

The cardio-regulatory centre in *C. obscurus* would appear to be rather resistant to certain stimuli: in the free-swimming fish, the rapid increase of movement which ensues when the fish is disturbed causes an increase in pulse rate of only 4/min (from 50 to 54 per min), and the pulse returns to resting-levels within 30 sec. This sluggish cardiac response was also noted in the free-swimming Stingray (*Dasyatis pastinaca*), when it was similarly disturbed.

Subject to what would appear in our experience to be changes due to injury, MS 222 did not affect the actual E.C.G. complex. There was occasional asystole for single beats, and, rarely, a few unifocal extra-systoles, which may have been due to the operative procedure itself. No conduction defect in the form of alteration of PR, QRS or QT distances was noted.

In a single instance, where we followed the administration of MS 222 with a single large intramuscular dose of 'Coramine', we obtained acute ST segment elevation within 30 sec, followed almost at once by a severe bradycardia (3/min) for some minutes; there was a return of the pulse to 10/min, and the animal then died. This may explain our failures in earlier attempts to resuscitate near-moribund MS 222-anæsthetized sharks, where intramuscular 'Coramine' in smaller doses caused initial improvement, which was invariably followed by death.

G. D. Campbell
D. H. Davies

Oceanographic Research Institute,
Durban, South Africa.

[1] Randall, D. J., *Nature*, **195**, 506 (1962).
[2] Gilbert, Perry, *Anat. Rec.*, **138**, 3, 351 (1960).
[3] Gilbert, Perry, *Science*, **126**, 212 (1957).