

the Hédon-Fleig solution is a more suitable physiological medium.

It is of interest to note that Prosser³ gives a formula for a physiological solution for *Helix* and attributes the formula to Bernard and Bonnet¹. However, the formula given by Prosser bears no resemblance to that of Bernard and Bonnet. Moreover, it yields a considerable amount of precipitate which persists at all temperatures from about 20° C. to boiling point. Prosser's formula was therefore regarded as an error and was not used in these experiments.

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¹ Bernard, A., and Bonnet, V., *C.R. Soc. Biol., Paris*, **103**, 1119 (1930).

² Bolles Lee, "The Microtometist's Vade Mecum", edit. by Gatenby and Beams, eleventh ed., 247 (Churchill, Ltd., London, 1950).

³ Prosser *et al.*, "Comparative Animal Physiology" (Saunders Co., Philadelphia and London, 1950; reprint 1952).

'Water Rigour' as an Aid when Operating on Marine Gastropoda

WHILE investigating the physiological aspects of the distribution of two species of the marine gastropod *Bullia*, one of us (A. C. B.) discovered that these snails can crawl only between certain limits of salinity. As the salinity of the sea-water is decreased by the addition of distilled water, movements become more and more restricted, eventually resulting in the total loss of locomotory powers while the animal becomes distended due to the osmotic inflow of water. Providing that dilution is accomplished reasonably slowly (taking 60 min. or more) the foot is not retracted at any stage, while the excitability of the muscles is greatly reduced or disappears completely. The animal remains motionless with stiff, swollen tissues. For *Bullia digitalis* Meuschen, this critical salinity lies in the region of 1.8 per cent, while in the case of *B. laevissima* (Gmelin) it is about 2.35 per cent. Loss of locomotory powers due to decreased salinity has previously been noted for some rocky-shore gastropods by Broekhuysen¹ and Brown². The phenomenon resembles the loss of contractility in vertebrate skeletal muscle subjected to 'water rigour'. However, in *Bullia* the process is completely reversible. The snails can be kept for some hours at these reduced salinities without apparent ill-effect; when returned to fresh, undiluted sea-water they soon recover and behave normally.

A fuller account of this work is soon to be published.

It is well known that most operations for experimental purposes on Gastropoda are extremely difficult, because of the powerful contractions of the animal's muscles. Anaesthetics, to which the snails are rather insensitive, do not prevent contractions satisfactorily, even if the animals are so heavily drugged that they do not recover. In connexion with research on the function of the osphradium in *Bullia*, we were interested in severing the osphradial nerve of the living snail. At first we were at a loss for a successful method of performing such a delicate operation, but then it occurred to us that animals subjected to 'water rigour' might prove to be suitable subjects. A number of snails were therefore brought into this condition. As expected, the snails could then be operated on without any difficulties arising from muscular contractions. A window was cut in the shell, over the osphradium and dorsal pallial artery,

and the osphradial nerve was then severed with a small eye-surgeon's scalpel. When returned to undiluted sea-water, more than half the snails operated on recovered and were used for experiments on sensory perception. None of these showed any abnormal behaviour which could be attributed to previous 'water rigour'.

Those snails which did not recover were later dissected in order to discover the cause of death. In all of them it was found that structures other than the osphradial nerve had been injured during the operation; in most cases the pallium had been damaged. In no snail was there any evidence that the reduced salinity conditions to which they had been subjected had produced ill-effects.

Other work in our Department (unpublished experiments by G. Noble) has confirmed that 'water rigour' is a very effective aid when operating on *Bullia*, not only for the operation described above but also in removal or cauterization of organs possibly connected with sensory perception, such as the inhalant siphon, tentacles, etc. It seems likely that the 'water rigour' technique might find wider application in the field of gastropod physiology.

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¹ Broekhuysen, G. J., *Trans. Roy. Soc. S. Afr.*, **28**, 255 (1940).

² Brown, A. C., *Portugal Acta Biol.* (in the press).

IMMUNOLOGY

Natural Occurrence of Delayed-type Iso-hypersensitiveness

EVIDENCE has been obtained for the existence, in certain normal guinea pigs, of a state of delayed iso-hypersensitiveness to a heritable factor present in the serum of other normal guinea pigs. This condition, which arises at about 2 months of age, appears analogous to the long known¹ natural existence of circulating iso-antibodies to heritable blood group antigens in other species. Delayed-type hypersensitivity to isophile antigens may, of course, be experimentally induced (*cf. ref. 2*), but its spontaneous occurrence seems not to have been suspected.

The occurrence of delayed skin reactions among guinea pigs inoculated for the first time with the sera of others is illustrated by a typical experiment. The sera of 12 adult albino guinea pigs (bred from Rockefeller Institute stock) were injected separately into the skin of the back of each of the animals; thus, every animal received 0.1 ml. of 1:3 dilution in saline of its own as well as of the 11 other sera. As shown in Table 1, certain of the guinea pigs (Nos. 1-3) responded to the sera of most of the other animals, though not to their own, with erythematous skin

Table 1. DELAYED SKIN REACTIONS GIVEN BY CERTAIN NORMAL GUINEA PIGS TO THE SERA OF OTHER NORMAL GUINEA PIGS

Test of serum from guinea pig No. :	Reaction of guinea pig No. :				
	1	2	3	4	5-12
1, 2, 3	0	0	0	0	0
4	±	0	0	0	0
5, 6, 7	+	+	+	0	0
8, 9	+	+	±	0	0
10	+	±	+	0	0
11, 12	+	±	±	0	0

0, ±, + = erythema 0-4, 5-9, 10-14 mm. in diameter, at 18-24 hr.