

Solution	Toxin mgm. (dry weight)	Total diluent added			Final toxin concentration (mgm./ml.)
		Plasma (ml.)	Water (ml.)	0.9% Saline (ml.)	
A	20	0	1	19	1
B	20	1	1	18	1*
C	20*	1	1	18	1*
D	20*	1	1	0	10*

\* Weight of toxin expected if it were all present in the supernatant solution (C) or in the precipitate (D).

and then further with 18 vol. of saline to a final toxin concentration of 1 mgm./ml. (solution B, Table 1). A precipitate formed when the plasma was combined with the toxin which did not dissolve when more saline was added. This precipitate was uniformly dispersed through the injection fluid and was included in the assay. The third aliquot was combined with an equal volume of turtle plasma and centrifuged to remove the precipitate. The supernatant liquid was assumed to contain all the toxin and was so diluted with saline as to bring the final toxin concentration to 1 mgm./ml. (solution C, Table 1). The precipitate was resuspended in a solution consisting of 1 ml. of turtle plasma and 1 ml. of glass-distilled water (solution D, Table 1). If all the toxic material were contained in the precipitate the concentration would have been 10 mgm./ml. Table 2 presents the results of assays of these solutions.

Solution	No. mice injected	Dose-range (mgm./kgm.)	Surviving after 2 hr.
A	10	2.55-2.61	0
B	10	2.56-2.59	0
C	11	2.57-2.61*	0
D	10	47.0-56.2*	10

\* Weight of toxin expected if it were all present in the supernatant solution (C) or in the precipitate (D).

Apparently all the toxicity originally present in solution A is still present in the toxin-plasma combination B. The precipitate which was separated and resuspended in saline D contained none of the activity. The supernatant solution C held all the toxicity of the original combination.

This experiment was repeated twice, with fewer mice, with entirely similar results. These results suggest that the loggerhead turtle lacks blood immune bodies which might be invoked to explain its apparent insensitivity to the toxin of *Physalia*.

Our interpretation does not exclude the possibility that the loggerhead might possess localized tissue antibodies. Phisalix<sup>7</sup> has shown that reptiles, and especially turtles, are less sensitive to the sting of bees than other vertebrates. It is possible that turtles are not susceptible to venoms of the type injected by *Physalia*.

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<sup>1</sup> Babcock, H. L., *Proc. Zool. Soc. Lond.*, A, 107, 595 (1937).

<sup>2</sup> Barbour, T., "Naturalist at Large" (Little, Brown and Co., Boston, 1943).

<sup>3</sup> Carr, A. F., "Handbook of Turtles" (Cornell University Press, Ithaca, New York, 1952).

<sup>4</sup> Murphy, R. C., *Copeia*, No. 2 (1914).

<sup>5</sup> Lane, C. E., and Eleanor Dodge, *Biol. Bull.*, 115, 219 (1953).

<sup>6</sup> Kunitz, M. J., *J. Gen. Physiol.*, 35, 423 (1952).

<sup>7</sup> Phisalix, Mme. M., *Bull. Mus. Nat. Hist. Paris*, 6, 166 (1934).

## Elucidation of the Life-cycle of *Fasciola hepatica*

DR. B. DAWES, in his recent communication<sup>1</sup>, has made an important contribution to our knowledge of the trematodes. In the interests of historical accuracy and in justice to a great nineteenth-century zoologist I would, however, like to point out that the life-histories of several trematodes, including that of *Fasciola hepatica*, were beautifully and accurately described by J. J. S. Steenstrup in his work "On the Alternation of Generations", translated into English and published as a Ray Society monograph in 1845, nearly forty years before the work of R. Leuckart and A. P. Thomas. Steenstrup solved one of the most difficult of all zoological problems, and his ideas on the alternation of generations were considered so revolutionary that G. Buck, his translator and editor, thought fit to write an apologetic introduction to assuage the anger of his readers.

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<sup>1</sup> *Nature*, 184, 1334 (1959).

I AGREE with H. S. Jefferies that historical accuracy is desirable and that the credit for scientific discovery should go where it is deserved. Parasitology owes much to the work of Steenstrup, who demonstrated the 'alternation of generations' which occurs in the lives of some invertebrate animals<sup>1</sup>. This expression was first used in 1819, however, by the Franco-German poet and naturalist, Louis Adelaide de Chamisso, in regard to the life-cycle of the pelagic tunicates known as salps, but the young Danish zoologists showed that it applies to the life-cycles of some coelenterates, trematodes and other invertebrate animals as well. Valuable discoveries were made also by other zoologists, and some of these were mentioned in Chapter 16 and elsewhere in my book<sup>2</sup>, and others have since been indicated by E. G. Reinhard, with special reference to the life-cycle of *Fasciola hepatica*<sup>3</sup>.

In using the term 'cercaria', Steenstrup recognized both a discovery and an invention made in 1773 by O. F. Muller. The forms which he called 'nurses' were later named 'rediae' by F. de Filippi in honour of Francesco Redi who, in 1668, made all discoveries in this field possible by destroying the false doctrine of spontaneous generation. Such 'nurses' were first seen in 1818 by Ludwig Bojanus, who called them 'royal-yellow worms' and emergent cercariae were first seen to encyst in 1807 by C. L. Nitzsch of Halle, who thus witnessed the formation of what Steenstrup later called 'pupae'. Some earlier discoveries made by two of C. A. Rudolphi's pupils did not enter into Steenstrup's considerations, though they concern the trematode life-cycle. In 1831, K. E. Mehlis described the hatching of 'ciliated embryos' from the eggs of trematodes, and in 1837, F. C. H. Creplin observed that such forms (miracidia) emerge from the eggs of *Fasciola hepatica*. Unluckily, Bojanus, Steenstrup and other persons mentioned here by name seem not to have made a study of the parasites of *Limnaea truncatula*, and for this reason did not observe the larval stages of *F. hepatica*. Steenstrup made a brief reference (5 lines) to the adult of this species and then turned to descriptions of echinostome and stylet cercariae. He did not even mention the larval stages of the liver-fluke, and in fact he did