Work is in progress to determine what part, if any, these factors play in cellular damage *in vivo*.

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Dietary Fats and Thrombosis

In order to study the influence of the fat content of food on the occurrence of thrombosis in the arterial and venous system, a group of 133 hospital patients aged 65–90 years were treated with a diet in which butterfat, margarine and lard were replaced by vegetable oils (unhydrogenated corn oil and soybean oil). The daily intake of vegetable oils was about 40 gm., or half the total fats. The time of treatment of the 133 patients ranged from 1 to 17 months (average 4·7 months) representing a total of 52½ years. For comparison, a control group of the same size and composition who received an ordinary hospital diet containing about 80 gm. animal fats (including about 40 gm. butter-fat, margarine and lard) was used. The occurrence of thrombosis and embolism in both groups was diagnosed clinically and post-mortem.

In the treated group only 4 cases of thromboembolism were observed: 1 case of myocardial infarction after 6 days of treatment, 1 case of myocardial infarction after 26 days of treatment, both verified by autopsy; 1 case of little stroke with transient aphasia of a few days' duration occurring after 30 days of treatment, and 1 case of pulmonary embolism after 4 months of treatment in a patient with bronchial cancer (verified by autopsy).

In the control group 15 cases of thrombosis were observed: 2 cases of thrombophlebitis (1 case complicated with pulmonary embolism and 1 case with thrombosis in the carotid artery), 2 cases of pulmonary embolism (both verified by autopsy), 5 cases of myocardial infarction (all typical, 2 verified by autopsy), 6 cases of cerebral thrombosis (3 verified by autopsy).

The difference in the occurrence of thrombosis in the two patient groups was statistically significant ($t = 5{\cdot}1$) and cannot be interpreted in terms of variations in age, weight and blood pressure in the groups.

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BIOLOGY

Interaction between the Plasma of the Loggerhead Turtle and Toxin of the Portuguese Man-of-War

The loggerhead turtle, *Caretta caretta*, is reported to eat the Portuguese man-of-war, *Physalia physalis*[1–3]. Where *Physalia* are assembled in windrows, loggerheads are often seen at the surface with eyes closed, eating this siphonophore. *Velella*, an allied species, has also been identified from stomach contents of a turtle which was identified as a loggerhead[4]. *Physalia* is equipped with a singularly potent toxin[5]. This is contained in nematocysts which can penetrate a rubber glove and presumably even the chitinous exoskeleton of a crab's leg. As Barbour pointed out, the dense impenetrable skin of the face and jaws might protect a turtle from some of the obvious hazards of such a feast. However, since tentacles of active colonies have been observed to cling tightly to the skin, gloves, glass containers and even to the polished steel surface of a pair of scissors, it is difficult to explain the ability of the turtle to swallow, much less to digest and survive this meal. We believe that the acid of the stomach contents would inactivate the nematocyst and its contained venom virtually upon contact. The unconcern of the adult turtle with the toxic effects of the nematocysts might be due to the presence of protective antibodies in the blood acquired by contact with *Physalia* early in life. It is the object of this communication to report the results of experiments designed to test this hypothesis.

A 15-kgm. loggerhead turtle which had been in captivity less than one week was obtained from the Miami Seaquarium. The jugular vein was cannulated and the animal was bled. Sufficient potassium oxalate was added to the blood to bring the concentration to 0·2 per cent and the blood was cooled. Later the blood was centrifuged and the separated plasma was frozen at —20° C. without preservative. Toxin of *Physalia* was prepared according to procedures already described[5], with the following modifications: (1) Washed quartz sand and glass-distilled water were used for homogenization of the washed purified nematocysts. (2) After centrifugation, the material was mixed with 4/3 volumes of calcium phosphate gel prepared by the method of Kunitz[6] and was recentrifuged. The water-clear supernatant solution apparently contains all the toxic material. (3) This supernatant solution was lyophilized in tared 5-ml. capsules. These were sealed without admitting air and stored at —20° C. until used.

Fractions prepared in this way have regularly assayed at *LD*90 of 2·5–3·0 mgm./kgm. body-weight of pure strain, 18–25 gm. *C*57 black male mice when the mice are injected intravenously. The lyophilized material dissolves easily and completely. We assume that the entire weight of lyophilized material is toxic for purposes of estimating concentrations. It should be noted that if any significant fraction of the lyophilized 'toxin' were inactive then the active moiety would considerably exceed 2·5 mgm./kgm. in toxicity. The toxin is quite thermolabile and its activity decreases during manipulation.

In one experiment lyophilized toxin was dissolved in glass-distilled water to produce a concentration of 20 mgm./ml. One aliquot was diluted with 19 vol. of 0·9 per cent saline to a final concentration of 1 mgm./ml. (solution *A*, Table 1). The second aliquot was diluted with one volume of turtle plasma

Table 1

Solution	Toxin mgm. (dry weight)	Total diluent added: Plasma (ml.)	Water (ml.)	0·9% Saline (ml.)	Final toxin concentration (mgm./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.

Table 2

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[7] 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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Elucidation of the Life-cycle of *Fasciola hepatica*

Dr. B. Dawes, in his recent communication[1], 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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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[1]. 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 cœlenterates, 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[2], and others have since been indicated by E. G. Reinhard, with special reference to the life-cycle of *Fasciola hepatica*[3].

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 cercariæ 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 cercariæ. He did not even mention the larval stages of the liver-fluke, and in fact he did