of the infection. The primary lesions in the aortic wall of the cat are, however, much less intense than they are in the dog, and it seems not unlikely that in the cat the infection becomes abortive before the worms are able to mature.

The complete life cycle of *Spirocerca sanguinolenta*, therefore, needs include only two hosts, the insect (primary larval) and the dog (definitive), although a wide variety of vertebrates which are insectivorous or which ingest insects accidentally may become intercalated as reservoir intermediate hosts in the cycle. With the exception of the wolf and the fox, the dog is the only known definitive host of this worm. It is not unlikely, however, that other Canidae may also be found to be natural definitive hosts.

ERNEST CARROLL FAUST
DEPARTMENT OF TROPICAL MEDICINE,
TULANE UNIVERSITY

VISCOSITY CHANGES DURING EARLY CLEAVAGE STAGES OF FUNDULUS EGGS

IN the course of a series of experiments carried on during the summer of 1927, it was noted that the removal of the protoplasmic material of one of the first two blastomeres of the egg of *Fundulus heteroclitus* is accomplished with little or no difficulty at certain times, while at other times it is practically impossible. The observations made on this point contribute to our knowledge of the relative viscosity of protoplasm[1] during the cleavage stages and should, I think, be reported in this place.

As is well known, the egg of Fundulus is relatively large and the first two blastomeres may very easily be seen under even the low power of the binocular dissecting microscope. Being large, the blastomeres of the early cleavage stages contain a considerable amount of protoplasm which bulges within the chorion. It was the purpose of the experiments to remove all the protoplasm of one of the first two blastomeres. This was found to be quite possible during the period commencing shortly after the completion of the first cleavage furrow and ending shortly prior to the formation of the second cleavage plane. Before and after these stages, it was impossible to remove all the protoplasm of the cell without injury to the other blastomere and without the loss of a considerable amount of yolk. A brief examination of the physical state of the cellular material showed the reason for this.

The experiments were performed with a fine glass needle by means of which one of the blastomeres was punctured, allowing the cytoplasm to flow out. Thus they resemble the microdissection studies carried on in a much more refined way by Chambers and his coworkers. The toughness of the cortical membrane of the fish egg precludes the use of the finer method in these experiments. Nor is it necessary. By means of the microscopically fine glass needle it is possible to puncture one blastomere of the egg in any part and to express the contained cytoplasm. The behavior of the extruded material and the rapidity of the outflow may be directly observed. These give definite information as to the relative viscosity of the protoplasm. Very briefly stated, the results of such tests follow.

A short time before the formation of the first cleavage plane, a puncture with the needle results in the outflow of all the contained protoplasmic material. When examined, it is quite evidently very fluid. This stage is soon replaced by another at which the cytoplasm in the cortical region begins to thicken. This gelation of the cellular material involves not only the actual cortex of the cytoplasmic mound, but also crosses the cell in the region of the future cleavage plane. In this way, the future line of division is foreshadowed even before the margins of the cell have indented. It is probable that the gelation is initiated in this mid-region. The cytoplasm at this time is so stiff in the outer region that the puncture of the cell results only in the extrusion of the medially located cytoplasm. This leaves a hollow shell of relatively rigid cytoplasm in the center of which may be a yolk or water-filled space. In other cases the entire cortex may crumple in. From the cortical area, this stiffening spreads centripetally, accompanying cleavage until both daughter cells show a high relative viscosity throughout. At this stage the removal of all the cytoplasm in one blastomere can not be accomplished without at the same time removing a large amount of yolk and in all probability injuring the sister blastomere.

The gelation effect is almost immediately reversed, so that the protoplasm of the cells again becomes very low in its relative viscosity. It is during this stage that it is expressed most easily, with very little loss of the yolk material, and no deleterious effects to the neighboring cell. The blastomeres remain in this state until just before the second cleavage, when the cortex again stiffens slightly. This is accompanied by a change in the cell itself similar to that found just prior to the first cleavage. The cytoplasm in the region to be occupied by the new line of division also stiffens. This region of higher viscosity is continuous with that of the cortex of the cells. There is every reason to believe that the cycle of changes just described for the so-called resting phases between the first and the second cleavages is repeated between

[1] For literature on this subject see L. V. Heilbrunn, *Quart. Rev. Biol.*, II, p. 230, 1927.

the second and the third, etc. Though the blastomeres become smaller and direct observation more difficult, it is perfectly evident after some experience that the most perfect elimination of the contents of the cells in the early stages may be accomplished after about two thirds of the total time elapsing between the two cleavages concerned has passed.

LEIGH HOADLEY

ZOOLOGICAL LABORATORY,
HARVARD UNIVERSITY

MANGANESE AS A FACTOR IN HEMOGLOBIN BUILDING

FOR the past year the authors have been studying the problem of nutritional anemia as produced when whole milk is fed as the sole diet.

Hart and coworkers have reported that the ash of lettuce and cabbage when added to whole milk and iron make a satisfactory diet for hemoglobin building. In our work we have found that the ash of alfalfa and other plants can be utilized in a like manner with almost if not quite as good results.

These facts, together with certain observations and conclusions of Bertrand, McHargue and others, that manganese is essential in plant growth and for the formation of chlorophyl, led us to the study of manganese as a factor in hemoglobin building.

In our experimental work we have attempted to use the rabbit, but with not very great success, due to the fact that the young rabbit when on a deficient diet is so susceptible to coccidiosis and other diseases that our losses have been excessive before we could produce a severe anemia. More recently we have been using the rat for this work as suggested by Waddell, Steinbock, Elvehjem and Hart and find this animal much more satisfactory.

In this preliminary paper we wish to report on the effectiveness of manganese in hemoglobin building, particularly as it affects rabbits. Some forty rabbits have been on this experiment but not all have received a purified diet. Two rabbits made anemic on a whole milk diet were fed five mgs of copper-free iron chloride and 0.5 mgs of copper-free manganese chloride.

Table I shows the hemoglobin content of the blood during the experiment.

TABLE I

Date 1928		Weight	Grams Hemoglobin per 100 cc blood	Diet
Rabbit No. 662				
Jan.	14	375	12.24	Whole milk only
	19	430	12.36	
	27	440	12.24	Added 5 mgs. iron in form of $FeCl_3$
Feb.	8	570	10.20	
	14	655	8.58	
	21			Added 0.5 mgs. manganese + 5 mgs. of iron, the manganese in form of $MnCl_2$
Mar.	12	1010	11.43	
	26	1135	13.26	
Apr.	30	1356	11.43	
May	15	1540	9.09	
June	20	1779	11.22	
July	26	1779	11.22	
Rabbit No. 678				
May	15	556	12.24	Whole milk only
	22	595	10.62	
	28	625	9.59	Added 5 mgs. iron as $FeCl_3$ + 0.5 mgs. manganese as $MnCl_2$
June	8	855	11.22	
	20	944	14.67	
July	9	—	14.60	
	26	1375	12.03	

The results shown above indicate that manganese has a beneficial effect when added to a milk-iron diet.

Manganese apparently exerts the same beneficial effects upon rats as upon rabbits, as is indicated by a number of these animals which we now have on experiment.

In our experimental work we have exercised every precaution to eliminate copper from the ration, since Hart and coworkers have shown this element to be effective in hemoglobin building.

The manganese used was a Baker & Adamson manganese carbonate, C. P. This salt was dissolved in the least quantity of hydrochloric acid, then treated with hydrogen sulphide under pressure for twelve hours. The solution was then filtered and the excess hydrogen sulphide removed by boiling. The iron used was prepared from a very high purity Bureau of Standards iron and treated with hydrogen sulphide to remove any copper.

The milk was handled and kept in aluminum cans and the animals were kept in galvanized wire cages on wire screens. Porcelain mortars were used for feeding dishes.

Further work is being done on this problem of the relation of manganese to hemoglobin building, the results of which will be reported in more detail a little later.

R. W. TITUS
H. W. CAVE

KANSAS STATE
AGRICULTURAL COLLEGE