PHYSIOLOGICAL ONTOGENY.

A. CHICKEN EMBRYOS.

XIV. THE HYDROGEN ION CONCENTRATION OF THE BLOOD OF CHICKEN EMBRYOS AS A FUNCTION OF TIME.

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These experiments continue to illustrate an interest in the processes of growth which are exhibited in earlier communications (1). We have turned now to the study of a more general function and have studied the changes which take place in the hydrogen ion concentration of the blood of chicken embryos. Chickens were selected in the expectation that the new data might form part of the information already accumulated and might serve the purpose of further inferences and wider generalization.

The ages of the embryos studied ranged between 8 and 20 days of incubation. It was impossible to secure sufficient volumes of blood before this age to permit measurements to be made, even when the vessels were successfully punctured by the needle of the syringe. We regret this omission from our data the more because we are aware of the fact that it is precisely at this early age, from 5 to 8 days, that information is desirable. It may be possible at another time to return to this period of the life cycle when methods other than those now available are at our disposal. During the period of hatching and in the first days of life, other difficulties beside that of drawing blood were encountered. Although no difficulty was experienced at the time of hatching in obtaining blood, it was found necessary to carry out this procedure either while the animals struggled or when they had been rendered quiet by the use of anesthetics such as amytal. In both cases, the blood must inevitably be unnatural as the result of dis-

turbance of the oxidation processes, due either to excess lactic acid formation during struggling, or in anesthesia to moderate asphyxia and to diminished rate of blood flow. We learned in the course of our experiments to avoid including measurements taken of the blood of embryos after they had been injured or when they were in a moribund state. In both cases there is a strong tendency for the blood to be more acid than is natural. We came in the end to recognize the presence of these states from examining the blood alone. It has occurred to us that the inclusion of such estimations might result in the publication of erroneous curves.

The incubation of the eggs of White Leghorn hens was arranged in the manner formerly described (2). The ages of the embryos were estimated with care, subject to the difficulties which are usually encountered even when eggs are brought promptly to the incubator after having been laid. The embryos were subsequently studied without removal from the incubator room. The glass electrodes (3), employed in measuring the hydrogen ion concentration, were also installed here. Embryos and their shed blood were, in short, constantly at $38^{\circ} \pm 0.5^{\circ}\text{C}$. The wires from the electrodes were led, appropriately sheathed, from the incubator room, through its wall to the adjoining room, where the electrometer was housed.

Blood was drawn either from a vein (arterial blood) or from an artery (venous blood) through a needle into the barrel of a syringe. Into the end of the syringe, a second one which contained the piston was fitted identical with the first. The syringe system from point of needle to piston was filled with paraffin oil. A second person manipulated the piston.

In opening the eggs it was necessary to avoid the occurrence of hemorrhage. Help toward this end was obtained by painting the shell membrane, after enough of the shell was chipped away, with paraffin oil. By this means two objects were attained; first, it was possible to locate blood vessels, and second, capillary hemorrhage was prevented. It is not certain how the effect in preventing hemorrhage took place nor whether the method was free from danger; capillaries may have closed because the membranes, coated with oil, became impermeable to the passage of gases. Usually the amount of the surface of the egg affected in this way was small. When, through

clearing the membrane, blood vessels became visible, it was often possible to insert a needle into them without further dissection—obviously a great advantage. Both oxygenated and reduced blood was obtained from embryos of all ages; in older embryos, both varieties were often drawn from the same animal.

Measurements of the reaction of the blood could be undertaken with volumes no greater than 0.25 cc. When withdrawn the blood

FIG. 1. A curve is shown (the solid line) indicating the course of the change in hydrogen ion concentration in the arterial (reduced) blood of chicken embryos. The circles represent the average pH of the blood at each day of incubation. In the middle of the curve, corresponding to the period between 10 and 14 days, there is a flattening of the curve. A curve (dotted line) published by Murray showing alterations with time of oxygen consumption, flattens at the same period; the ends of the two curves follow similar courses.

was plunged into Ringer solution already under oil in such proportions that the dilution of blood to solution was as 1 to 3 or 4. A small amount of sodium fluoride was likewise present in the test-tube to inhibit glycolysis. The effect of dilution to this extent in the value of the measurement, since it was not sufficiently known, has been studied. Preparations were made of undiluted and of diluted blood and estimated. The difference in value between the two was found

to be no more than a few hundredths of a pH. So diluted and prepared by vigorous stirring to prevent coagulation, the blood was transferred to the cup of the glass electrode. Measurements required so little time that the rate at which they could be made depended on the dissection of the embryos and the withdrawal of blood rather than on the manipulation of the apparatus.

We found a consistent change in the hydrogen ion concentration of the blood throughout that period of embryonic life which we examined. The general trend of the change was from acid to alkaline (Fig. 1 and Table I). At 8 days the pH averaged 6.92 and at 20 days 7.22. The lowest figure which was encountered was 6.75 in an 8 day embryo. We may point out that the lowest pH encountered so far in human beings is 6.95* and was found in a patient in a moribund state (4). It should be pointed out that the value in the venous blood of an adult animal, when the blood was withdrawn from an animal lying quite still without the use of anesthesia was 7.30. It is important to notice the low value in embryos of younger age. It suggests inferences that may be drawn from investigations published by Warburg and his collaborators (5) in connection with measurements of rapidly growing tissues in general, in the case either of tumors or of embryonic tissues in the period when their mass is undergoing great increase. We may recall that in this connection Warburg's measurements dealt with the rate of glycolysis measured in terms of the rate of lactic acid formation. There is to be found in this process not only increase in acid formation but actually an increase in the acid reaction. We are not in position to say that these two phenomena are necessarily mutually dependent. We shall return to this point.

We have for comparison with the results in fowl, studied the blood both arterial and venous, of the fetuses of cats. We were unable, unfortunately, to secure a continuous series so that data could be systematically collected according to time. The fetuses that were studied differed in age but the dating was too uncertain to allow us to draw a curve of the rate of change which takes place. The measurements which were made covered the range from pH 7.04 to 7.31. These it will be perceived lie within that found in chicken embryos. It

* In a personal communication Dr. D. D. Van Slyke tells us that this is the lowest pH of which he is aware.

appears that the reaction which is found in chicken embryos is in no way peculiar to this family, but is characteristic of embryonic and perhaps of rapid growth in general. Okuneff (6) has in point of fact shown that plant tissue in the stage of regeneration after injury likewise exhibits a low pH.

TABLE I.
*The Changes in Hydrogen Ion Concentration of the Blood in Chicken Embryos.**

Incubation age	Reduced blood		Oxygenated blood	
	Average pH	No. of observations	Average pH	No. of observations
<i>days</i>				
8	6.92	13	7.03	8
9	7.12	4	7.18	5
10	7.06	6	7.23	3
11	7.15	4	7.19	1
12	7.11	4		
14	7.16	2	7.19	1
15	7.11	5		
16	7.22	4	7.22	3
17	7.24	3		
18	7.19	5	7.23	4
19	7.20	4	7.20	2
20	7.22	5	7.23	2
Adult	7.30		7.34	

* The standard deviations from the averages are not given. They are wider than would be encountered in the normal resting human subject. At the 8th day for instance, the range is 6.75 to 7.11. We have not given the figures in detail because in our opinion they are in a sense misleading. We believe the wide range is due to the impossibility not only of dating accurately the age of the embryos but also of excluding the possibility of injury. There seems now no reason to doubt that the pH of embryos, if they could be known to be of the same age, would be similar within a narrow range.

On our curve of change in the hydrogen ion concentration, we have inserted the points indicative of the change in oxygen consumption obtained by Murray (7) as expressive of change in the rate of metabolism. It is difficult to escape the impression that the two observe, while not a parallel, at all events a somewhat similar course. As a result of this comparison arises the suggestion either that both are

expressions of the same underlying process, or that they stand in some causal relation to each other. That oxygen consumption and lactic acid formation are both expressions of the metabolic function, is already known. The fact that the curve expressing the change in hydrogen ion concentration takes the form it does in respect to Murray's curve of oxygen utilization raises the question of the possibility of the existence of a relation between lactic acid production and the concentration of hydrogen ions. We are not in possession of measurements which place the formation of lactic acid in a determining position in respect to reaction but have been led to think of this possibility on account of the facts which have just been described.

SUMMARY AND CONCLUSION.

We have studied by means of glass electrodes the hydrogen ion concentration of the blood of chicken embryos from 8 to 20 days. When plotted as a curve, the average data show that a constant change takes place in the measurement, being acid at the beginning of this period and becoming alkaline toward its end. The acid reaction we think was characteristic not only of the embryos of fowl, but of the fetuses of cats and indeed as Warburg has shown of rapidly growing tissue in general. We have suggested the possibility, though we have no data to substantiate the suggestion, that the hydrogen ion concentration may under conditions like these, seeing that the curve of change resembles that of oxygen consumption, be expressive of changes in the rate of metabolism.

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