amino acid solution per tube, supplemented with 0.7 mg of L-glutamine (heat labile) per tube, was found to substitute partially (Table II) for the peptone (concentrations given in mg %): DL-alanine (51.9), L-arginine monohydrochloride (51.9), L-asparagine monohydrate (5.18), L-cysteine hydrochloride (10.4), glycine (5.18), L-histidine monohydrochloride monohydrate (5.18), DL-isoleucine (51.9), L-leucine (26.0), DL-lysine monohydrochloride (10.4), DL-methionine (5.18), DL-norleucine (10.4), DL-norvaline (10.4), DL-phenylalanine (26.0), L-proline (5.18), DL-serine (10.4), DL-threonine (10.4), DL-tryptophan (5.18), L-tyrosine (10.4), and DL-valine (38.9). The solution just described will be referred to hereafter as Solution A, and unless otherwise indicated the amount used per tube was 1.0 ml.

Growth equivalent to that obtained with peptone (Table II) was obtained with the amino acid mixture (Solution A) when it was further supplemented with 0.7 ml of the following vitamin-purine-pyrimidine-salt solution per tube (concentrations given in micrograms per cent): thiamine hydrochloride (6.7), pyridoxine hydrochloride (10.7), pyridoxal hydrochloride (0.67), pyridoxamine dihydrochloride (0.67), calcium DL-pantothenate (13.4), riboflavin (13.4), niacinamide (13.4), biotin (0.034), *p*-aminobenzoic acid (0.67), folic acid (0.034), choline chloride (51.1), inositol (168), adenine sulfate (80), guanine (80), uracil (80), xanthine (80), $MgSO_4$ (658), $FeSO_4 \cdot 7H_2O$ (67), and $MnSO_4 \cdot H_2O$ (51.1). The solution just described will be referred to hereafter as Solution B, and unless otherwise indicated the amount used per tube was 0.7 ml.

The basal medium supplemented with dialysed rabbit serum and Witte's peptone was found to support growth of *Leptospira canicola* through a prolonged series of transfers. When the peptone was replaced by Solutions A and B plus glutamine good growth was obtained in the first transfer, but growth was reduced in subsequent transfers. It was apparent, nevertheless, that the semisynthetic medium consisting of the basal medium supplemented with dialysed rabbit serum, glutamine, and Solutions A and B might serve as a basis for determining many of the amino acid and vitamin requirements of *Leptospira canicola*. Experiments to determine these requirements as well as further studies on the nature of the serum factor and the peptone factors required for good growth in serial subcultures are in progress. The results of these experiments are to be given in subsequent papers from the authors' laboratories.

Summary. Nutritional experiments with *Leptospira canicola* have been described. A semisynthetic medium containing dialysed rabbit serum, salts, vitamins, amino acids, and purine and pyrimidine bases was developed and appeared to be adequate as a basis for determining amino acid and vitamin requirements of this organism.

Received August 7, 1950. P.S.E.B.M., 1950, v75.

Respiration of Embryonic Blood. (18148)

CHARLES C. BOYER. (Introduced by J. O. Foley.)

From the Department of Anatomy, Medical College and School of Dentistry, University of Alabama, Birmingham, Ala.

Embryonic respiration has long been a matter of great interest to physiologists. A review of extensive literature on the subject will not be attempted. Hall(1) briefly reviews contemporary works on the respiratory role of the blood in his report on hemoglobin function in the blood of the chick embryo. These works, however, have been largely qualitative and concerned with the changes in the character and function of hemoglobin

1. Hall, F. G., *J. Physiol.*, 1934, v83, 222.

throughout development. The data here reported are the results of preliminary investigations into the quantitative changes in respiratory rate of the chick embryo, day by day, throughout the period of incubation. Since methylene blue is known to be a catalyst in respiration of adult blood it was deemed feasible to ascertain its effect on respiration of embryonic blood.

Material and methods. Determinations of oxygen consumption were made on samples of whole blood. Eggs from white leghorn fowls were incubated in an electric incubator under carefully controlled conditions of temperature (100°F) and humidity (65% at 100°F). Under optimum conditions a hatch in excess of 90% was obtained. Blood samples were taken at ages of 14, 16, 18, 19, 20, 21, 22, 23, 24, 26, 28, and 29 days after the onset of incubation. In the case of the younger embryos the blood was drawn from the vitelline artery into an hypodermic syringe. From those 18 or 19 days of age and older the blood was taken by direct cardiac puncture. Samples 2 cc in volume were used throughout, coagulation being prevented by the addition of 1 mg of heparin. It was found necessary to pool the blood from several embryos to obtain the required volume for a determination, but according to Hall(1), the variation among embryos is so slight as to introduce no significant error in so doing. The apparatus employed was that of Warburg. The vessels used with the apparatus were those having an approximate volume of 20 cc with an "inset-cup" in the center and a side-arm. Brodie solution was used in the manometers. The water bath was maintained at a constant temperature of 37.7 ± .05°C by an ether and mercury contact thermo-regulator. The manometers were shaken in the bath through an angle of about 25° at the rate of 126 oscillations per minute.

In most cases 2 cc of whole blood were placed in the main chamber of the vessel, .5 cc of 10% KOH in the inset cup to absorb the CO_2 evolved, and .75 cc of .06% methylene blue in .8% NaCl solution in the side arm. In 2 determinations only .25 cc of KOH and .50 cc of methylene blue were used, the total volume of the contents of the vessel being made up, as always, to 3.25 cc by the addition of .5 cc of .01 M solution of KCN. The manometers were shaken in the water bath for 20 minutes with the stopcocks open to the atmosphere. Equilibration was found to be complete at this time. The stopcocks were then closed and the manometers shaken for 30 minutes before the first reading was made. The manometers were then read at the end of ten-minute periods for, in some cases, one hour, and, in others, one and one-half hours. The curves for samples of the same age followed the same course over periods of either duration. At the end of this time the methylene blue was poured in from the side-arm and readings again made every ten minutes for periods of the same duration as before. Three samples were run concurrently, a fourth manometer containing distilled water in place of the blood was used in every case as a thermobarometer.

Cell volume was determined only in 2 or 3 cases. For obvious reasons it could not be satisfactorily determined at the end of the experiment after methylene blue had been added, and because of the difficulty in obtaining sufficient quantities of blood, it was not

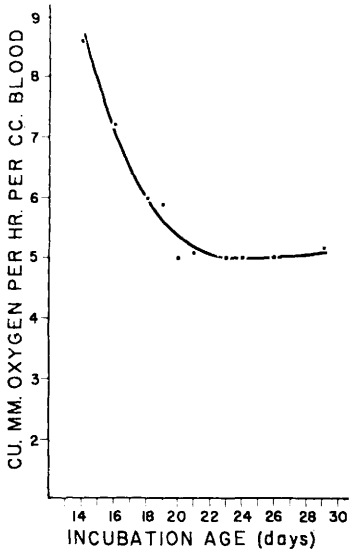

FIG. 1.
Summary curve showing quantitatively the rate of respiration of embryonic blood during incubation.

TABLE I. Cell Volume and Oxygen Consumption.

Age	Cell volume	O_2 consumption per cc blood per hr
28 days	29.5%	5.1 cu mm
24 "	30.0	5.0
23 "	33.3	5.25
22 "	31.9	5.1
21 "	34.3	5.1
20 "	30.8	5.0
19 "	34.9	5.9
18 "	32.1	6.0
—	19.0-35.7*	—

* Figures of Tipton[2] for chicken blood.

investigated at the beginning. Tipton(2) demonstrated an apparent lack of correlation between red cell count, red cell size and red cell volume, on the one hand, and oxygen consumption, on the other, in the blood of the adult chicken. Cell counts were not made in this experiment but cell volume determinations made on the blood of embryos, by means of the Wintrobe(3) hematocrit tube, showed a lack of correlation with rate of oxygen consumption similar to that found by Tipton(2).

Results. The relative rate of respiration of the embryonic blood of the chick was found to decrease steadily from 14 to 19 days after the onset of incubation. From this time onward until 29 days after the onset of incubation the rate is virtually constant (Fig. 1). Cell volume at various ages was determined by means of the Wintrobe hematocrit. The percentage cell volume was found to fluctuate within rather wide limits although, in general, it becomes less as the age increases. However, there seems to be no direct correlation between cell volume and the rate of respiration in embryonic blood (Table I).

Barron and Harrop(4) and Tipton(2) have previously pointed out that methylene blue has no appreciable effect on the respiration of chick erythrocytes. (These findings are here confirmed.) Although this is true, methylene blue does demonstrate its usual capacity to neutralize the effect of cyanides. In 2 determinations .5 cc of a .01 M solution of KCN was added to the usual sample of blood. This was not a sufficient quantity to completely suppress respiratory activity but it did show a marked inhibitory effect on it (Table II).

Upon the addition of methylene blue to blood containing cyanides it acts only to overcome the effect of the cyanide. The rate of respiration of the sample returns toward that found for normal unadulterated blood or blood containing methylene blue but no cyanide. Thus, although methylene blue shows no effect on normal respiration it does neutralize the effects of KCN and raises the oxygen consumption close to but not above normal at 29 days. At 20 days the effects of KCN are neutralized but the recovery in oxygen consumption was found to be slight. Reasons for this differing effect with age have not been established.

Discussion. The respiratory rate of embryonic blood of the chick, determined by the oxygen consumption of samples of a given volume of blood, is found to decrease gradually from 14 to about 19 days after the beginning of incubation. From this time onward there appears to be little change. Hall(1) postulates a change in the character of the hemoglobin molecule from the embryonic to the adult condition. When the embryo consumes only the oxygen that can diffuse through the egg shell it is respiring from an atmosphere of low oxygen tension. When this condition prevails, a hemoglobin with a high affinity for oxygen is needed. When the egg shell becomes permeable to gases, as it does with age, and later when the chick tears the shell membrane and breathes from the air-space and eventually from the atmosphere directly, the oxygen tension of its atmosphere is greater and a hemoglobin with a somewhat lesser affinity for oxygen is supplied. It is the changing ratio of the quantities of these types of hemoglobin that accounts for the shift of the dissociation curve for embryonic blood of the chick as age progresses so that the position of the adult dissociation curve is gradually approximated (Hall)(1).

It is during the earlier stages of development, when the blood volume and the oxygen

2. Tipton, S. R., *J. Cell. and Comp. Physiol.*, 1933, v3, 313.

3. Wintrobe, M. M., *J. Lab. and Clin. Med.*, 1929, v15, 287.

4. Barron, E. S. G., and Harrop, G. A., Jr., *J. Biol. Chem.*, 1928, v79, 65.

TABLE II. Effect of KCN and Methylene Blue on Oxygen Consumption, Age, O_2 Consumption per cc Blood per Hour.

Age	Normal blood	Blood plus KCN and M.B.	Blood plus KCN	Increase with KCN and M.B. over KCN without M.B.
20 days	5.0 cu mm	1.4 cu mm	1.2 cu mm	.20 cu mm
29 "	5.2	5.0	2.2	2.80

tension are low, that hemoglobin with the highest affinity for oxygen is present. At this stage a given volume of blood shows a high oxygen uptake. In later stages, when the blood volume and oxygen tension are greater, there is present a greater number of molecules of hemoglobin having a lesser affinity for oxygen. At this stage the same volume of blood has a smaller oxygen uptake. However, this is only true if the percentage cell volume and the needs of the embryo are considered. As pointed out above, there seems to be no very exact correlation between cell volume and rate of oxygen consumption of blood (Table I).

The growth rate from 14 days of incubation is very small and steadily becomes less. This seems to indicate that the needs of the embryo are becoming relatively constant during this period.

Previous workers [Wright(5) and Tipton (2)] have demonstrated the fact that young blood cells produced during anemia have a much higher respiratory rate than have mature red blood cells. These "primitive" cells, however, are formed in a different locale than are the young cells found in embryonic blood. Thus, though the two be structurally similar, they may be physiologically dissimilar. Primitive cells produced during anemia are much larger than mature red blood cells (Tipton) (2). These may show their high respiratory rate because of the fact that their production is initiated in a previously established hemopoietic organ by a complex mechanism set in motion by the lack of a sufficient quantity of cells. Thus, their high respiratory rate may be due to a process of rebuilding or differentiation.

Young embryonic cells, on the other hand, arising from the generalized mesenchyme, because of their rapid growth, would be expected to have a high metabolism and therefore their oxygen uptake should be greater, as shown.

The growth rate of the embryo is ever decreasing from at least 7 days of incubation onward. Since fewer and fewer young cells are being added to the circulation, the ratio of mature or maturing red blood cells to young or primitive red blood cells would seem to be going toward the side favoring the older cells. If then the respiratory rate of younger cells is greater than that of the mature cells it would seem that the gradual decrease in the rate of respiration of embryonic blood found to take place from 14 to 19 days of incubation might conceivably be due in part to the fact that there is a gradual diminution in the number of young red blood cells in comparison with the number of mature cells in the circulation. Supporting this, to bring about the drop in respiratory rate with age, there might also be considered the change in the relative quantities of the two types of hemoglobin present in the developing and mature chick, as postulated by Hall(1).

Summary. 1. The rate of respiration of a given volume of embryonic blood gradually decreases from the 14th to the 20th day of incubation. From this time until 29 days after the onset of incubation (the greatest age investigated) it remains virtually constant. 2. It is suggested that the decrease in respiratory rate is due to a decrease in the number of young cells having a high respiratory rate and a hemoglobin with a lesser affinity for oxygen. 3. Methylene blue shows no catalytic effect on the rate of respiration of embryonic blood of the chick. It will, however, counteract the inhibitory effect of KCN when present.

5. Wright, G. Payling, *J. Gen. Physiol.*, 1930, v14, 179.

Received August 22, 1950. P.S.E.B.M., 1950, v75.