

PHYSIOLOGICAL ONTOGENY.

A. CHICKEN EMBRYOS.

XII. THE METABOLISM AS A FUNCTION OF AGE.

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The rates of absorption, storage, and elimination of energy are perhaps the best indices that we possess of the vitality of a living organism. Since it has been frequently verified that: $[A]$ absorbed energy = $[S]$ stored energy + $[E]$ eliminated energy; and since, by the chemical analyses of embryos at successive ages, the rate of storage has been ascertained (1), it remains only to obtain the catabolic activity of the embryo in terms of age. This has been done by the manometer method for the estimation of oxygen, and the present communication reports the results. The initial estimations of CO_2 production reported elsewhere (2) were admittedly subject to a number of unknown variables determinable with difficulty, such as variations in the concentration of CO_2 in the embryo and in the albumin and yolk, together with the contribution made by the carbonates dissolved from the shell. These values are functional to the carbon dioxide tension about the egg, and since, moreover, they cannot be estimated with great precision, a statistical analysis of the data from a very large number of eggs would be necessary. For these reasons we chose to measure metabolism by the oxygen consumption.

Method.

A Warburg manometer was used, attached to a special glass vessel to contain the hen's egg. The egg rested upon glass tips projecting from the walls to suspend it above the bottom which was layered with 5 cc. of a 1.0 N NaOH solution.

The volume of each apparatus, and thus its constant, was obtained by the method of Warburg (3). Brodie's fluid was used in the manometer. The volume occupied by the egg contents in the vessel, *i.e.* the whole egg minus the air sac, was assumed to be equal to the weight of the egg minus the weight of the shell (approximately 6 gm.). In general, eggs of the same size and shape were selected.

A control was made with each test; at first in the form of a fertile egg of 1 day incubation, but later, when this was found unnecessary, with a vessel empty except for the alkali. The experiments were done in a constant temperature room, eliminating thereby the use of a water bath.

A thermometer ground into each glass cover registered the temperature, which averaged approximately 39.0°C. The small fluctuations which did occur were not found to affect appreciably the results. Moreover, it was found that the large surface of alkali exposed provided for maximum absorption of CO₂ without the necessity of shaking. Shaking for a minute prior to reading the manometer made no difference in the result. Nor was it found, when thin rubber tubes in which cold water flowed were run along one side of the vessel to cool the wall over this area and thereby to initiate by convection a regular circulation, as in Barach's (4) recently constructed human oxygen chamber, that any acceleration of CO₂ absorption took place.

About $\frac{1}{2}$ hour (the length of time being judged by the behavior of the control) was allowed for conditions to reach equilibrium. After this time readings were taken at varying intervals during periods of 2 to 6 hours until repetition of approximately similar results made one confident of their reliability. During the intervals between tests, the manometer and vessel were connected with an oxygen bag, so that the concentration of oxygen within the vessel remained always the same.

To obtain values for the rate of oxygen absorption per gm. of body weight the following figures are necessary, (1) the constant for the vessel (previously calculated); (2) the manometer readings, (3) the weight of the whole egg, and (4) the weight of the embryo.

One phenomenon was observed which we have not been able to explain. The embryo of an incubation age over 16 days, even when connected with the oxygen bag, did not survive in the apparatus over 12

hours. Their metabolism after 3 to 6 hours gradually fell. If the vessel was fully opened to the air for a few minutes the embryo would revive. Apparently it had nothing to do with a lack of oxygen, accumulation of CO₂, or changes in the humidity; neither was there an accumulation of ammonia.

TABLE I.
Metabolism of Chicken Embryos as a Function of Age.

1	2	3	4	5	6	7	8	9	10	11
Age.	No. of observations.	By experiment. O ₂ per day per gm. wet weight.	Standard deviation. =	From curve. O ₂ per day per gm. wet weight.	O ₂ per day per gm. dry weight.	Solid oxidized per day per gm. dry weight.	Solid stored per day per gm. dry weight.	Absorption per day per gm. dry weight.	CO ₂ per day per gm. wet weight.	Resp. quot.
days		cc.		cc.	cc.	gm.	gm.	gm.	cc.	
6	8	50.0	2.1	50.0	896	0.444	0.665	1.11	29.9	0.60
7	5	38.4	2.3	43.0	735	0.364	0.584	0.95	29.6	0.69
8	3	36.7	1.5	39.0	628	0.311	0.510	0.82	29.3	0.75
9	10	39.2	1.3	36.5	562	0.278	0.478	0.75	29.0	0.79
10	7	32.8	1.2	35.0	500	0.248	0.465	0.71	28.5	0.81
11	7	33.9	1.2	34.0	442	0.219	0.465	0.68	28.0	0.82
12	9	33.9	1.2	33.2	378	0.187	0.465	0.65	27.0	0.81
13	5	32.4	0.7	32.5	322	0.159	0.465	0.62	25.7	0.79
14	5	30.3	0.6	31.7	259	0.128	0.447	0.57	24.0	0.76
15	7	34.3	1.3	30.5	209	0.103	0.395	0.50	22.0	0.72
16	11	29.0	1.4	28.7	175	0.087	0.320	0.41	20.1	0.70
17	5	26.1	1.0	26.2	152	0.075	0.250	0.33	18.1	0.69
18	6	21.1	1.3	23.2	131	0.065	0.215	0.28	16.2	0.70
19	3	20.5	0.3	20.0	113	0.056			14.2	0.71

Column 6 = figures calculated by the aid of values for the percentage of solid substance previously determined (Murray (1)).

Column 7 = values in Column 2 divided by 2019.3 (amount of oxygen absorbed when 1 gm. of fat is burned).

Column 8 = figures previously obtained (Murray (1), Table III).

Column 10 = figures read from smooth curve previously obtained (Murray (5)).

RESULTS.

The results of the oxygen determinations (Table I) may be seen (Fig. 1) to demonstrate a decrease in metabolic rate per gm. of body weight with age. This conclusion confirms that reached when the carbon dioxide was determined; except that in a more precise analysis

and comparison of the results it appears that the oxygen estimations show a sharp fall of metabolism during the first days of the period under observation, whereas the carbon dioxide figures do not (5). As has been mentioned in the introductory remarks, however, numerous complications arising from the variability of unknown factors cast doubt upon the value of CO_2 elimination as a measure of catabolic change.

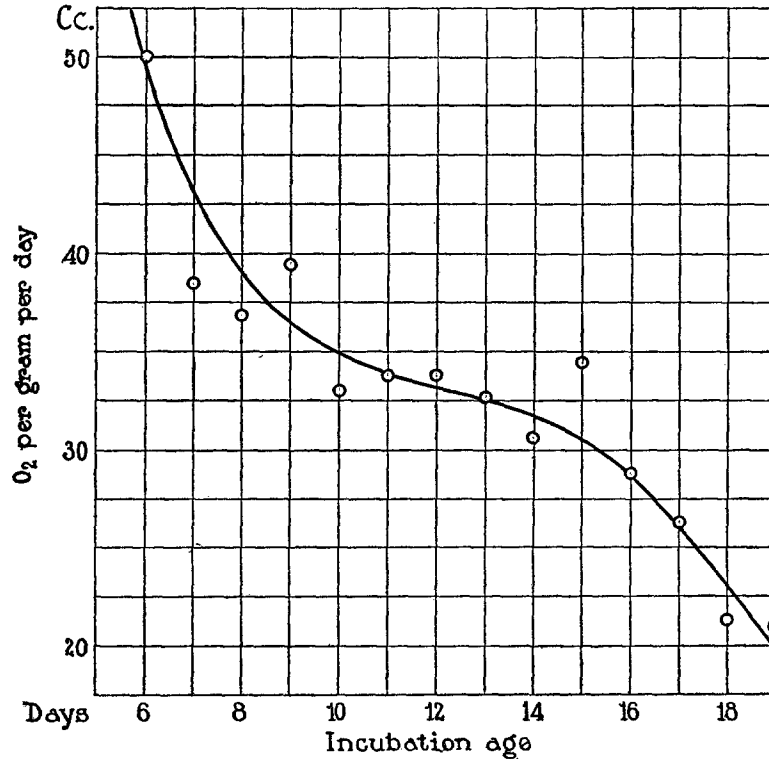


FIG. 1. Oxygen consumption in cc. per gm. of wet weight of chick embryo per day, as a function of age.

The oxygen determinations on the other hand were well controlled and presented no obvious factor to vitiate their use as indices of metabolic activity.

To oxidize 1 gm. of fat approximately 2000 cc. of O_2 are absorbed; whereas to oxidize 1 gm. of protein (966.3 cc.) or starch (828.8 cc.) about 900 cc. of O_2 are used (6). The total oxygen consumption for

the first 19 days, estimated by graphical integration, comes to 2988 cc., which on the basis that only fat is burned during incubation leads to the conclusion that 1.48 gm. of dry substance (*i.e.* fat) is oxidized during that period. If only protein and starch were burned, it would require over 3.28 gm. to use the observed amount of oxygen. Previous chemical analyses have shown that approximately 1.62 gm. of substance is burned during the first 19 days, a figure which may now be accounted for on the assumption that 92 per cent of the metabolism is oxidation of fat, and the rest of protein and carbohydrate. This value is to be compared to 98 per cent fat oxidation found by measuring the CO₂ output. The former figure is probably more accurate.

During the last 5 days of incubation, when about four-fifths of the total oxidation takes place, the respiratory quotient is approximately 0.71, which points to fat consumption, during this period. The earlier values for the respiratory quotient are somewhat higher (up to 0.81); but they are variable and it is uncertain whether they deserve consideration. The results point to some error during the first 3 days when the CO₂ figures, and thus the quotient, also seem to be definitely too low.

If we discard the carbon dioxide estimations in favor of these later O₂ determinations and assume as we may without undue error that catabolism is at the expense of fat, we arrive at some notion of the changes in the metabolic rate with age.

Regarding the organism energetically and dynamically, the amount of energy exchange measures its activity or vitality. Hence, the amount of energy stored plus the amount set free might be used as a criterion of aliveness. By adding the rate of storage in terms of weight (previously obtained) to the rate of elimination, likewise in terms of weight as measured by oxygen usage, one obtains the desired value; namely, the rate of dry mass absorption per gm. of body weight per day (Table I). It may be seen (Fig. 2) that there is a marked fall with age in the rate of absorption expressed in these terms. Reasons have been enumerated for believing that during the first half of incubation, when the amount of metabolism is small relative to the total metabolism during incubation but large relative to the weight of the embryo, there is a not inappreciable amount of protein and carbohydrate oxidized. If this were a fact a straight line rather than an S-shaped curve as graphically represented might be indicated.

The consumption of food during the early days is enormous. On the sixth day for instance the embryo absorbs over its own mass of dry substance. Assuming that the water content of the diet is ap-

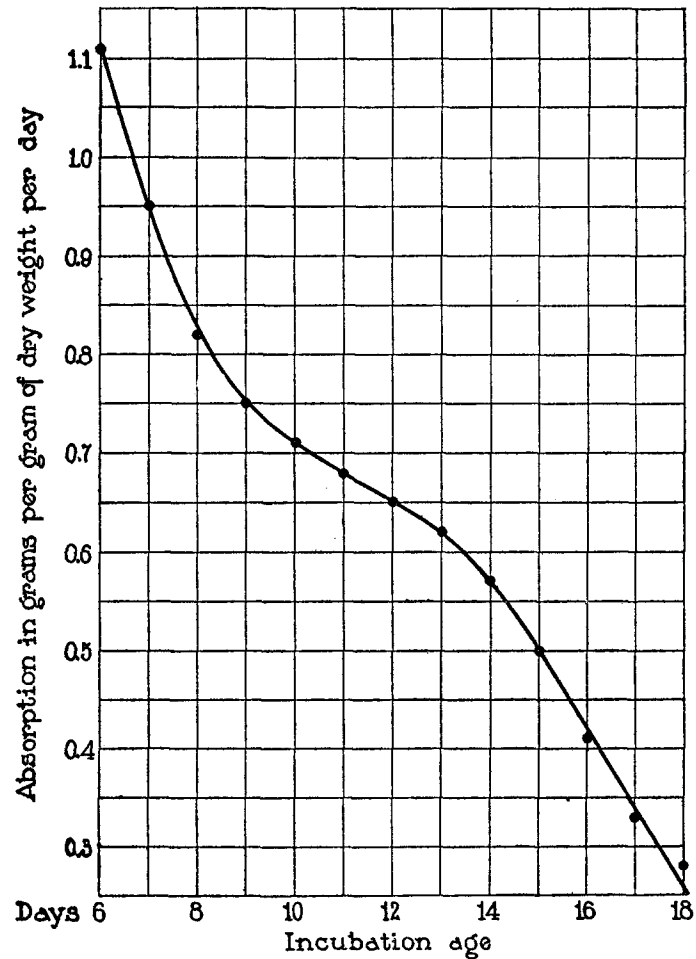


FIG. 2. Absorption of solid matter in gm. per gm. of dry weight of chick embryo per day, as a function of age.

proximately that of the tissues, this would be equivalent to a mature man eating about 150 pounds of food per day. During the 12 days under observation, however, the percentage rate of absorption falls

to about 25 per cent (one-fourth its earlier value). According to Lotka, a mature meadowlark consumes about 6.6 per cent of its own weight a day (7) which would suggest a fall in absorption rate during the postembryonic period of a degree comparable to that which occurs during the 12 days before hatching.

SUMMARY.

1. The previous findings that the rate of metabolism per gm. of body weight decreased with age, and that during the incubation period catabolism was mostly at the expense of fat, have been confirmed.
2. These determinations of the rate of oxygen uptake have afforded more precise values for the catabolic rate and thus permit estimations of the changes with age in the rate of absorption.

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