Oxidative Enzymes in Embryos from Dams Fed Synthetic and Practical Type Laying Diets.* (28410)

C. R. CREGER, R. E. DAVIES† AND J. R. COUCH
Departments of Biochemistry and Nutrition and Poultry Science, A & M College of Texas, College Station

Unidentified factors essential in the nutrition of the domestic fowl have been demonstrated by many workers(1,2,3,4,5). The biochemical significance of unidentified factors that stimulate the hatchability of fertile eggs in the domestic fowl is not known. It appears reasonable to assume, however, that the substances influence the metabolic activities of the embryo and that such changes may be reflected in the activity of various enzymes. The following studies were made to determine if there were enzymatic changes in the embryos associated with the changes observed in hatchability of fertile eggs. The enzymes selected for study were cytochrome *c* reductase, succinic dehydrogenase, lactic dehydrogenase and cytochrome oxidase.

Procedure. The basal purified type glucose monohydrate and isolated soybean protein diet used had been previously reported (6) to be inadequate for support of normal hatchability even though it contained adequate levels of all vitamins, minerals and essential amino acids known to be required by the domestic fowl. However, when the basal diet was supplemented with either soybean oil meal or corn, hatchability of fertile eggs was markedly increased. For this study day-old inbred hybrid white Leghorn chicks were reared to maturity on a glucose monohydrate and isolated soybean protein diet calculated to contain 26% protein, 1.294% calcium and 0.70% phosphorus. At no time did the chicks receive any known source of unidentified factors. At sexual maturity the birds were divided into 4 groups of 12 birds each and transferred to individual laying cages. The experimental plan is shown in Table I. Supplementation of the laying diets with corn, corn steepwater or a combination of soybean oil meal and corn was made at the expense of glucose monohydrate and isolated soybean protein. All diets were calculated to be isocaloric and isonitrogenous. Feed and water were supplied *ad libitum.* Management, of egg collection, storage, insemination, incubation and embryonic mortality records was similar to that previously reported(5).

Four day-old embryos were obtained from eggs selected at random from each of the 4 experimental groups of hens 30 weeks after initiation of the hatchability studies. The embryos were homogenized in a Potter-Elvehjem glass homogenizer while suspended in a 1.5 molar phosphate buffer, pH 7.4, at 0°C. For each enzyme determination, 5 embryos from each group were individually homogenized and the samples pooled. Five separate determinations were made, involving a total of 25 embryos per group. The assays of enzyme activities were performed with the Beckman Model D.U. Spectrophotometer. Zero time was taken at inversion of the cuvette after addition of the last reactant, which was the embryo homogenate. Enzyme activities were determined as shown in Table I.

Results and discussion. Hatchability of fertile eggs. There was a marked difference in the hatchability of fertile eggs between the various experimental groups. There was also a marked decrease in hatchability of fertile eggs from the beginning to the termination of experiment in the group fed the basal diet. At the beginning of experiment, all groups had approximately the same percentage hatch of fertile eggs. However, at termination of the experiment, the hatch of fertile eggs in the basal group had dropped to 35%. This suggests that as the hens produced more eggs, there was a continuous depletion of the factors necessary for maximum hatchability of fertile eggs. It should be emphasized that the birds had been reared

* Contribution of Texas Agri. Exp. Station.

† Present address: Dept. of Dermatology, Skin and Cancer Hospital, Philadelphia, Pa.

TABLE I. Enzymatic Activities of Embryos from Dams Fed Practical and Synthetic Type Laying Diets.

Group	Supplement to basal diet	% hatch of fertile eggs	Cytochrome *c* reductase activity*	Succinic dehydrogenase activity†	Pyruvic reductase activity‡	Cytochrome oxidase activity§
1	None	60.3	89 ± 9	5.13 ± 1.01	9.87 ± 3.61	243 ± 18
2	64% yellow corn	83.5	32 ± 6	2.87 ± 0.94	18.74 ± 2.54	175 ± 22
3	20% corn steep water	80.0	29 ± 4	1.89 ± 0.99	21.34 ± 2.97	168 ± 14
4	46% yellow corn + 32% soybean oil meal	90.9	35 ± 11	2.17 ± 0.76	23.21 ± 3.98	178 ± 17

* One unit represents amount of enzyme that will reduce 1×10^{-9} moles cytochrome *c* reductase/sec/mg tissue.

† One unit represents amount of enzyme that will reduce 1×10^{-9} moles of DPN/sec/mg tissue.

‡ One unit represents amount of enzyme reduced/mg tissue/sec $\times 10^{-3}$.

§ One unit represents amount of enzyme that will oxidize 1×10^{-3} moles cytochrome/sec/mg tissue.

Cytochrome c reductase. Reaction mixtures contained in cuvettes were added in the following concentrations and sequence: 1.0 ml of 0.15 M KH_2PO_4—NaH_2PO_4 buffer, pH 7.4; 0.4 ml H_2O; 0.1 ml of 3.0×10^{-3} M NaCN; 1.0 ml of 0.5 mg of pure cytochrome *c* per 1.0 ml H_2O; 0.2 ml of 0.82 M nicotinamide; 0.2 ml reduced DPN; 0.1 ml of 207 solution of embryo homogenate.

Succinic dehydrogenase. Reaction mixtures contained in cuvettes were added in the following concentrations and sequence: 1.0 ml of 0.15 M KH_2PO_4—Na_2HPO_4 buffer, pH 7.4; 0.10 ml of 0.05 M sodium succinate; 0.7 ml H_2O; 0.1 ml of 3×10^{-3} M NaCN; 1.0 ml of 0.911×10^{-3} M of oxidized cytochrome *c*; 0.1 ml of a 20% solution embryo homogenate.

Pyruvic reductase. Reaction mixtures contained in cuvettes were added in the following concentrations and sequence: 0.5 M KH_2PO_4—Na_2HPO_4 buffer; pH 7.4; 0.1 ml of 3×10^{-3} M NaCN; 2.0 ml H_2O (distilled); 0.1 ml Na pyruvate; 0.1 ml reduced DPN and 0.1 ml of a 20% solution of embryo homogenate. Oxidation of reduced DPN was followed at 341 mu with a slit width of 0.1 mm.

Cytochrome oxidase. Reaction mixtures contained in cuvettes were added in the following concentrations and sequence: 2.0 ml of ferrocytochrome *c* (0.5 mg pure cytochrome *c* per 1.0 ml of H_2O plus 1.0 ml of 0.15 M KH_2PO_4—Na_2HPO_4 buffer, pH 7.4; sufficient water to yield a total reaction volume of 3.0 ml; 0.1 ml of a 20% solution of embryo homogenate. Oxidation of ferrocytochrome *c* was followed at 550 mu with a slit width of 0.01 mm.

to maturity on the synthetic basal diet and had not received any known source of unidentified hatchability factors in their diets. Groups 2 and 3 showed an 83.5 and a 80.0% hatch of fertile eggs, respectively. These results were 23 and 20 percentage points greater than the hatchability of fertile eggs from the basal group. Group 4 (corn and soybean oil meal) produced eggs with the highest hatchability of any of the experimental groups, 30.6 percentage points more than from Group 1. Addition of 32% soybean oil meal to the diet, in combination with 46% corn, increased the hatchability of fertile eggs by 7.4 percentage points over that of the group fed the diet containing 64% corn and 10.9 percentage points over that of the group fed the diet containing 20% corn steepwater.

Lactic dehydrogenase. The activity of lactic dehydrogenase was much greater in embryos from hens fed diets containing natural ingredients. Activity in each of these 3 groups was approximately twice as great as in the embryos from the control group. Differences within the 3 supplemented groups were smaller, although there appeared to be higher activity in the case of the combined soybean meal-corn supplement than in the case of the corn diet. The activity of this enzyme system followed closely the trend observed in the case of hatchability.

Succinic dehydrogenase. A deficiency of unidentified factor sources resulted in a distinct increase in activity of succinic dehydrogenase. Activity in the 3 supplemented groups was fairly similar, and roughly one-half as great as that observed in embryos from birds fed the purified basal diet.

Cytochrome oxidase. The activity of this enzyme, like that of succinic dehydrogenase, was greatly elevated in the absence of a source of unidentified factors. Once again the 3 supplemented groups were essentially identical with respect to enzyme activity. The increase in activity in the deficient embryos was approximately one-third, as compared to the supplemented groups.

Cytochrome c *reductase.* The activity of the enzyme was affected in a similar manner to that found with the metabolically related cytochrome oxidase. Once again the unidentified factor-deficient embryos demonstrated greatly elevated activity, in this case approximately 3 times as great as that of the supplemented groups. As observed with the other enzyme systems, there was little difference in activity among the 3 supplemented groups.

Discussion. Of the 4 enzyme systems studied, one (lactic dehydrogenase) is related to anaerobic metabolism, one (succinic dehydrogenase) is associated with acetate oxidation *via* the Krebs cycle, and the remaining 2 are involved in the electron transport systems of aerobic metabolism. Since the earliest stages of embryonic development are dependent on energy derived from anaerobic (glycolytic) metabolism, it might have been reasonable to suppose that retarded embryonic development might result from a deficiency of unidentified factors, and that as a consequence the deficient embryos might exhibit relatively greater activity of anaerobic pathways and less activity of aerobic systems. In fact, the opposite situation was observed. One explanation might be that an actual acceleration of the development of aerobic systems might result from the deficiency condition.

Another possible explanation which would be more susceptible to testing, involves the assumption that the primary energy source at this stage of development (4 days) is fat. If this is correct, then the primary function of the pathway of glucose metabolism would be glyconeogenesis rather than glycolysis (in such a condition the function of lactic dehydrogenase would be interconversion of the synthetic, intermediate, pyruvate, and the inactive reservoir form, lactate).

If it is assumed that a deficiency of unidentified factors results in a decrease in efficiency of the energy trapping mechanism associated with oxidative phosphorylation, a possible explanation for the observed changes becomes apparent. The oxidative enzyme systems of the deficient embryos would be developed to the fullest in an attempt to meet the deficit of high energy phosphate compounds. Moreover, the proportion of available acetate which could be diverted to glyconeogenesis would be decreased by the elevated functioning of the degradative pathways, hence the demands on the glycolytic pathway would be relatively small. It appears to be reasonable that if a defect of oxidative phosphorylation existed, the equilibria associated with oxidative metabolism would be displaced in a catabolic direction. Such a hypothesis would account for relative increases in activity of the Krebs cycle and EOT enzymes with a concurrent decrease in activity of enzymes associated with glycolysis.

Summary. Enzymatic activity of embryos obtained from hens fed a synthetic-type laying diet, was compared to that of embryos from hens fed various supplements to the basal laying ration. Low hatchability of fertile eggs occurred in eggs of hens whose embryos had high cytochrome *c* reductase activity, high succinic dehydrogenase activity and high cytochrome oxidase activity. In all cases addition of corn, corn steepwater or a combination of soybean oil meal and corn to the hen basal diet improved hatchability of fertile eggs, and embryos from such groups exhibited lowered enzyme activity. Pyruvic reductase activity of embryos was increased when sources of unidentified factors were added to the basal diet of the dam. Increased hatchability of fertile eggs was also obtained from the dams fed these supplements. The embryos that hatched poorly seemed to be suffering from general debility rather than from any specific deficiency symptom.

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Received April 25, 1963. P.S.E.B.M., 1963, v113.