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The Relationship of Hyperthermia to Liver Growth and Liver Glycogen in the Chick Embryo

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INTRODUCTION

NUMEROUS studies have indicated that elevated incubation temperature for the chick embryo results in visceral overgrowth and protrusion of the viscera into the body stalk (celosomia), various other congenital malformations and growth acceleration. Ancel and Lallemand (1941) reported the production of visceral overgrowth and celosomia in chick embryos incubated at 39.0°C. during the third through the sixth day of incubation. This same malformation was produced in the chick embryo by Delphia and Eveleth (1961) from continuous incubation temperature of 40.0°C. Alsop (1919) and de la Cruz *et al.* (1966) recorded numerous congenital malformations in chick embryos incubated at 40.0°C. Rott (1957a, b) observed that incubation of the chick embryo at 38.5°C. accelerated growth during the first half of incubation. Delphia and Elliott (1965) using a temperature of 40.0°C. during the third through the sixth day and continuously, found lowered myocardial glycogen levels.

The present study is concerned with the effect of elevated incubation temperature upon the growth of the liver and the accumulation of glycogen in the liver during the period when hyperthermia results in visceral overgrowth and celosomia.

MATERIALS AND METHODS

Specimens for the Control Group were incubated at 37.5°C. Two Experimental Groups were designed. Experimental Group 1 consisted of embryos incubated at 40.0°C. during the third through the sixth day of incubation with normal incubation temperature (37.5°C.) before and after the high temperature period. Experimental Group 2 consisted of embryos incubated continuously at 40.0°C. Temperature was the only environmental factor altered in this study.

General observations were made concerning the effects of high temperature incubation as described for Experimental Groups 1 and 2 (above) on visceral enlargement and expression of celosomia and embryonic mortality. This investigation involved 50 embryos in each Control Group and Experimental Group for the following days of incubation: six, seven, eight, ten, and twelve.

The effect of elevated incubation temperature as described for Experimental Groups

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1 and 2 upon stage of growth, liver weight and liver glycogen was undertaken. Ten to twenty-two living specimens were used for the Control Group and each Experimental Group for the following days of incubation: six, seven, eight, ten, and twelve.

The differences in liver weight and in mean percent glycogen between the Control Group and each Experimental Group and between the two Experimental Groups were analyzed by means of the student T test.

The embryo was removed from the shell; the liver was removed immediately and weighed rapidly upon a Mettler balance. This liver was stored on dry ice until time of digestion. The head region, limb buds and integument were used in the determination of the Hamburger and Hamilton (1951) stage of development.

Glycogen analysis of the livers in this study was based on the KOH-Anthrone method of Carroll *et al.* (1956) with slight modifications. The weighed, frozen tissue was placed in 1.5 ml. 30% KOH. The centrifuge tube containing the tissue and KOH was placed in a water bath adjusted to 97°C. for twenty-three minutes. The tube was cooled to room temperature; 5.0 ml. 95% ethyl alcohol was added. The mixture was shaken well and brought to boiling by way of a water bath. The tubes were centrifuged for ten minutes. The supernatant was poured off carefully and the tubes were inverted for ten minutes to drain. Two ml. distilled H_2O were added and the tube was shaken to get the glycogen into solution. At this time 5.5 ml. Anthrone reagent (100 mg. Anthrone in 5 ml. distilled H_2O to which 50 ml. concentrated H_2SO_4 has been added) was placed in each tube. The mixture was mixed thoroughly and placed in a water bath adjusted to 97.0°C. for three and one-half minutes. The tube was cooled. The contents of the tube were transferred to a cuvette and a reading was taken on a Ruoy-Leitz photrometer with an Anthrone reagent blank set 100% light transmission. The wavelength of light was kept at 610. Glycogen determinations were made from a graph prepared from known quantities. The percentage of glycogen in the liver tissue was then calculated.

OBSERVATIONS

The Control Group had a mortality rate from 6.0% and 8.0% at six and twelve days respectively. The mortality rate in Experimental Groups 1 and 2 progressed from 14% and 18% respectively at six days incubation to 78.0% and 82.0% at twelve days incubation. All specimens in Experimental Groups 1 and 2 showed visible enlargement of the visceral organs. This enlargement was visible as celosomia (protrusion of the visceral organs into the body stalk) in 61.0% of all specimens in Experimental Group 1 and in 64.0% of all specimens in Experimental Group 2.

An investigation of the effects of an incubation temperature of 40.0°C. upon stage of growth, mean liver weight and mean percent liver glycogen is shown in Table 1.

Experimental Group 1 shows an advancement by one stage of growth over that of the Control Group through ten days incubation. The Control Group and Experimental Group 1 have the same average stage of development at twelve days incubation. Experimental Group 2 is advanced over the Control Group by two growth stages (approximately one day) through ten days incubation. This Experimental Group is advanced over the Control Group by one growth stage at twelve days incubation.

The mean liver weight of Experimental Group 2 is slightly larger than that of the Control Group at six days incubation. This increase is significant at the 5% level (Table 1). The liver weight increases markedly in all Groups between six and seven days incubation. The mean liver

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TABLE 1.—*Influence of incubation temperatures on weight of liver and percent liver glycogen in chick embryos*

Day of incubation	Group	No. of living specimens	Stage of growth	Weight of liver (mg.)		Liver glycogen (%)	
6	Control	15	29[1]	4.44 ±	0.52[2]	0.205 ±	0.038[2]
	Exp. 1	14	30	4.96	0.43	0.106*	0.029
	Exp. 2	19	31	5.75*	0.34	0.306* ′	0.042
7	Control	13	31	9.22	0.54	0.104	0.020
	Exp. 1	15	32	13.56**	0.66	0.047**	0.009
	Exp. 2	17	33	17.95** ″	0.70	0.024**	0.009
8	Control	15	33	15.23	0.56	0.247	0.034
	Exp. 1	20	34	21.33	0.96	0.114**	0.018
	Exp. 2	18	35	30.73** ″	1.19	0.056** ′	0.009
10	Control	17	35	40.99 ±	2.13	0.288 ±	0.039
	Exp. I	15	36	56.63**	2.06	0.301	0.036
	Exp. II	22	37	59.32**	2.75	0.285	0.028
12	Control	19	38	102.93	2.10	0.298	0.025
	Exp. I	11	38	101.03	5.43	0.292	0.045
	Exp. II	10	39	115.51* ′	8.35	0.304	0.024

[1] As described by Hamburger and Hamilton (1951).
[2] Mean ± S.E.
Control: continuous incubation at 37.5°C.
Exp. 1: elevated incubation temperature (40.0°C.) during 3rd through 6th day of incubation.
Exp. 2: continuous incubation at 40.0°C.
* and ** Student T test shows difference between mean of Control and either Exp. Group to be significant; respectively significant at the 5% or 1% level respectively.
′ and ″ Student T test shows difference between means of the Experimental Groups to be significant at the 5% or 1% level respectively.

weight in Experimental Group 1 and Experimental Group 2 is increased significantly over that of the Control Group at seven days incubation. Experimental Group 2 has a significantly larger mean liver weight than does Experimental Group 1 at this time.

The mean liver weights of all Groups continue to show a large daily increase from seven to eight days incubation. The mean liver weight of the Control Group at this time (eight days) is slightly smaller than that of Experimental Group 2 at seven days incubation. This advancement of the weight of the liver in Experimental Group 2 by one day's growth corresponds to the advancement in this Group by two growth stages, or one day's growth.

Both Experimental Groups show significantly enlarged livers (by weight) at ten days incubation. Experimental Group 1 and the Control Group have similar liver weights by twelve days incubation. The growth stages are also the same.

The mean liver weight of Experimental Group 2 is significantly larger than that of the Control Group and Experimental Group 1 at twelve days incubation. Experimental Group 2 is advanced in growth stage over the Control Group and Experimental Group 1 by one growth stage at this time. The mean percent liver glycogen at six days incubation is highest in Experimental Group 2. This increase is significant at the 5% level. The decrease in percent liver glycogen in Experimental Group 1 below that of the Control Group is also significant at the 5% level.

The mean percent liver glycogen decreases sharply in the Control Group and both Experimental Groups by seven days incubation. It can be seen that the de-

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creases in mean percent liver glycogen of both Experimental Groups below that of The Control Group are both highly significant. Consideration of the average amount of glycogen (mean percent liver glycogen × mean liver weight) for the Control Group and Experimental Group 1 demonstrates that the quantity of glycogen present at six days incubation in the Control Group and Experimental Group 1 has not decreased from six to seven days incubation. Rather, glycogen storage has decreased. Using the same method, the actual amount of glycogen is decreased tremendously in Experimental Group 2 from six to seven days incubation.

The mean percent liver glycogen increases in the Control Group and both Experimental Groups from seven to eight days incubation. It can be observed that Experimental Group 2 has the lowest mean percent liver glycogen, the largest mean liver weight and the lowest quantity of stored glycogen of all Groups studied at eight days incubation.

No difference was observed in the mean percent liver glycogen for the Control Group and each Experimental Group at ten and twelve days incubation.

DISCUSSION

The data concerning the visceral enlargement and celosomia resulting from elevated incubation temperature in Experimental Groups 1 and 2 are in agreement with the findings of Ancel and Lallemand (1941) and Delphia and Eveleth (1961). The progressive increase in embryonic mortality in Experimental Groups 1 and 2 is consistent with the observations of the two groups of workers cited above. Either method of high temperature incubation (40.0°C. during the third through the sixth day of incubation or continuously) also accelerates the rate of growth. This is evidenced by the advancement in the stages of growth and the mean liver weights shown in Table 1.

The observation that the mean liver weight in Experimental Group 2 is at seven days incubation increased by at least one day of incubation coincides with the observation that at this time the specimens in Experimental Group 2 are advanced morphologically by two growth stages (one day's growth at this age). In other words, liver weight is an accurate measurement of growth of the individual during the period when high temperature causes visceral overgrowth. The increase in liver weight for Experimental Groups 1 and 2 (Table 1) is consistent with the observations of Rott (1957 a, b) for the heart. The present study also agrees with the observation of Rott (1957 a, b) that continuous incubation at an elevated temperature accelerates growth during the first half of incubation.

It is apparent that continuous incubation at 40.0°C. brings about not only an advancement in stage of growth and an increase in liver weight but also an increase in glycogen storage in the liver at six days incubation. The data concerning seven days incubation indicate that the previously stored glycogen in Experimental Group II must be depleted. This depletion necessitates the ability of the liver cells to undergo glycogenolysis. The present data suggest that glycogenolysis does take place in the liver during continuous high temperature incubation at 40.0°C. between six and seven days incubation. The ability of liver in Experimental Group 2 to undergo glycogenolysis between six and seven days incubation can be explained by its advancement in stage of growth, and in actual weight by at least one day of incubation. The histochemical studies of Guha and Wegmann (1961) showed that the enzymes necessary in glycogenolysis exist in the normal chick embryo of seven days incubation. The present data suggest that continuous incubation at 40.0°C. not only

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advances the liver of the six day embryo by a normal day's growth in weight but also brings about the earlier production of the enzyme system necessary for glycogenolysis in the liver.

The glycogen storage increases in all Groups at eight days incubation. However, the increased percent glycogen in the livers of the embryos in the livers of the embryos in the Control Group over that of both Experimental Groups at this time indicates that some factor is lessening the accumulation of glycogen in the livers of the two Experimental Groups. It is suggested that there is a greater demand for glucose placed upon the embryos in Experimental Groups 1 and 2; thus, less carbohydrate is available for conversion into glycogen. The mean liver weight of Experimental Group 2 at this age suggests that the liver in this Group is undergoing the greatest amount of growth. Therefore Experimental Group 2 would also have the least material available for glycogen storage, as is shown in the mean percent glycogen value for this Group.

The present study indicates that the rate of liver growth and growth acceleration declines after eight days incubation in both Experimental Groups while the ability to store glycogen in the liver in these Experimental Groups at this time reaches normal proportions.

SUMMARY

Two experimental groups were designed to investigate the relationship of hyperthermia to liver growth and glycogen storage in the chick embryo. One Group was incubated at 40.0°C. during the third through the sixth day of incubation. The other Group was incubated at a temperature of 40.0°C. continuously. A third (Control) Group was incubated at 37.5°C.

Both methods of elevated temperature incubation (above) accelerate the general stage of growth of the embryo. Both methods bring about advancement in size of the liver as evidenced by the weight of the liver.

Specimens incubated continuously at high temperature (40.0°C.) show evidence of glycogenolysis one day earlier than in normal temperature specimens. The glycogen storage in the liver of the chick embryo is inhibited between seven and eight days incubation by both methods of high temperature incubation.

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Pancreas and Intestine Weights of Turkey Poults Fed Corn and Barley Grain-based Rations and the Effect of Oleandomycin and Enzyme Supplements[1]

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IT IS well known that Western barley seriously depresses the growth of chicks and poults. This growth depression is apparently limited to the young bird (Moran and McGinnis, 1966) and can be significantly altered by adding "effective" antibiotic and/or bacterial (*B. subtilis*) enzyme concentrates to the diet (Moran and McGinnis, 1965). Burnett (1966) concluded that the poor nutritional value of growth depressing barleys is related to the glucan and β-glucan hemicellulose components which give rise to stable highly viscous gels in the small intestine. Because of the beneficial results obtained with antibiotics, Moran and McGinnis (1965) suggested an "unfavorable" gastrointestinal microflora as the primary reason for the growth depression and the β-glucan component of the carbohydrate as the predisposing factor for the condition.

Arscott *et al.* (1965) have observed a "pancreatic enlargement" with chicks given barley rations a result which they suggest was due either to a protein or carbohydrate fraction in the grain. One might rationalize this observation in terms of a reduced enzymic efficiency due to highly viscous conditions and consequently increased pancreatic activity; however, the intestine should also be affected. The present study was initiated to determine whether the turkey shows a "pancreatic enlargement" and if the intestine size is also affected.

EXPERIMENTAL

Each experimental ration was fed to 5 replicate pens of 8 poults per pen. Sex distribution was assumed to be equal because distribution of the poults to pens was random and even in number. The birds were maintained in raised-wire floor pens of an electrically heated battery brooder where feed and water were offered *ad libitum*.

The composition of the experimental rations is shown on Table 1. Both diets were pelleted and crumbled to reduce density differences. Oleandomycin (2.2 mg./kg.) was the only antibiotic used and "Bacterial Amylase A"[3] (440 mg./kg.) the only enzyme supplement.

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[3] Bacterial Amylase A, a crude enzyme concentrate of *Bacillus subtilis* origin, was obtained from Chas, Pfizer and Co., Terre Haute, Indiana (Lab.

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