Effect of Environmental Stress on Chick Weight[1]

W. C. GUNTHER and ROBERT K. JONES, Valparaiso University
and Purdue University

Introduction

It has been demonstrated that subjecting chick eggs to varying degrees of non-optimal incubating temperatures has a deleterious effect on the behavior of the hatched birds (3, 4). While observing this, it soon became apparent that the size of the experimental birds was quite different from that of the normal birds (Figs. 1, 2). There seemed to be a consistent

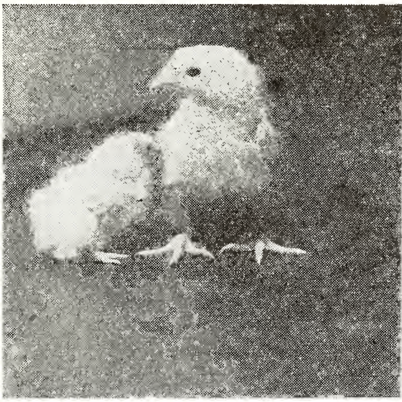

Fig. 1. Newly hatched chicks from eggs incubated at normal temperature (right) and at 41° for the first three days of incubation (left).

Fig. 2. Five-week-old roosters hatched from eggs incubated at normal temperature (left) and at 41° for the first three days of incubation (right).

decrease in body weight with an increase in incubating temperature. Before proceeding with further analysis of this phenomenon, it seemed desirable to test these data statistically in order to determine the reliability of the weight differences. This report is concerned with the results of this statistical treatment.

Materials and Methods

A total of 864 chicken eggs was incubated. The eggs were divided into 3 groups of 288. Each group was further subdivided into 48-egg lots. Group I (White Leghorn, De Kalb Strain) consisted of 5 lots of 48 eggs, each lot incubated respectively for 1, 2, 3, 4, and 5 days at 42°C. (all temperatures herein reported are centigrade) and then placed in a normal temperature incubator (37.5°) for the remainder of the incubation period. Group II (White Rock, unknown strain) consisted of 5 lots of 48 eggs, each lot incubated respectively in a normal temperature incubator for 16, 17, 18, 19, and 20 days and then placed in an incubator at 42° until hatched. Group III (White Leghorn, De Kalb Strain) consisted of 5 lots of 48 eggs, each lot incubated respectively for 1, 2, 3, 4, and 5 days at 41° and then placed in a normal temperature incubator for the remainder of the incubation period. A sixth lot (Control) of 48 eggs in each group was incubated for the entire incubation period at normal temperature.

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Incubators were of the standard Montgomery Ward 416-egg, forced-air type. Humidity and temperature were carefully controlled, the incubators being modified with Fenwal thermo-regulators. Continuous recordings of temperature and humidity were made by means of Dickson Minicorders and Short and Mason recording hygrometers. Temperatures varied within the limits of $\pm\frac{1}{2}^{\circ}$ during all incubation periods, and humidities were kept constant at a level recommended by the manufacturers of the incubators. Eggs were regularly turned at 8-hour intervals. The room housing the incubators was maintained at a constant temperature of 23° and at a humidity level of 55-59% R.H. Room temperature and humidity were recorded by means of a Dickson Minicorder and a Short and Mason hygrometer.

At hatching, the chicks were carefully weighed to the nearest tenth of a gram and tagged with wing bands. The various lots were then placed in separate compartments of a hatching brooder. Food (Purina Starter Mash) and water were available ad lib. All birds were weighed once a week on the same day.

Results

Group I

The data relevant to the hatching of the eggs are summarized in Table 1. It is readily seen that an incubation temperature of 42° is lethal

TABLE 1

Hatching data of Group I

Lot*	No. of eggs incubated	No. of days incubation at 42°	No. of chicks hatched	Total incubation period (days)
Control	48	0	24	20–22
1-day	48	1	12	19–22
2-day	48	2	2	21
3-day	48	3	1	22
4-day	48	4	0	—
5-day	48	5	0	—

*Reference is made in the text to the different lots of eggs and to the hatched chicks by means of these designations.

when eggs are initially exposed for 4 or more days, and that the number of chicks hatched drops sharply upon exposure of the eggs to this temperature for 24 or more hours. However, the total incubation time of the hatched eggs does not vary appreciably among the 6 lots. Since only one animal was obtained in the 3-day lot, the data for this bird are not considered in subsequent statistical analyses.

The mean weight of each lot was computed at hatching and at the end of the first, second, and third weeks after hatching. These mean weights appear in Table 2, which also includes the results of Kruskall-Wallis one-way analyses of variance (8) which were performed on the data at hatching and at the end of each post-hatching week. The nonparametric Kruskall-Wallis procedure was deemed most appropriate for the evaluation of lot mean differences because of the small number of animals in the 2-day lot, and also because the results of Bartlett's tests (9) run on the data at hatching and at the end of each post-hatching week indicated the error variances at each of these periods to be nonhomogeneous.

TABLE 2

Mean weights and analyses of variance of lots of Group I at hatching and at the end of each post-hatching week

Mean weight (grams)

Lot	Hatching	1*st* Week	2*nd* Week	3*d* Week
Control	44.8	62.1	115.2	201.7
1-day	44.5	60.7	125.0	198.3
2-day	49.5	30.8	64.9	124.3

Kruskall-Wallis one-way analyses of variance

	H	*df*	*P*
Hatching	3.31	2	.19
1*st* week	6.43	2	.04*
2*nd* week	6.06	2	.05*
3*d* week	3.73	2	.16

*Significant

An inspection of the mean weights reveals a general decline in weight with increase in time of exposure to the temperature insult. If $P = .05$ is adopted as a minimal level of statistical significance, the results of the Kruskall-Wallis analyses reveal significant differences in mean weights among the three lots at first and second weeks after hatching ($P = .04$ and $P = .05$, respectively), while the values of H obtained at hatching and at the third post-hatching week have associated P values of .19 and .16. The Kruskall-Wallis test was also used to identify significant differences between pairs of lot means at the first and second post-hatching weeks. The results indicated the Control and 1-day animals to be significantly heavier than the 2-day birds at both the first and second weeks after hatching ($P \leqslant .04$ for all comparisons). The control and 1-day mean differences failed to attain significance at either of these periods.

Group II

The hatching data for this group are summarized in Table 3. Unlike the procedure employed with Group I, the temperature insult of 42° was

TABLE 3

Hatching data of Group II

Lot*	No. of eggs incubated	No. of days incubation at 42°	No. of chicks hatched	Total incubation period (days)
Control	48	0	33	20–21
20-day	48	1	23	19–20
19-day	48	2	11	20–21
18-day	48	3	4	20
17-day	48	4	0	—
16-day	48	5	0	—

*Reference is made in the text to the different lots of eggs and to the hatched chicks by means of these designations.

imposed during the last five days of incubation in this case. A sharp decline in number of chicks hatched with increased exposure is again evident. Two of the four 18-day chicks were badly crippled and the remaining 2 fared so poorly that the data for these 4 animals was not included in this report. As in Group I, the total incubation time varies very little for those lots in which some eggs hatched.

In Table 4 are presented the mean weights of the lots of this group at hatching and at the end of each post-hatching week, and also the

TABLE 4

Mean weights of lots of Group II at hatching and at the end of each past hatching week and summary of repeated measures analysis of variance.

Mean weight (grams)

Lot	Hatching	1*st* week	2*nd* week	3*d* week	4*th* week
Control	42.7	55.2	98.7	174.1	267.9
20-day	42.7	51.7	93.7	164.1	256.5
19-day	40.2	44.1	78.9	143.3	244.3

Repeated measures analysis of variance of data of Group II

Source	*df*	*SS*	*MS*	*F*
Between Lots	2	9,792.82	4,896.41	3.888*
Animals within Lots	59	74,311.63	1,259.52	5.314**
Weeks	4	2,056,292.32	514,073.08	2,168.810**
Weeks × Lots	8	3,941.68	492.71	2.079*
Animals × Weeks within Lots	236	55,938.05	237.03	
Total	309	2,200,276.50		

*P < .05
**P < .001

summary of a repeated measures analysis of variance performed on the complete data for the group. Bartlett's tests for homogeneity of variance were run on both the correlated and uncorrelated error variances of the analysis summarized in Table 4 and on the error variances of all analyses of variance reported below in this section. The results of these tests indicated the assumption of homogeneity to be tenable in each case.

An inspection of the mean weights in Table 4 reveals that these weights decrease steadily from the Control to the 19-day lots from hatching to the fourth week after hatching. The analysis of variance summary indicates that a significant difference (P < .05) exists between lot means, as reflected in the F for between Lots. The low P associated with the F for animals within Lots reflects the presence of significant individual differences between animals, while the P associated with the F for the Weeks term indicates significant weight gain for the entire group of animals across the four-week period. In this analysis, interest is directed to the value of P associated with the interaction term, Weeks × Lots, which indicates significant differential rates of weight gain among the lots.

Table 5 includes the results of analyses of variance of the data at hatching and at the end of each post-hatching week, and also the results of tests of lot mean differences at these periods. The "least significant difference" method, described by Steel and Torrie (9), was employed to evaluate differences between lot means. The values of F at hatching and at the fourth post-hatching week do not attain significance, although highly significant differences between lot means are evident at the first,

TABLE 5

Analyses of variance of Group II data at hatching at the end of each post-hatching week and results of tests of mean differences.

Analyses of variance

	Source	*df*	*SS*	*MS*	*F*
Hatching	Between Lots	2	56.10	28.05	1.888 (NS)
	Within Lots	64	951.30	14.86	
	Total	66	1,007.40		
1st week	Between Lots	2	899.99	450.00	7.700*
	Within Lots	61	3,564.90	58.44	
	Total	63	4,464.89		
2nd week	Between Lots	2	2,535.93	1,267.96	7.067*
	Within Lots	59	10,585.80	179.42	
	Total	61	13,121.73		
3rd week	Between Lots	2	6,352.65	3,176.32	5.977*
	Within Lots	59	31,354.96	531.44	
	Total	61	37,707.61		
4th week	Between Lots	2	4,223.56	2,111.78	1.478 (NS)
	Within Lots	59	84,302.17	1,428.85	
	Total	61	88,525.73		

$^*P \leqslant .005$
NS: $P > .05$

Results of tests of mean differences ("least significant difference" method) for lots of Group II. Table entries are lot mean differences.

Comparison	Hatching	1*st* week	2*nd* week	3*rd* week	4*th* week
Control—20-day	0.1	3.5	5.1	10.1	11.4
Control—19-day	2.4	11.2**	19.9**	30.9**	23.6
20-day—19-day	2.5	6.1*	14.8**	19.1*	12.2

$^*P \leqslant .05$
$^{**}P \leqslant .01$

second, and third post-hatching weeks. The results of the tests of mean differences reflect no significant differences between the Control and 20-day lots. At the first, second, and third weeks after hatching the mean weights of the 19-day animals were significantly lower than those of the Controls, and the 19-day means were significantly lower than those of the 20-day lot.

A trend analysis performed on the lot means for all weekly weighing periods elicited significant differences in linear trend ($P < .01$) between all possible pairs of the three lots, the growth curve of the Controls displaying the largest linear trend component and that of the 19-day lot the smallest.

In order to identify the periods during which differential rates of gain occurred among the lots, repeated measures analyses of variance were run on all lots for each interval of one week. The results of these analyses indicated that significantly different rates of gain between the lots were in evidence for the interval between hatching and the first post-

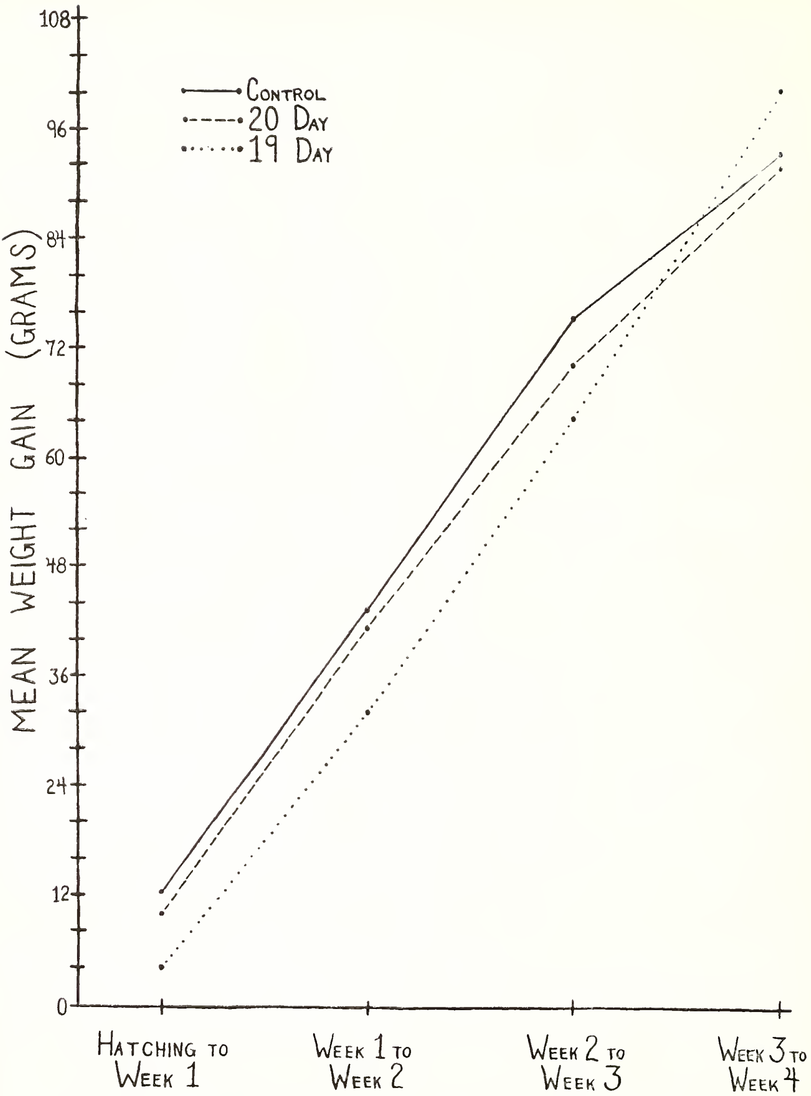

Fig. 3. Mean weight gain of the three lots of chicks in Group II during four weekly intervals.

hatching week ($P < .005$), between the first and second post-hatching weeks ($P < .01$), and between the second and third post-hatching weeks ($P < .05$). During the interval between the third and fourth post-hatching weeks the lots did not differ significantly in terms of rate of weight gain.

Fig. 3 is a plot showing mean weight gain of the three lots during the four weekly intervals. Analyses of variance run on the weight differences for those intervals during which significant differential rates of gain were found, viz., Hatching to Week 1, Week 1 to Week 2, and Week 2 to Week 3, elicited significant values of F ($P < .005$, $P < .025$, and $P < .05$, respectively). Tests of mean differences ("least significant difference" method) were performed to identify those lots which differ significantly in mean rate of gain at these weekly intervals. These results indicated that the mean rate of gain of the Controls was significantly greater than that of the 19-day lot during all three intervals (all P's $< .05$) and that the mean increase of the 20-day lot was significantly greater than that of the 19-day lot during the intervals Hatching to Week 1 and Week 1 to Week 2 ($P < .05$). Of particular interest is the rapid gain in weight of the 19-day birds during the interval Week 3 to Week 4.

Group III

Hatching data for this group are summarized in Table 6. The eggs were exposed to the non-optimally high temperature of 41° during the

TABLE 6

Hatching data of Group III

Lot*	No. of eggs incubated	No. of days incubation at 41°	No. of chicks hatched	Total incubation period (days)
Control	48	0	27	21–23
1-day	48	1	29	20–22
2-day	48	2	22	21–22
3-day	48	3	12	21–23
4-day	48	4	8	21–23
5-day	48	5	0	—

*Reference is made in the text to the different lots of eggs and to the hatched chicks by means of these designations.

first five days of the incubation period. It may be seen that the number of chicks hatched from eggs exposed to the high temperature for only the first 24 hours compares favorably with the number hatching in the Control lot. A slight decline in the numbers hatched is evident at two days' exposure, and a marked decline exists for the remaining lots.

Mean weights and the summary of a repeated measures analysis of variance for the complete data of this group are shown in Table 7. Except for the first post-hatching week, the mean weights of the chicks declined steadily with increased number of days' exposure of the eggs to the 41° temperature. The results of Bartlett's tests run on the correlated and uncorrelated error variances of this analysis indicated these variances to be nonhomogeneous. In view of this, the degrees of freedom associated with the error terms, Animals within Lots and Animals × Weeks within Lots, were multiplied by one-half before the P values associated with the F ratios were determined. The Between Lots term is significant ($P < .03$), indicating differences in mean weights among the lots. The value of F for Animals within Lots ($P < .001$) reflects the presence of significant individual differences between animals. The F for the Weeks term indicates significant weight gain for the entire group of animals across the four-week period. The F for Weeks × Lots has an associated $P < .001$, again reflecting highly significant differential rates of weight gain among the lots.

TABLE 7

Mean weights of lots of Group III at hatching and at the end of each post-hatching week and summary of repeated measures analysis of variance

Mean weight (grams)

Lot	Hatching	1*st* week	2*nd* week	3*d* week	4*th* week
Control	44.2	58.9	84.5	140.1	238.8
1-day	42.9	59.6	81.7	134.0	228.8
2-day	41.7	60.1	79.6	129.7	216.1
3-day	41.0	55.3	72.1	122.6	210.8
4-day	37.6	52.7	58.6	104.9	175.5

Repeated measures analysis of variance of data of Group III

Source	*df*	*SS*	*MS*	*F*
Between Lots	4	11,563.40	2,890.85	3.452*
Animals within Lots	83	69,503.41	837.39	5.472**
Weeks	4	1,929,282.07	482,320.52	3,152.009**
Weeks × Lots	16	8,385.41	524.09	3.425**
Animals × Weeks within Lots	332	50,802.73	153.02	
Total	439	2,069,537.02		

*P < .03 P values were obtained after multiplying degrees of freedom
**P < .001 for error terms by one-half.

Analyses of variance of the weekly weight data are shown in Table 8, along with the results of tests of lot mean differences. Bartlett's tests indicated that the error variances of the analyses summarized in Table 8, as well as those of analyses reported below in this section, may be regarded as homogeneous. The analyses of variance elicit significant values of F at hatching and at the second, third, and fourth post-hatching weeks. The F for the first post-hatching week does not attain a significant level.

The results of tests of mean differences ("least significant difference" method) disclose highly significant differences between the Controls and 4-day animals and between the 1-day and 4-day birds in all analyses, with the exception of the first post-hatching week. Significant differences are found between the Controls and 3-day animals at each week (except for the first post-hatching week) and between the Controls and the 2-day birds at hatching and at the end of the fourth week after hatching. Other between-lot differences attain significance also, but these are sporadic. In the above comparisons the mean weights of the Control lot are greater in each case.

A trend analysis was run on the lot means across all weekly weighing periods. Significant differences in linear trend ($P < .01$) were found between the growth curves of all possible pairs of the five lots with the exception of the comparison of the 2-day and 3-day lots. As in the Group II data, an inverse relationship exists between the magnitude of the linear trend component of the growth curve of each lot and the length of the period of exposure to the temperature insult, the curve for the Control lot displaying the greatest linear trend and that of the 4-day lot the least.

TABLE 8

Analyses of variance of Group III data at hatching and at the end of each post-hatching Week and results of tests of mean differences

Analyses of variance

	Source	*df*	*SS*	*MS*	*F*
Hatching	Between Lots	4	316.49	79.12	9.105**
	Within Lots	92	799.19	8.69	
	Total	96	1,115.68		
1*st* week	Between Lots	4	243.66	60.92	1.571 (NS)
	Within Lots	83	3,218.77	38.78	
	Total	87	3,462.43		
2*nd* week	Between Lots	4	2,100.06	525.02	4.270*
	Within Lots	83	10,204.56	122.95	
	Total	87	12,304.62		
3*d* week	Between Lots	4	4,050.01	1,012.50	2.923*
	Within Lots	83	28,746.12	346.34	
	Total	87	32,796.13		
4*th* week	Between Lots	4	13,903.02	3,475.76	3.648*
	Within Lots	83	79,084.88	952.83	
	Total	87	92,987.90		

*$P < .03$
**$P \leqslant .001$
NS: $P > .05$

Results of tests of mean differences

("least significant difference" method) for lots of Group III.
Table entries are lot mean differences.

Comparison	Hatching	1*st* week	2*nd* week	3*rd* week	4*th* week
Control—1-day	1.3	0.7	2.8	6.1	10.0
Control—2-day	2.5**	1.2	4.9	10.4	22.7**
Control—3-day	3.2**	3.6	12.4**	17.5*	28.0*
Control—4-day	6.6**	6.2	25.9**	34.2*	63.3**
1-day—2-day	1.2	0.5	2.1	4.3	12.7
1-day—3-day	1.9	4.3	9.6	11.4	18.0
1-day—4-day	5.3**	6.9	23.1**	28.1*	53.3*
2-day—3-day	0.7	4.8	7.5	7.1	5.3
2-day—4-day	4.1**	7.4	21.0**	23.8	40.6
3-day—4-day	3.4*	2.6	13.5	16.7	35.3

*$P \leqslant .05$
**$P \leqslant .01$

To determine the periods during which the various lots differed in terms of mean rate of weight gain, repeated measures analyses of variance were run on the data for each interval of one week. These results disclosed significant differences in rate of gain between the first and second post-hatching weeks ($P < .01$) and between the third and fourth post-hatching weeks ($P < .025$).

Fig. 4 shows the mean weight gains of the five lots for the four weekly intervals. Analyses of variance run on the weight differences for the

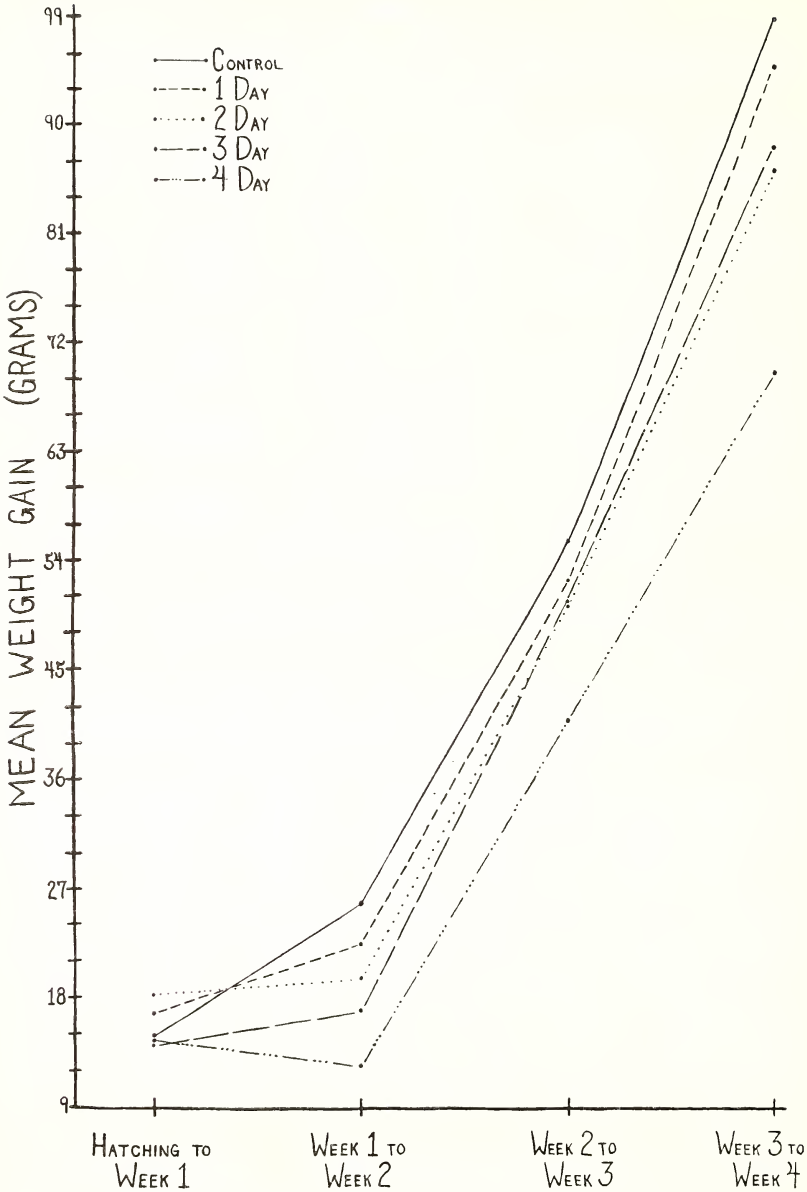

Fig. 4. Mean weight gain of the five lots of chicks in Group III during four weekly intervals.

intervals Week 1 to Week 2 and Week 3 to Week 4 elicited significant values of F ($P < .01$ and $P < .005$, respectively). The results of tests of mean differences at these intervals indicated that during the interval

Week 1 to Week 2 the mean gain of the Control lot was significantly greater than those of the 1-day, 2-day, 3-day, and 4-day lots (all P's $< .05$) and that the mean gain of the 1-day birds was significantly greater than those of the 3-day and 4-day animals (both P's $< .05$). During the interval Week 3 to Week 4 the rate of gain of the Controls was significantly greater than that of the 2-day animals ($P < .01$) and 4-day animals ($P < .05$); also, the 1-day animals gained at a significantly faster rate than did the 4-day birds ($P < .05$). It may be seen in Fig. 4 that the rate of weight gain of the 3-day birds during the interval Week 3 to Week 4 was greater (but not significantly so) than that of the 2-day animals. Furthermore, even though the mean weight gain of the Controls was smaller than those of the 1-day and 2-day birds during the first post-hatching week (non-significant differences), the Controls gained weight more rapidly than the experimental animals and maintained their lead for the entire period of observation.

Discussion

On the basis of the results of statistical analyses, it appears incontrovertible that increased incubation temperature for varying numbers of days resulted in a reduction of total body weight in these chicks. Coupled with this is the fact of increased mortality of the embryos with increased temperature and length of exposure.

Of interest is the fact that a decrease of only one degree of incubation temperature (from 42° to 41°) results in a marked decrease in mortality of the embryos. Total incubation time is not appreciably different, regardless of temperature or length of exposure under these conditions. Mortality rate and total incubation time are not considered further in this report since primary concern is with phenomena associated with weight. Extensive work has been done on mortality rate and total incubation time (7, 10), although that work does not coincide exactly with the results of this research.

It was surprising that significant differences in weight of the hatched chicks occurred both when the eggs were incubated for five days at 42° initially (Group I) and when another group (Group II) was incubated at 42° for the last five days of incubation. According to Romanoff (6), the temperature effect is greatest during the early stages of incubation, but comparison of Tables 2 and 5 indicates that the temperature effect on weights persisted through the first and second post-hatching weeks in Group I and through the third post-hatching week in Group II. Moreover, in both of these groups significant differences in weight did not occur until after hatching. Because of lack of space the Group I birds were not available for weighing after the third post-hatching week. The Group II birds, however, were weighed at the fourth post-hatching week, and Table 5 reveals that no significant differences in mean weights were found at that time, although the mean weights are seen to be appreciably less the longer the exposure to the 42° temperature (Table 4).

A different type of situation exists in Group III, in which the eggs were incubated at 41° initially. This is one degree lower than the non-optimal temperatures used with Groups I and II. Here it is seen that, except for the first post-hatching week, highly significant differences in weight of the hatched birds persisted through the fourth week (Table 8).

In other words, there was no apparent reduction of the temperature effect as the birds grew older. It could be speculated that the 42° temperature used in Groups I and II was fatal to all but extremely well-adapted or healthy birds and that these consequently were able to overcome the effects of the abnormal temperatures. At 41° (Group III) chicks not so well adapted and not so healthy may have survived and were not able to overcome the ill effects of the non-optimally high temperature.

When the data on the differential rates of weight increase are examined critically (Figs. 3, 4), it is apparent that there is basically no difference in the causes underlying the disparities in the results between Groups II and III. The best interpretation here is that the birds in Group III simply partially recovered from the effects of the temperature insult of 41° more rapidly than the birds of Group II did from the temperature insult of 42°. Insofar as the differences in mean weight gain are concerned, the point to be made here is that these differences occur between the lots of birds almost in an inverse step-wise fashion and that they are statistically meaningful. As will be seen in the following paragraphs, these results fit in rather well with an assumption of protein denaturization.

The physiological basis for these differences in mean weight is unknown. Studies aimed at determining this are currently being conducted in our laboratory. It has been suggested that at higher temperatures the eggs lose too much water and appear to dry up (10). Barott (1) found that when eggs were incubated at 38.8° the hatched chicks were smaller, less lively, and less alert than those hatched from normally incubated eggs (4). Recent work indicates that the quantity of polysaccharides is diminished in liver and kidney cells of chick embryos when the eggs are exposed to high temperatures (5). This suggests an interference with the normal conversion of sugars to glycogen. On the other hand, this lack might be the result of a more rapid use of sugars because of the higher temperature.

With respect to dehydration as a result of the higher temperatures, it would seem necessary to assume that, if drying occurred, weight differences at hatching should be significant. As is apparent in Tables 2 and 5, no significant differences were found in weights of the newly hatched chicks in Groups I and II. However, Table 8 reveals highly significant differences in hatching weights among the birds in Group III. Furthermore, unlike the situation in Groups I and II, these weight differences remained significant through the fourth week, although no significant differences were found at the first post-hatching week. One might assume that in spite of the lack of weight differences in the newly hatched birds of Groups I and II dehydration had occurred, and that it would be expected that with continued growth the weight differences would shortly disappear. This was found to be the case. In the case of the Group III chicks it could be said that no dehydration occurred in spite of the highly significant weight differences at hatching, and that the differences continued during the period under observation because of some factor other than dehydration. If it were to be argued that dehydration occurred in Group III but not in Groups I and II, an explanation would have to be forthcoming for the continued weight difference in Group III and for the evening out of the weight differences in Groups I and II.

In view of the conflicting nature of the evidence it might be more profitable to assume that dehydration was not a factor, and to seek an answer in the effects of temperature on biological systems. Already mentioned has been the work of Preda and Cracium (5). The rapid depletion of carbohydrate stores would force an early dependence on protein and fat reserves. By measuring the respiratory and thermal quotients it has been found that carbohydrates are oxidized mainly during the first 2 days of incubation, proteins mainly from days 3-10, and finally fat from day 11 on. Some evidence is accumulating to the effect that carbohydrates may be principally utilized up to the tenth day of incubation (11). The point is that an earlier dependence on the reserves in the egg could cause a loss of the total dry weight of these substances, and, instead of being used for tissue building, these would be lost in the form of waste metabolites. This would account for the significant difference in the weights of Group III (incubated initially at 41°) at hatching, but not for its continuance beyond the fourth week, since the birds should have had ample time to recuperate by that time. The above analysis would not hold for Groups I and II (incubated at 42°), since no significant differences in the mean weights were found between lots in these two groups at hatching.

A third possibility exists. The answer to the cause of the weight loss might be found in the area of cell physiology. Specifically, protein denaturization of one or several enzymes can so affect the system that weight losses occur at different times and in different ways. The thermal inactivation of enzymes may, in one system, be irreversible, as might have been the case in Group III (incubated at 41°). Even within groups (Group III in particular at the first post-hatching week) some reactions might readily be either irreversible or reversible. According to Giese (2), even if a denatured enzyme is not irreversibly altered, a cell might die because of prolonged sub-lethal heat treatment. This has the effect of suspending life processes necessary for the continued existence of a cell long enough to do irreversible damage. In other words, the heat treatment may not be at a temperature sufficiently high to cause irreversible denaturization ot enzymes but may be of sufficient duration to cause an irreversible alteration. Heat injury may also be caused by a derangement of the lipids of the cell, and it has been suggested that the disruption of these important cellular components could easily lead to death. It has also been suggested that heat releases calcium from the cell, which liberates a clotting enzyme that could cause the protoplasm to gel. It would appear that these latter two reactions are irreversible and that this could not account for the observed differences in the chicks' weights, especially in those groups in which weights returned to normal. It seems more likely that reversible enzymatic denaturization occurred in Groups I and II and that in Group III an irreversible reaction occurred. Different enzyme systems become affected at different times in development, and at different temperatures.

Whatever the actual cause it seems reasonable to assume that, on the basis of the statistical evidence, these weight differences are real, and that further investigation of the problem is fully warranted. Such investigation is being continued in our laboratory.

Summary

Chick eggs were incubated at higher than normal temperatures for varying periods of time up to five days, both at the beginning and at the end of the incubation period. The eggs were then permitted to hatch. The chicks were weighed at hatching and at the end of each early post-hatching week.

Statistical analyses disclosed significantly greater mean weight for control animals hatched from eggs incubated at normal temperatures as compared with the weight of experimental birds. In two groups of chicks (eggs incubated at 42° for the first five days and for the last five days of incubation, respectively) the differences in mean weights failed to attain significance at hatching and at the third and fourth weeks after hatching, although significant differences appeared between these times. In a third group (eggs incubated at 41° for the first five days of incubation) significant differences in mean weights were found at hatching and at the second, third, and fourth weeks after hatching but not at the first week after hatching. Highly significant differential rates of weight gain also appeared among the different lots of chicks within these groups, the control birds gaining weight from week to week at a higher rate than the experimental animals.

The possible causes of the observed weight differences are discussed in terms of dehydration, protein denaturization, thermal injury to lipid organization, and release of coagulating enzymes.

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