GROWTH AND METABOLIC ACTIVITIES OF DEVELOPING WINGS AND LEGS OF THE CHICK EMBRYO

I. ESTABLISHMENT OF MULTIPLICATIVE GROWTH RATES ON THE BASIS OF DESOXYRIBOSENUCLEIC ACID*

by

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At the earliest stages of ontogenesis of the chick embryo, the size of the forelimb and hindlimb is approximately the same, but in the course of further development, the legs become appreciable larger. This difference is clearly illustrated in the figures 122, 130, 142, 153, etc. of DUVAL's atlas[1] of the embryology of the chick. SCHMALHAUSEN[2,3] was the first to establish these differences in a mathematical form by calculating the growth rates on a wet weight basis. Recently, HAMBURGER AND HAMILTON[4] have stressed the heteroauxesis of the two limb buds as a basis for classification of the developmental stages of the chick embryo. Because of these differential growth characteristics, the developing appendages present particularly suitable material for the study of enzymic activities related to growth.

In the present investigation, the object was to establish the growth rates of the wings and legs as the basis for enzymic studies. However, overall growth consists of three different processes (NEEDHAM[5]): increase in cell number (multiplicative), increase in cell size (auxetic), and increase in extracellular substance (accretionary). It was decided to study the multiplicative growth and compare it with the overall growth as expressed by the wet weight.

In order to measure the multiplicative growth of the wings and legs of 5- to 12-day old embryos, the desoxyribosenucleic acid (DNA) content was chosen as reference (NOWINSKI AND YUSHOK[6]). The relationship between DNA and multiplicative growth was first shown by MANDEL, BIETH AND STOLL[7]. It also was established that the DNA content of an average nucleus in many diploid somatic cells of the same species is the same (BOIVIN, VENDRELY AND VENDRELY[8]; VENDRELY AND VENDRELY[9]; MIRSKY AND RIS[10]; DAVIDSON *et al.*[11]). On the basis of these results, DAVIDSON AND LESLIE[12,13] used DNA as a measure of cell multiplication and as a standard reference for revealing changes in metabolic patterns of cell activity. According to MANDEL AND BIETH[14] the increase in DNA is the best approximation of multiplicative growth available at present.

* This investigation was supported by a research grant (No. RG-2167) from the Research Grant Division of the U.S. Public Health Service.

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EXPERIMENTAL

Materials and methods

Throughout these studies, a cross of White Leghorn hens and New Hampshire Red cockerels was used. This cross, recommended to us by Prof. J. R. COUCH as being a vigorous hybrid, proved to be extremely suitable for our purpose because of its viability and uniformity*.

In the preliminary phase of the work on nuclear counts and DNA, still-air incubators were used. However, because of the temperature gradient and uncontrolled humidity conditions, the variability in growth was so great that it was necessary to switch over to the Jamesway forced draft incubator**. In this incubator, the eggs were kept at a temperature of 37.5° C (± 0.1° C) and relative humidity of 60 to 65%. The eggs were turned automatically every two hours. By keeping these environmental conditions constant, the variability of embryonic material was reduced to a minimum.

The embryos were removed from the eggs and immediately immersed in ice-cold saline solution and rinsed. After taking off the membranes, the embryos were rinsed again. The wings and legs were carefully removed as near the body as possible by means of fine, curved forceps, and kept in ice-cold saline***. Prior to weighing, the pooled wings and pooled legs were placed in a small Buchner funnel (Coors #0000), with a close fitting filter paper disk (Whatman #2) on top of which was put a piece of cellophane with tiny perforations. This cellophane disk served as a protection against loss of tissue from adhesion to the filter paper. The funnel was then fixed in a filter tube and the excess of water removed by gentle suction.

After the appendages were weighed on a Roller-Smith torsion balance, they were homogenized in a Potter-Elvehjem homogenizer with ice cold 0.04 *M* citric acid, as recommended by DOUNCE[15]. One drop of caprylic alcohol-ethanol mixture (1:4 by volume) was added from a fine capillary to reduce occasional foaming.

In order to prevent possible loss of DNA and to simplify the procedure, a special tube was designed in which the purification and extraction were run. An ordinary Pyrex culture tube 100 × 12 mm was constricted in the middle and accurately calibrated at the constriction to contain 5 ml.

The estimations were done in duplicate. To each tube, 2 ml of 5% tissue homogenate and 5 ml of ice-cold 10% trichloractic acid (TCA) were added, mixed thoroughly and allowed to stand with occasional stirring for 10 minutes in an ice bath. The tubes were then centrifuged at full speed for a 10-minute period in a Precision micro-centrifuge, refrigerated by a special mantle, described elsewhere (NOWINSKI AND YUSHOK[16]). After decanting the supernatant, the precipitate was re-suspended in 5 ml of 75% ethyl alcohol, and centrifuged at room temperature for 10 minutes.The precipitate was extracted with 4 ml of 5% TCA, according to SCHNEIDER[17]. In order to prevent foaming, which may cause loss of extracted material, one drop of caprylic alcohol-ethanol mixture was again added. After extraction, the tubes were cooled to room temperature and the content was brought up with 5% TCA to the 5 ml mark and centrifuged for 10 minutes. The supernatant was syphoned into a test tube without disturbing the loose precipitate.

The DNA was estimated according to DISCHE[18]. To a 2 ml aliquot (in duplicate) of the extract, 4 ml of diphenylamine reagent† was added. The tubes were kept in a boiling water bath for exactly 10 minutes and immediately cooled to room temperature in tap water. The absorbancy at 590 mμ was measured in a Beckman DU spectrophotometer.

The mixture of diphenylamine reagent and the TCA extract was found to be sensitive to visible light of an intensity present in our laboratory on a bright sunny day. Exposure of the samples to light resulted in an appreciable positive error. Therefore, from the moment the reagent was added, special care was taken to keep the samples in the dark except during the time they were being transferred from one container to another. When these precautions were followed, the absorbancy remained constant for at least one hour (the longest period checked). Whether the magnitude of the light effect is related to the presence of variable quantities of photosensitizing substances remains to be investigated.

For the appendages of 5- and 6-day embryos, a semi-ultramicro method was worked out to permit duplicate determinations on 50 mg of tissue. All the quantities in the entire procedure were reduced to one tenth of that of the original method. The absorbancy was measured in Lowry-Bessey microcells.

Sodium desoxyribosenucleate (Nutritional Biochemicals Corp.) was used as standard. Its purity was determined on the basis of phosphorus content. The theoretical P content of pure NaDNA

* We are grateful to Dr. J. R. COUCH and Dr. J. H. QUISENBERRY, both of Texas A. and M. College, for supplying the eggs.

** Supplied by Jamesway Manufacturing Co., Fort Atkinson, Wisconsin.

*** The technical assistance of Mrs. DOROTHA ROARK is gratefully acknowledged.

† The diphenylamine (Eastman Kodak) was purified by distillation through an air condenser; only the middle third portion of the distillate was used.

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was taken to be 9.28% (MIRSKY AND POLLISTER[19]). A sample of desoxyribosenucleic acid (Schwartz Laboratories, Inc.), calculated on an equal P content, as well as a sample of highly polymerized DNA preparation (Worthington Biochemical Sales Co.) gave the same absorbancy.

For nuclear counts, a sample of 1 ml of 5% homogenate was placed in a 5 ml volumetric flask, and 0.2 ml Delafield's hematoxylin was added. The mixture was allowed to stand for approximately 30 minutes at room temperature and was then diluted to the mark with 0.04 *M* citric acid, containing 0.3% Saponin. The latter was added in order to hemolyze the erythrocytes (DOUNCE AND LAN[20]) and to facilitate uniform spreading of the suspension in the counting chamber. The number of nuclei was determined in a Spencer Bright-Line hemocytometer: 10 groups of 16 small squares per group were counted. Counts in duplicate were made on two aliquots. The deviations in the counts of nuclei were similar to those of red blood cells as reported by MAGATH, BERKSON AND HURN[21].

RESULTS

Increases in wet weights. The wet weight data presented in Table I were obtained throughout a one-year period. The weighings were performed on pooled appendages: for example, from 20 to 50 wings or legs were taken on the 5th day; from 15 to 34 on the 6th day; and progressively less until the 12th day when 3 to 8 appendages were pooled.

TABLE I

WET WEIGHTS OF WINGS AND LEGS: MEAN AND STANDARD DEVIATION

Days of incubation	*No. of samples of appendages**		*Weight mg per appendage*	
	Wing Pools	*Leg Pools*	*Wing*	*Leg*
5	30	29	1.92 ± 0.26	2.30 ± 0.23
6	43	42	3.51 ± 0.45	5.54 ± 0.66
7	34	34	6.56 ± 0.54	12.4 ± 1.2
8	34	36	11.9 ± 1.0	25.3 ± 2.6
9	38	35	21.6 ± 2.1	46.8 ± 4.6
10	48	47	38.7 ± 3.6	90.4 ± 11.8
11	38	36	66.4 ± 7.1	169 ± 20.0
12	17	17	108 ± 15.6	266 ± 29.0

* of pooled samples.

On the 5th day of incubation, the average weight of a leg was 20% greater than that of a wing. The statistical evaluation showed this difference to be significant: $t = 5.9$ (for "Student's" t values see FISHER[22]). On the 6th day, the difference increased to 58% ($t = 16.7$). During further development, the differences became more and more pronounced and reached 146% on the 12th day.

Smooth exponential curves were formed (Fig. 1) when the weights of an appendage was plotted against days of incubation. Approximately straight lines were obtained on a semi-logarithmic scale (Fig. 2).

Instantaneous rate of weight increase (K_w) were calculated on a percentage basis, according to the formula used by BRODY[23,24]:

$$K_w = \frac{100\,(\log_e W_2 - \log_e W_1)}{t} \qquad (1)$$

where W_2 and W_1 are the final and initial weights, respectively, and t is the time interval.

According to the data presented in Table II, the K_w values for wet weights of

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wings from the 5th through the 10th day of incubation were approximately 60% per day. During the 11–12 day period the rate dropped to 49%. The K_w of the legs was 88% during the 5–6 period and progressively decreased until the 8–9 day, from which time until the 10–11 day, it remained approximately stationary. On the 11–12 day period, it dropped sharply to 45%.

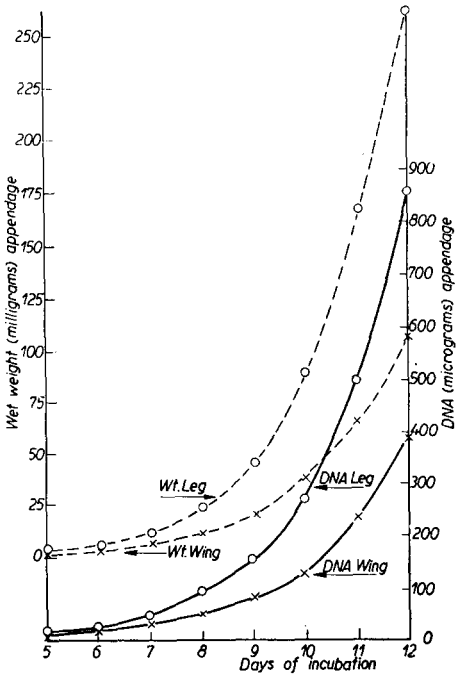

Fig. 1. Multiplicative growth (DNA) and overall growth (wet weight) of wings and legs.

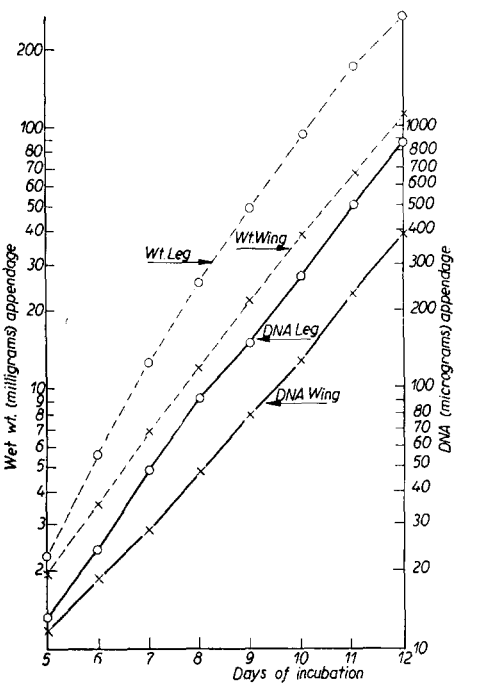

Fig. 2. Multiplicative growth (DNA) and overall growth (wet weight) of wings and legs plotted on a semilogarithmic scale.

TABLE II

WET WEIGHT PER WING AND PER LEG; INSTANTANEOUS RATE OF INCREASE (K_w), RATIO OF RATES (a), AND TIME OF DOUBLING (t_{2w})

Incubation period	*Rate of weight increase in % per day*		*Ratio of rates*	*Time of doubling of weights in hours*	
day	*Wings*	*Legs*	*Leg/wing*	*Wings*	*Legs*
5–6	60	88	1.5	28	19
6–7	62	81	1.3	27	21
7–8	60	71	1.2	28	23
8–9	60	62	1.0	28	27
9–10	58	66	1.1	29	25
10–11	54	63	1.2	31	26
11–12	49	45	0.92	34	37

Ratios of the growth rates (legs/wings) were also calculated (Table II). They correspond to HUXLEY's constant k for relative growth (HUXLEY[25], NEEDHAM[26]) which

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we prefer to call the allometric constant a after VON BERTALANFFY[27]. During the 5–6 day period, the ratio was 1.5, and it decreased progressively up to the 8–9 day interval, when it reached 1.0, *i.e.* the growth rates of both appendages were the same. However, it increased again until the 10–11 day and dropped in the 11–12 day period.

The comparison of the time required for the weights of the appendages to double gives further insight into the allometric processes. The formula for calculation of the doubling time (t_{2w}) was derived from the equation (1):

$$K_w = \frac{\log_e 2w - \log_e W}{t} \tag{2}$$

which becomes:

$$K_w = \frac{\log_e 2}{t} \tag{3}$$

the rearrangement of equation (3) gives:

$$t_{2w} = \frac{\log_e 2}{K_w} \tag{4}$$

The results of calculations based on this formula are given in Table II. From the 5th through the 10th day, the t_{2w} for wings was fairly constant, 27 to 29 hours. In the 5–6 day period, the t_{2w} for legs was much lower, 19 hours; it increased gradually to 27 hours on the 8–9 day. From this period through the 11th day, the t_{2w} did not change appreciably, but in the last interval investigated it was significantly higher than in any preceding stage.

DNA content of nuclei. Before using DNA as an expression of multiplicative growth, it was necessary to establish the mean DNA content of nuclei at the investigated stages of development. Table III summarizes the results, based on the estimations of 907 wings and 889 legs. It was found that the overall average content of DNA per million nuclei was the same for both appendages, and corresponded to 8.2 μg with practically identical standard deviations (wings, 1.2; legs, 1.4).

TABLE III

DNA CONTENT OF NUCLEI OF WINGS AND LEGS

Days of incubation	*No. of experiments (No. of appendages expt.)*		*μg DNA million nuclei**	
	Legs	*Wings*	*Legs*	*Wings*
5	2 (43–61)	2 (51–61)	9.2 (9.2–9.3)	9.1 (8.3–9.9)
6	3 (48–129)	4 (51–130)	7.7 (7.1–8.2)	7.7 (7.2–8.5)
7	6 (34–45)	7 (30–50)	7.6 (6.6–8.2)	8.1 (7.3–9.3)
8	6 (20–25)	6 (11–16)	8.1 (6.5–9.9)	7.6 (5.8–9.5)
9	5 (20–40)	5 (12–30)	8.4 (6.2–9.9)	8.3 (6.4–10.0)
10	5 (10–25)	5 (6–16)	8.8 (7.9–10.6)	9.1 (8.0–9.9)
All stages	27 (907)**	29 (889)**	8.2 $\pm$ 1.2***	8.2 $\pm$ 1.4***

* Mean (range of experimental values).
** Total number of appendages.
*** Mean $\pm$ standard deviation of pooled samples.

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At each stage investigated, the average DNA content per million nuclei of the wings was similar to that of the legs. However, considering the large experimental errors in the separation of nuclei and in the nuclear counts, as well as a slight error in the DNA method, it appears to be improbable that a significant difference could be established between the two limbs.

TABLE IV

CONCENTRATION AND ACCUMULATION OF DNA IN WINGS AND LEGS

Days of incubation	*No. of estimations (No. appendages/estimation)*		*DNA concentration* (μg/mg wet weight)*		*μg DNA appendage**	
	Wings	*Legs*	*Wings*	*Legs*	*Wings*	*Legs*
5	10 (26–38)	10 (26–38)	5.32 ± 0.17	5.24 ± 0.16	11.8 ± 0.66	13.3 ± 0.60
6	10 (19–34)	10 (19–33)	5.38 ± 0.076	4.66 ± 0.12	18.6 ± 0.76	24.8 ± 0.70
7	10 (38–44)	10 (38–44)	4.58 ± 0.10	4.01 ± 0.060	28.5 ± 1.1	48.1 ± 1.5
8	8 (21–30)	10 (22–30)	3.99 ± 0.067	3.65 ± 0.057	48.1 ± 1.4	92.6 ± 4.0
9	10 (13–18)	9 (14–18)	3.89 ± 0.035	3.38 ± 0.024	81.5 ± 2.7	152 ± 3.2
10	10 (7–12)	10 (7–12)	3.79 ± 0.060	3.35 ± 0.047	127 ± 1.5	271 ± 7.0
11	12 (4–8)	12 (4–8)	3.88 ± 0.043	3.46 ± 0.084	230 ± 8.7	508 ± 8.4
12	10 (3–8)	9 (3–6)	3.95 ± 0.070	3.45 ± 0.073	389 ± 11	863 ± 35

* Mean ± standard error of the mean of pooled samples.

Concentration and accumulation of DNA. The wings of 5- and 6-day old embryos had a high concentration of DNA (Table IV): 5.32 μg and 5.38 μg/mg wet tissue, respectively. The concentration dropped to 4.58 on the 7th day and further to 3.99 on the 8th day. From then on through the 12th day, no statistically significant differences were found between any two stages. In the legs, the concentration of DNA was highest on the 5th day, 5.24 μg/mg wet tissue, and gradually decreased to 3.35 on the 10th day. The DNA concentrations in the 11- and 12-day legs were not significantly different from that in the 10th day.

On the 5th day of incubation (Table IV), the average wing contained essentially the same amount of DNA as the average leg, $t = 1.7$. On the 6th day, the quantity of DNA per leg was already significantly greater than that of the wing ($t = 6.0$), in spite of the higher concentration of DNA on the basis of mg wet tissue of the latter. The difference was increasing steadily throughout the later stages, reaching 863 μg for the leg and 389 μg for the wing on the 12th day.

When DNA per single appendage was plotted against days of incubation, exponential curves were obtained for the wings and the legs (Fig. 1). On a semi-logarithmic scale (Fig. 2), the increase in DNA showed a linear trend.

DNA: Rates of accumulation and time of doubling. The instantaneous rates of DNA accumulation (K_{dna}) were calculated according to equation (1), in which the DNA was substituted for weight. The data in Table V reveal that from the 5th through the 8th day the legs accumulated DNA at an appreciably greater rate than the wings. During the 8–9 day period, the K_{dna} of the legs and the wings were approximately equal. The greatest difference in the rate of DNA accumulation was during the 6th to 7th day period, which was indicated by the ratio 1.5 of legs/wings.

Table V also shows the times of doubling in the quantity of DNA (t_{2dna}), calculated according to formula (4). During the 5–6 day period, t_{2dna} of the legs was 27 hours,

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and that of the wings, 36 hours. From the 6th to the 7th day of incubation, the doubling time of DNA of the legs was 25 hours; of the wings, 39 hours. In the 7–8 day period, the t_{2dna} of the wings decreased to 32 hours, but that of the legs did not change. The doubling times for both appendages were essentially the same in the 8–9 day interval. On the next stage, t_{2dna} increased for the wings (38 hours) and decreased for the legs (29 hours); however, during the last two periods there was no difference in the doubling time between the two appendages.

TABLE V

DNA PER APPENDAGE: INSTANTANEOUS RATE OF ACCUMULATION (K_{dna}), RATIO OF RATES (a), AND TIME OF DOUBLING (t_{2dna})

Incubation period	*Rate of DNA accumulation in % per day*		*Ratio of rates*	*Time of doubling of DNA in hours*	
day	*Wings*	*Legs*	*Leg/wing*	*Wings*	*Legs*
5–6	46	62	1.4	36	27
6–7	43	66	1.5	39	25
7–8	52	66	1.3	32	25
8–9	53	50	0.94	31	33
9–10	44	58	1.3	38	29
10–11	59	63	1.1	28	26
11–12	53	53	1.0	31	31

DISCUSSION

If DNA is to be used as an expression of multiplicative growth, the DNA content per nucleus should be established on a statistical average basis. However, in many somatic tissues of a given species of vertebrates, the DNA content per average nucleus has been reported to be constant (BOIVIN, VENDRELY AND VENDRELY[8]; VENDRELY AND VENDRELY[9,28]; MIRSKY AND RIS[10]; DAVIDSON *et al.*[11]; LESLIE AND DAVIDSON[29]). This claim of constancy may be valid for an organ whose cells contain a diploid number of chromosomes exclusively and have a negligible rate of division. In cells of organs which exhibit polyploidy, constancy of DNA per nucleus can not be expected. The situation is even more complicated in growing organs, such as the wings and legs, which have high rates of cell multiplication. Studies on signle nuclei reveal that the quantity of DNA changes during the complete mitotic cycle (SWIFT[30]; POLLISTER, SWIFT AND ALFERT[31]; BLOCH AND PATAU[32]). In the early prophase, the DNA content is twice as great as that in the telophase, which indicates that it is synthesized during the interphase. In a given animal species, the DNA content during telophase is the same as that in the non-dividing nucleus of diploid chromosome number.

In the present studies the value of 8.2 μg per million nuclei of wings or legs represents the overall average of cells in various intermitotic and mitotic stages but not those in which the nuclear membrane is absent. This mean value is not expected to be equal to the DNA content of non-dividing cells of diploid chromosome number. If the nuclei of the wings and legs attain a tetraploid quantity of DNA during the late interphase, then the DNA per average nucleus should be somewhere between the diploid and tetraploid quantity. This value can be affected by two factors: (1) proportion of cells synthesizing DNA to non-dividing cells, and (2) the rate of DNA accumulation

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during interphase. The extent to which these factors vary in embryonic tissues cannot be determined by the existing methods. However, the calculated ranges in which 95% of the DNA values were found for all the stages from the 5th through the 10th day were 5.8 to 10.6 μg per million nuclei of the wings and 5.4 to 11.0 μg of the legs. An absolute constancy in DNA per nucleus can be assumed only by attributing all the variation to the experimental methods used. Since absolute constancy cannot be expected where the rates of cell division and of DNA synthesis are high, the DNA value per nucleus can be expressed only as a statistical average of a given population.

In the logarithmic phase of growth of the appendages, 100 μg of DNA correspond to approximately 12 million cells. This statistical cell population can be used as a basis for relating metabolic activities to multiplicative growth (NOWINSKI AND YUSHOK[6]; YUSHOK AND NOWINSKI[33]). When these activities are expressed on a unit DNA basis, comparisons between cell populations can be made. In the subsequent papers of this series, metabolic rates will be evaluated on this basis.

The rates of accumulation of DNA are equivalent to those of cell number and are independent of the absolute DNA content of a given cell. The comparison of the instantaneous rates reveals that, from the 5th through the 10th day, wet weight increases are greater than those of DNA (Fig. 2). This is to be expected because the change in wet weight (volume) represents not only the increase in number of cells but also the increase in cell size and in extracellular space.

The time of doubling in DNA offers the best available approximation of the time required for a new generation of cells to be formed. In the 6–8 day legs, the number of cells approximately doubles within a period of 25 hours. In the ideal case, if every cell of the legs undergoes division, the duration of a complete mitotic cycle of a cell would be 25 hours. Approximately 96% of the cells would divide in 24 hours. When the same assumption is made for 6–7 day wings, it is calculated that approximately 60% of the cells would undergo mitosis in 24 hours. Actually, the calculation of doubling time refers to the interval in which the whole cell population doubles irrespective of the rates of division of the different cell types and of a possible multiplicative growth gradient from the proximal to distal ends of the appendage.

If the duration of the mitotic stages (telophase, prophase, metaphase, and anaphase) of an average cell is assumed to be one hour and the time of doubling in cell number is 25 hours (as in 6–8 day legs), approximately 4% of the cell population would be in the mitotic stages and 96% in the interphase at a given instant. Assuming that the duration of the four mitotic phases is also one hour in 6–7 day wings ($t_{2dna} = 39$ hours), the percentage of the cell population in mitotic stages would be approximately 2.5; in interphase, 97.5.

SUMMARY

1. The multiplicative growth rates of wings and legs of 5- to 12-day chick embryos were calculated on the basis of desoxyribosenucleic acid (DNA) which was extracted according to SCHNEIDER's method and estimated with DISCHE's diphenylamine reagent.

2. The overall average and range of DNA content per million nuclei were the same for the legs and the wings.

3. On a semi-logarithmic scale, the increases in DNA and wet weight showed a linear trend for both appendages.

4. On the 5th day of incubation, the mean DNA content per wing was not significantly different from that of a leg. During further development, the legs accumulated DNA at an appreciably higher

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rate than the wings. The instantaneous rate of DNA increase were calculated. The ratio of these rates (leg/wing) was highest at the 6 to 7 day interval.

5. During the 5–8 day periods, the times required for the quantity of DNA per leg to double were between 25 to 27 hours; those per wing, between 32 to 39 hours.

6. The rates of increase of DNA (cell number) were compared with those of wet weight (overall growth) and were found to be lower.

7. The unit DNA as an expression of a given cell population was suggested as a reference for metabolic studies.

RÉSUMÉ

1. Les vitesses de multiplication cellulaire des ailes et des pattes d'embryons de poulet de 5 à 12 jours ont été calculées d'après les quantités d'acide désoxyribonucléique (DNA) extrait par la méthode de Schneider et déterminé colorimétriquement par le réactif à la diphénylamine de Dische.

2. La quantité globale de DNA pour 10^6 noyaux est du même ordre de grandeur pour les pattes et pour les ailes.

3. Sur un graphique semi-logarithmique, l'accroissement de l'acide désoxyribonucléique (DNA) et du poids sec est approximativement linéaire pour les deux groupes d'organes.

4. Au 5e jour d'incubation, la quantité moyenne du DNA des ailes n'est pas significativement différente de celle des pattes. Par la suite, les pattes accumulent l'acide désoxyribonucléique à une vitesse sensiblement supérieure aux ailes. Les vitesses d'accroissement du DNA ont été calculées; le rapport de ces vitesses (patte/aile) est maximum vers le 6–7e jour.

5. Au cours de la période allant de 5 à 8 jours, les temps requis pour doubler la quantité de DNA est de 25 à 27 heures pour les pattes; ce temps est de 32 à 39 heures pour les ailes.

6. Les vitesses d'accroissement du DNA (nombre de cellules) ont été comparées à celles du poids sec (croissance totale) et ont été trouvées inférieures.

7. Il est suggéré d'utiliser comme référence au cours d'études du métabolisme l'unité acide désoxyribonucléique comme expression d'une population cellulaire donnée.

ZUSAMMENFASSUNG

1. Die Vermehrungswachstumsgeschwindigkeit von Flügeln und Beinen von 5–12 Tage alten Kückenembryonen wurde auf Grund der Desoxyribonucleinsäure (DNS) berechnet, die nach der Methode von Schneider extrahiert und mit Dische's Diphenylaminreagens bestimmt wurde.

2. Der Gesamtdurchschnitt und der Bereich des DNS-Gehaltes pro 1,000,000 Kerne war derselbe in den Beinen und in den Flügeln.

3. Bei beiden Extremitäten zeigte der Anstieg der DNS und des Nassgewichts im halblogarithmischen Masstab einen linearen Verlauf.

4. Am 5. Tag der Bebrütung war der mittlere DNS-Gehalt pro Flügel nicht bedeutend verschieden von dem eines Beines. Während der weiteren Entwicklung wurde in den Beinen DNS mit beträchlich grösserer Geschwindigkeit angesammelt als in den Flügeln. Die augenblickliche Geschwindigkeitszunahme wurde berechnet. Das Verhältnis der Geschwindigkeit von Bein zu Flügel war am grössten nach einem Zeitraum von 6–7 Tagen.

5. Während des Zeitraumes von 5–8 Tagen war die zur Verdopplung der Menge DNS pro Bein erforderliche Zeit 25–27 Stunden, die pro Flügel 32–39 Stunden.

6. Die Geschwindigkeit der Zunahme der DNS (Zellenzahl) wurde mit der des Nassgewichtes (Gesamtwachstum) verglichen und wurde als niedriger gefunden.

7. Die Einheit DNS als Ausdruck einer gegebenen Zell-Population wurde als Bezugseinheit für Stoffwechseluntersuchungen vorgeschlagen.

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Received February 19th, 1953