NUCLEIC ACID METABOLISM OF THE DEVELOPING CHICK EMBRYO

by

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Studies of the incorporation of labelled precursors into the nucleic acids of adult animal tissues have been steadily pursued during the past few years, and it appears possible to state that more incorporation occurs into the ribonucleic acid (RNA) than into the desoxyribonucleic acid (DNA) and that the higher the mitotic index (number of mitoses per 1000 cells) of the system, the greater is the uptake into the DNA. For example, after labelled sodium phosphate had been administered to rats, the specific activity of the RNA phosphorus greatly exceeded that of the DNA phosphorus. At the same time, tissues where active cell division was taking place, such as intestinal mucosa, regenerating liver or tumours, showed a much greater uptake of the label into the DNA than did the mitotically inactive organs such as adult liver and brain[1–10]. This tendency for a system only to take up a precursor into DNA if cells were dividing was shown also when the precursor was ammonia[3], formate[11,12], adenine [11,13–15], and orotic acid [16,17].

In the case of the uptake into RNA however, no such dependence on mitotic activity can be seen, and the amount of isotope incorporated seems to depend more on the actual compound carrying the label than on any metabolic or functional state of the system. Thus, studies on various rat organs showed that the ratio of the specific activities of the RNA purines of liver and intestine was about 1.6 when adenine was the precursor[13], but that the ratio dropped to less than 0.2 when the incorporation of formate was similarly studied[18]. This difference was confirmed by an experiment where both compounds were offered at the same time[11]. Glycine resembled formate in showing a high uptake into the RNA of intestinal mucosa[19,20].

So many new compounds are being isolated from the acid soluble fractions of tissues[21–25] that it is too early to expect full metabolic studies to have been reported, but a few results on the incorporation of adenine into the acid soluble fractions of rat organs showed no obvious relation to mitotic activity[26].

Although the literature on the nucleic acid metabolism of adult organs is extensive, much less is known about embryonic tissues. In foetal rat liver, the DNA, as would be expected, incorporated labelled sodium phosphate so extensively that after 2 hours, the isolated DNA phosphorus had a higher specific activity than the RNA phosphorus[3,27]. Rat embryos showed some ability to incorporate labelled adenine which had been injected into the mother[28]. The uptake of formate into the nucleic acids of developing chick embryos has been studied by LAVIK[29] who was primarily interested in the effects of radiation on the process. The incorporation of thymidine

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into the DNA of the chorio-allantois of the chick has been followed by FRIEDKIN *et al.*[30] and of adenine into the DNA of tissue cultures of embryonic chick heart by LU KIAO-HUNG AND WINNICK[31].

STEVENS[32] injected labelled sodium phosphate into 9–16 day chick embryos and incubated them for six hours. He isolated and counted the RNA phosphorus and DNA phosphorus and included in his calculations the pool of unlabelled sodium phosphate available in the egg. He concluded that the incorporation of phosphate reached a peak on about the 13th day of development and he considered that this was related to the peak of RNA and DNA concentration in the embryo which NOVIKOFF AND POTTER[33] had shown to occur at about this time. From this he concluded that after the 13th day, a profound change in nucleic acid metabolism occurred. STEVENS injected his material into the yolk sac accompanied by penicillin and streptomycin, but other results[34, 35] suggest that penicillin is not without action on nucleic acid metabolism. It was thought desirable therefore to investigate the nucleic acid metabolism of the developing chick embryo by studying, in the first instance, the incorporation of labelled formate [36, 37] into the nucleic acid purines and into the acid soluble adenine of whole chick embryos, hoping by this means to evolve a picture of nucleic acid metabolism from a much earlier stage than had been done previously.

METHODS AND MATERIALS

Injections of labelled formate solution

Preliminary experiments had shown that embryos were able to tolerate 10 mg of sodium formate without any apparent damage, so a dose of 5 mg per egg was decided upon. This dose would ensure that the amount of labelled formate available to the embryo was not likely to be a limiting factor in nucleic acid formation from formate at least in the early stages. 0.5 mc ^{14}C sodium formate (Radiochemical Centre, Amersham) was diluted with carrier so that the solution eventually contained 250 mg sodium formate in 25 ml distilled water. 0.5 ml of the above solution was injected into the air sac with no aseptic precautions, and the eggs stood in egg cups with the air sac upmost in the incubator. When liquids are injected into the air sac of an egg containing a living embryo, they are absorbed within a few hours into the vascular network underneath. This, in early stages belongs to the yolk sac; after 8–9 days it is that of the chorio-allantoic membrane. This method of administration avoids mixing the injected solution with the yolk, from which a variable proportion may remain unabsorbed during the experimental period. After 12 hours, the embryos were dissected out, freed as far as possible from extra-embryonic membranes and briefly examined at a low magnification. Dead embryos were discarded and the stage of each of the others was determined by comparison with the standards given by LILLIE[38]. As is usual, the figure for the age of development obtained in this way is from 12–24 hours less than the actual time of incubation. The embryos were then treated as described below. The number of eggs injected depended on the age of development and was 24 for the 3 day stage (of which 4 were later discarded as non-viable), 9 at 4 days, 5 at 5 days, 3 at 6 and 7 days, 2 at 8 and 9 days, and 1 each for all the later stages.

Separation of the constituents from the embryo

Immediately after dissection, the embryos were homogenised with about 3 volumes of ice cold 0.6 *N* perchloric acid and the residue spun down in the cold. The supernatant was evaporated to dryness and 72% perchloric acid (2 ml/g wet weight) added. The acid soluble extract was then hydrolysed at 100° for 45 min[39] and further isolation of the liberated adenine was carried out as described by ROLL, WELIKI AND BROWN[40]. Final separation was carried out by chromatography on Whatman No. 1 filter paper in *iso*-propanol/HCl[41].

The tissue residue was washed twice with water, once with alcohol, and suspended in 10 ml *N* KOH overnight at room temperature. DNA was precipitated by addition of HCl and trichloracetic acid[42], while barium ribonucleotides were precipitated from the supernatant[40]. Both the washed DNA and barium ribonucleotides were hydrolysed in 10 ml *N* HCl at 100° for 1 hour, the hydrolysates cleared by filtration and evaporated to dryness. The liberated purines were separated on paper as before.

Elution and radioactive measurement of the separated purines were carried out as previously described[26].

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RESULTS

The results are presented in Figs. 1–3 which show the variation with age of the embryo of the specific activities of the acid soluble adenine (Fig. 1), of the DNA purines (Fig. 2) and of the RNA purines (Fig. 3). The measured specific activities have been plotted backwards for 12 hours from the nominal age of the embryo to allow for the lag in development which has been mentioned previously. The slopes of the curves decrease with age and, at any particular age, the activity of the acid soluble adenine exceeds that of the RNA purines which, in turn, exceeds that of the DNA purines.

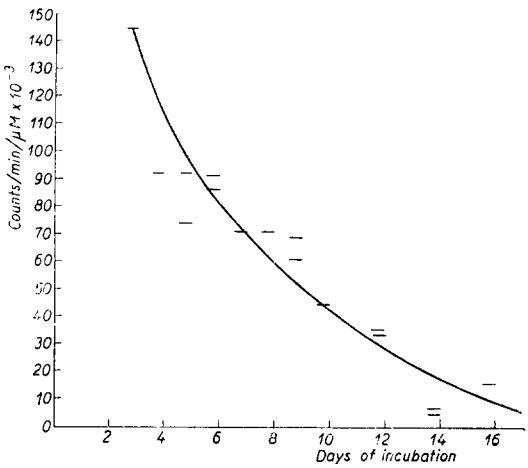

Fig. 1. Specific activity of acid soluble adenine isolated from chick embryos which had incorporated ^{14}C sodium formate for 12 hours at various ages of incubation.

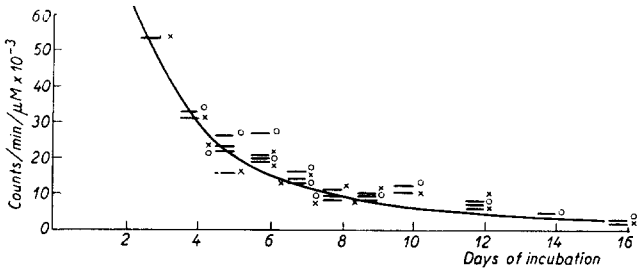

Fig. 2. Specific activity of desoxyribonucleic acid purines (× : adenine, ○: guanine) isolated from chick embryos which had incorporated ^{14}C sodium formate for 12 hours at various ages of incubation.

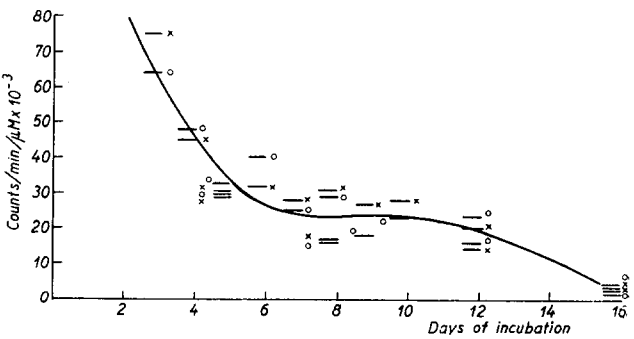

Fig. 3. Specific activity of ribonucleic acid purines (× : adenine, ○: guanine) isolated from chick embryos which had incorporated ^{14}C sodium formate for 12 hours at various ages of incubation.

DISCUSSION

In seeking for a closer understanding of the metabolism of the nucleic acids of the embryo, we would first wish to compare the results which have emerged from the present work with other developmental variables. Of these, the most important are the concentration and total amount of the nucleic acids present, the mitotic rate, and the progress of differentiation of the embryonic tissues. It should be mentioned that the

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ratio of the weight of the embryo to that of its membranes is variable[43]. The yolk sac is much the greater part of the whole system at first, but at about 8 days, the weight of the embryo becomes equal to that of its membranes, and afterwards rapidly overtakes it. Since the administered formate must become distributed throughout both embryo and membranes and since only the embryo has been investigated, clearly a varying proportion of the synthesising system has been taken at the different ages. An exhaustive enquiry of this kind would demand separate data for each of the main systems of tissues. It is hoped to continue this work by comparison of different organs with respect to these features, but meanwhile certain general conclusions can be drawn.

Relationship of the specific activities of the nucleic acid purines to the concentration of nucleic acids in the embryo

The changes in the concentration of nucleic acids in the whole embryo have been studied by NOVIKOFF AND POTTER[33] from the 2nd to 15th day, and by REDDY, LOMBARDO AND CERECEDO[44], from the 7th to the 19th day. Although there at first appears to be a considerable discrepancy between these investigations (*cf.* ref. 33, Fig. 3 and ref. 44, Fig. 2) this is for the most part due to an error in the caption to NOVIKOFF AND POTTER's graph which should read as a plot of nucleic acid concentration in mg % *wet* weight (confirmed by personal communication from the authors). Recalculation of the results of REDDY *et al.* on a wet weight basis[45] gives a curve not widely different from that given by NOVIKOFF AND POTTER. (These are shown together on Fig. 4). It is clear that the amount of synthesis of the nucleic acids in the chick embryo as determined by the incorporation of sodium formate is unrelated to the changes in concentration of either RNA or DNA. The specific activity of both sets of purines falls away with increasing age of the embryo and it is noteworthy that over the period studied, no regular difference between the specific activities of the purines existed, either in the RNA or in the DNA.

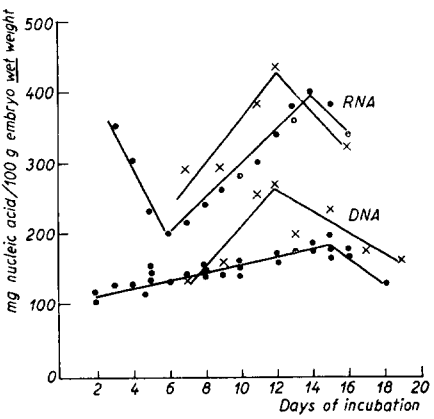

Fig. 4. Variation in the nucleic acid content (mg % wet weight) according to REDDY, LOMBARDO AND CERECEDO[44] (×) and according to the corrected estimates of NOVIKOFF AND POTTER (●) (see text). Top curves – Ribonucleic acid; Bottom curves – Desoxyribonucleic acid.

Relationship of the specific activities of the nucleic acid purines to the total amount of nucleic acid present

If the DNA is metabolically inert once formed [2,13,14,54], then there must be a connection between the specific activity of the DNA purines and the proportion of the DNA which has been synthesised during the experimental period. The mathematical relationship can be deduced knowing the amount of DNA present in the system, and will be discussed more fully in a subsequent paper. So far, no correlation can be detected in the case of RNA.

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Relationship of the specific activities of the nucleic acid purines to the mitotic index of the embryo

No figures exist for the mitotic index of the whole embryo save for the first 3 days of development[46], but data have been obtained for several organs of the embryo, namely spinal cord[47], heart [48], and liver[49,50]. Although there is a general tendency for the mitotic index to decrease with time, the rate of decline varies from tissue to tissue. Thus HAMBURGER[47] has shown that mitotic figures become very rare in the spinal cord after the 8th day when the rate of division in the liver and heart is still relatively high. In Fig. 5 are reproduced published figures for the mitotic index of various embryonic organs from 2–21 days and it should be observed that even in the heart at 21 days the index is not zero, while there are always plenty of mitoses occurring in the intestines. From Fig. 5 the approximate mitotic index for the whole embryo may be taken as about 20/1000 at 4 days and about 10/1000 at 9 days. Crick's formula quoted by HUGHES[51] shows that the whole mitotic cycle of interphase and division at these ages will take about 35 and 70 hours respectively (assuming time of mitosis to be 1 hour), so that during 12 hours (the duration of the experiments) the 4-day embryo will have undergone about 7 mitoses/1000 cells and the 9-day embryo about 1.7 mitoses/1000 cells. It is possibly significant that the ratio of these mitotic figures (*i.e.*, 4) is not widely different from the ratio of the specific activities of the DNA purines at these ages taken from Fig. 2 (32,000/11,000 = 3). A relationship between mitoses in rat liver and intestine and synthesis of DNA has been advanced by STEVENS, DAOUST AND LEBLOND[52] who, on the basis of ^{32}P uptake of labelled sodium phosphate, suggested that each cell division required the synthesis of 2 units of DNA phosphorus, the old material being discarded. It is not possible to relate those results to those presented here since STEVENS *et al.* injected at zero time and sacrificed at varying intervals afterwards, while the present work is concerned with the amount of synthesis taking place over a constant period of time at different stages in development of the system.

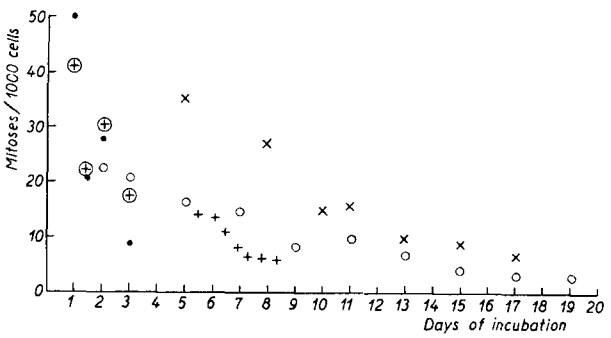

Fig. 5. Mitotic indices for various organs of the developing chick embryo. ⊕ Average of 3 germ layers – SCHULTZ[46]; ● Mesoderm – SCHULTZ[46]; ○ Heart – OLIVO AND SLAVITCH[48]; + Liver – O'CONNOR[49]; × Liver – PERRY[50]

Finally, the specific activities of the isolated purines always decreased in the order acid soluble adenine, RNA purines, DNA purines and this is not inconsistent with the possibility that the acid soluble materials may serve as nucleic acid precursors, as has been suggested in previous work[53].

SUMMARY

The incorporation of ^{14}C labelled sodium formate into the acid soluble adenine and into the nucleic acid purines of the developing chick embryo has been studied from the 3rd to the 16th day of incubation.

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RÉSUMÉ

L'incorporation du formiate de soude, marqué à l'aide de ^{14}C, dans l'adénine de la fraction acide-soluble et dans les purines de l'acide nucléique du poussin embryonnaire en développement a été étudiée du troisième au seizième jour de sa croissance.

ZUSAMMENFASSUNG

Die Einverleibung von mit ^{14}C markiertem ameisensauren Natrium in das säure lösliche Adenin und in die Nucleinsäure-Purine des wachsenden Hühnerembryos wurde vom dritten bis zum sechzehnten Tage der Entwicklung untersucht.

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