SYNTHESIS OF NUCLEIC ACIDS BY CHICK BLASTODERMS GROWN ON SYNTHETIC MEDIUM

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HOFF-JØRGENSEN[1] found that there is a constant level of deoxyribonucleic acid (DNA) in the whole hen's egg during the first three days of incubation. However, during this period considerable cell division and morphogenesis of the rapidly growing blastoderm occur, and this is accompanied by a rapid rate of increase of ribonucleic acid (RNA) and DNA[2]. The hen's egg yolk and white has recently been found to contain relatively large amounts of DNA[3], compared with the requirements of the early embryo. HOFF-JØRGENSEN's results may thus indicate that the developing blastoderm is obtaining DNA from the egg white and yolk. However, it is also possible that the chick blastoderm can synthesise its own supply of DNA during embryogenesis. SPRATT[4,5] has shown that the explanted chick blastoderm will undergo morphogenesis when grown on synthetic medium containing glucose as the sole carbon source. This technique has been used in this work to demonstrate that the explanted blastoderm is capable of synthesising RNA and DNA during embryogenesis.

METHODS

The technique for growing blastoderms *in vitro* is the same as that used by SPRATT[5,6]. The culture medium consisted of 34 ml chick Ringer solution (sodium chloride 0.9 g, potassium chloride 0.042 g and calcium chloride 0.024 g per 100 ml bidistilled water), 2 ml of phosphate buffer solution (anhydrous disodium hydrogen phosphate 0.116 g and potassium dihydrogen phosphate 0.052 g per 100 ml), 2 ml of sodium bicarbonate solution (1.1 g per 100 ml) saturated with carbon dioxide and sterilised by filtration, 2 ml of glucose solution (0.44 g per 100 ml), 2 ml of phenol red solution (0.02 g per 100 ml) and about 0.2 g powdered agar. All solutions were stored at 4° C. The chick Ringer, glucose, phenol red solution and agar were pooled, and after neutralisation with sodium bicarbonate, were autoclaved with the phosphate buffer solution. The phosphate and bicarbonate buffer solutions were then added and the medium pipetted (2 ml portions) into watch glasses, which were placed in Petri dishes containing sterile pads of moistened cotton wool and allowed to cool to 38° C. All glass apparatus was sterilised in the autoclave for 20 min and dissecting instruments were dry-sterilised for 4 h at 110° C.

Eggs from Rhode Island X Light Sussex fowls were incubated at 38° C for 20–36 hours. The blastoderms were then removed from the yolk, the vitelline membrane removed and the blastoderms rinsed several times in warm saline (37° C) to remove yolk platelets. Blastoderms were identified morphologically according to the stages of HAMILTON AND HAMBURGER[7]. The blastoderms were transferred to the surface of the agar and incubated at 38° C for 18–26 hours. The blastoderms were again examined morphologically, and if normal, classified according to the stages of HAMILTON AND HAMBURGER[7]. Blastoderms showing similar morphological development were pooled and stored at —20° C.

Nucleic acids were extracted from homogenates of each group of blastoderms by the perchloric acid method previously described[2]. The average number of nuclei in some homogenates

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was determined by staining with methyl green and counting in a haemocytometer[8]. RNA was measured by the orcinol method[9] for ribose after breakdown of free pentoses and hexoses with alkali[10], and by an isotope dilution method for uracil[11]. DNA was measured by indole reaction with deoxyribose[11] and by an isotope dilution method for thymine[2]. Both colorimetric methods were modified so that the colour was measured in 1 ml and a correction applied to deoxyribose determinations in the presence of RNA (10 μg RNA $\equiv$ 1 μg DNA). Protein was determined by the method of SUTHERLAND *et al.*[12].

RESULTS AND DISCUSSION

Five groups of blastoderms (batches 1–5), in which the notochord was just visible (HAMILTON AND HAMBURGER'S stage 5), were explanted on glucose medium and incubated at 38° C for 18 or 26 hours. The increase in amounts of RNA, DNA and protein per blastoderm during this period are shown in Table I. It can be seen that there is no correlation between the time of incubation *in vitro* and the extent of morphogenesis (as shown by the average number of somite pairs in each batch) and morphogenesis is usually slower than normal (*cf.* WADDINGTON[13] and SPRATT[5]). The amounts of RNA, DNA and protein in the blastoderm after incubation *in vitro* are compared with amounts previously found in blastoderms grown *in ovo* after the same incubation period. The amounts of DNA in blastoderms grown *in vitro* are generally

TABLE I

NUCLEIC ACID SYNTHESIS BY 20-HOUR BLASTODERMS GROWN ON GLUCOSE MEDIA

Batch No.	*1*	*2*	*3*	*4*	*5*
No. of blastoderms	2	4	7	4	3
Duration of incubation time *in vitro* (h)	26	26	26	26	18
Average no. of somite pairs after growth *in vitro*	6	7	10	11	13
Average no. of somite pairs after growth *in ovo* (controls)	16	16	16	16	11
DNA					
μg per blastoderm grown *in vitro* (by deoxyribose)	10.6	8.7	9.4	7.7	5.0
(by thymine)	—	—	8.8	—	—
μg per blastoderm grown *in ovo* (controls)	17.6	18.7	17.6	18.7	9.0
% controls	60	47	53	41	56
μg synthesised per blastoderm	7.5	5.3	6.0	4.3	3.2
Average no. of cells per blastoderm $\times 10^6$	—	—	—	—	6.4 (7.2)*
RNA					
μg per blastoderm grown *in vitro* (by ribose)	117	75	81	60	42
(by uracil)	107	71	—	—	—
μg per blastoderm grown *in ovo* (controls)	102	108	102	108	62
% control	110	68	79	56	68
μg synthesised per blastoderm	84	46	53	31	17
Protein					
μg per blastoderm grown *in vitro* $\times 10^2$	5.2	4.7	8.7	4.9	6.6
μg per blastoderm grown *in ovo* (controls) $\times 10^2$	24	26	24	26	11
% control	22	18	36	19	60

* control *in ovo*.

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lower than those grown *in ovo* (41–60%) but there is a definite synthesis of DNA *in vitro* as shown by the increased amounts of DNA per blastoderm (3.2–7.5 μg) above those of the explanted blastoderm[2]. Blastoderms (20 h) growing on glucose medium synthesise comparatively large amounts of RNA (17–84 μg per blastoderm), which are generally slightly less than the amounts produced by blastoderms grown *in ovo* (56–110% of normal). However, the amounts of protein synthesised appear to be small (18–60% of normal), which implies that a redistribution of protein within the blastoderm is taking place so that embryogenesis may proceed.

Nucleic acid synthesis in later stages of embryogenesis was studied by growing blastoderms with 5–10 somite pairs on glucose medium for 20 h (Table II). During this period of incubation, morphogenesis proceeded nearly as rapidly as in the egg (except for batch 6). As would be expected, the amounts of DNA synthesised *in vitro* (6.9–18 μg per blastoderm) are greater than when starting with 20-hour blastoderms, and the amounts per blastoderm are correspondingly higher (48–122% of normal). However, the amounts of RNA synthesised are lower (except for batch 8) than during the earlier stages of embryogenesis.

TABLE II

NUCLEIC ACID SYNTHESIS BY 5–10 SOMITE BLASTODERMS GROWN ON GLUCOSE MEDIA FOR 20 HOURS

Batch No.	6	7	8	9	10
No. of blastoderms	3	2	2	5	1
Average no. of somite pairs after growth *in vitro*	13	19	19	20	22
Average no. of somite pairs after growth *in ovo* (controls)	26	30	17	17	30
DNA					
μg per blastoderm grown *in vitro* (by deoxyribose)	15.3	23	23	16.7	15.4
(by thymine)	—	—	—	21.3	—
μg per blastoderm grown *in ovo* (controls)	26	32	18.8	18.8	32
% control	59	72	122	101	48
μg synthesised per blastoderm	8.3	14.5	18	14	6.9
Average no. of cells per blastoderm × 10^6	—	—	—	13 (15)*	12.4 (26.0)*
RNA					
μg per blastoderm grown *in vitro* (by ribose)	82	—	164	42	70
(by uracil)	—	—	—	65	55
μg per blastoderm grown *in ovo* (controls)	140	—	109	109	163
% control	58	—	150	49	39
μg synthesised per blastoderm	32	—	124	14	12

* control *in ovo*.

In three batches, the average number of nuclei per blastoderm was determined and in each case was found to be below normal. The average amounts of DNA per nucleus were $0.8 \cdot 10^{-6}$, $1.3 \cdot 10^{-6}$ and $1.2 \cdot 10^{-6}$ μg in batches 5, 9 and 10, respectively; this is near the range found for blastoderms growing *in ovo*[2] ($1.2 \cdot 10^{-6} - 1.8 \cdot 10^{-6}$ μg DNA per nucleus).

The RNA/DNA ratios of blastoderms grown *in vitro* can be compared with values

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of blastoderms grown *in ovo* based on incubation time and on the extent of morphogenesis (Table III). Slightly higher values for blastoderms grown *in vitro* are found in batches 1–5 in relation to either time or morphogenesis; the values in batches 6 and 10 are similar to the normal values, but in batches 8 and 9 they differ from the normal.

It has thus been shown that the chick blastoderm can synthesise its own nucleic acids to enable embryogenesis to proceed *in vitro*. Under normal conditions, in the egg, the blastoderm probably ingests precursors of nucleic acids from the yolk, as has been shown by the incorporation of radioactive formate[14] and thymidine[15] by chick embryos during later stages of development. It has been suggested[2,3] that the nucleic acids present in the yolk form a storage reserve of these precursors, which are kept in the form of nucleic acids until degraded by enzymes in the yolk.

TABLE III

RNA/DNA RATIOS OF BLASTODERMS GROWN *in vitro*

	RNA/DNA		
		in ovo	
Batch No.	*in vitro*	*based on incubation time*	*based on "corrected incubation time"*
1	11.0	5.8	8.3
2	8.7	5.8	7.2
3	8.7	5.8	6.9
4	7.8	5.8	6.8
5	8.4	6.9	6.5
6	5.4	5.4	6.5
8	7.0	5.8	5.7
9	2.5	5.8	5.7
10	4.5	5.1	5.6

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SUMMARY

The synthesis of RNA and DNA by explanted chick blastoderms grown on a synthetic medium containing glucose as the sole carbon source has been demonstrated.

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FRACTIONATION OF IMMUNE RABBIT SERUM GAMMA-GLOBULIN

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It has been recently reported that it is possible to separate electrophoretically several subfractions of human serum albumin by using buffers of low ionic strength at pH values close to the isoelectric point[1]. Human γ-globulin (Cohn fraction III) has also been fractionated in three boundaries by this method[2]. SAIFER AND COREY[3] suggest that these results are due to a "microheterogeneity" of the protein molecules present in albumin and in γ-globulin, *i.e.* to the existence in them of patterns of molecules having similar but not identical physical and chemical properties.

The principle described by SAIFER AND COREY has been used by HUMPHREY AND PORTER[4] for studying by partitional column chromatography the immune globulin of rabbits at different stages of immunization with various antigens. The detection of three subfractions was possible with rabbit serum globulin also, but the antibodies were not present in all subfractions. The specific antibody was found either in the slow migrating boundary or in the middle one, depending upon the time after the immunization and the type of the antigen. The authors suggest that different types of cells may produce several types of γ-globulins differing slightly one from another.

In the present paper an account is given of an attempt to separate different subfractions of serum γ-globulin of both normal and immunized rabbits by means of paper electrophoresis, and to detect the specific antibody in these subfractions.

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