THE CHEMICAL COMPOSITION OF THE CHICK EMBRYONIC CELL

by

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INTRODUCTION

Of the numerous biochemical studies which have been made on the developing chick embryo the great majority have been concerned with chemical changes occuring in the embryo as a whole[24,27]. By comparison, only a few investigations have been made on the chemical changes within individual organs during development. This is unfortunate since it is frequently difficult to assess the significance of the results derived from so complex a system as the whole embryo, particularly when changes are described in terms of concentrations per unit weight of tissue. Not only are the various organs developing in different ways and at different rates during the various phases of embryonic life, but their chemical composition also changes as they develop towards their adult functional state.

Even when the organs are studied separately, difficulties arise in the interpretation of results, especially when analytical figures are expressed in terms of concentration of a constituent per unit weight of tissue, since the weight itself refers to a constantly changing complex of variables. There are, furthermore, the errors introduced by biological variations as instanced by the problem of deciding embryonic age[20].

Some of these difficulties may be overcome by following the approach to the biochemistry of growth which we described recently[10,11]. The essence of this method is to follow the chemical development of tissues in terms of the changes in cell number and in the amounts of the various constituents per cell. This has become possible as the result of the observation that the deoxyribonucleic acid (DNA) content of the cell nucleus is constant in amount in all the somatic tissues of any one species[4,13,23,32,33]. When the amount of DNA per nucleus is known for the particular species under investigation, cell number can be found by determining the total DNA in the organ, and the average cell composition can be obtained by expressing other constituents in relation to the amount of DNA.

For the fowl, the results obtained by various research groups agree that the content of DNA per nucleus is of the order of 2.3 to $2.4 \cdot 10^{-6}$ μg and this figure has been shown to hold for the chick embryo at 14 and 19 days[11]. In the present study the results are based on the approximate mean figure of $2.35 \cdot 10^{-7}$ μg DNA phosphorus (DNAP) per nucleus for chick brain, heart, liver and skeletal muscle. This apparent constancy in

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the amount of DNA per nucleus throughout development is in agreement with the concept held by geneticists that most somatic nuclei contain the set of chromosomes characteristic of the genotype at all stages of development. Since DNA appears from cytochemical evidence to be an integral part of chromosome structure, the amount found in different nuclei of the same species should be the same. On the other hand cells containing the haploid number of chromosomes (sperm) might be expected to contain half the amount of DNA found in somatic nuclei and this has been proved experimentally in a number of cases. An increase in the amount of DNA per nucleus would be expected in cells where the chromosomal material has been increased by polyteny or polyploidy.

The use of DNA instead of wet or dry weight as a standard of reference in biochemical studies of growth has several advantages. The weight of an organ can rise by increase in cell number or cell size, or by a combination of both. By reference to DNA the two processes can be distinguished, for it becomes possible to follow changes in average cell weight throughout development when the weight of the tissue is related to the amount of DNA per cell. Changes in cell weight, in turn, can be correlated with the determinations of cell number and cell composition. Another advantage is that results expressed as amounts per cell of any constituent are independent of the proportions of the other constituents of the tissue, and their significance can be judged on the basis of whether the cell constituent increases, decreases, or remains constant per cell during growth and development. This also applies to the determination of growth rates of cell constituents relative to that of DNA. In the allometric method originally proposed by TEISSIER[30] and NEEDHAM[25, 26] growth rates were based on the wet or dry weight of the whole, and their significance was to some extent obscured because the constituent was part, and sometimes an important part, of the complex of variables which represented the weight. On the other hand, when based on DNA, the relative growth rates show quantitatively how a constituent varies with increasing cell number over distinct phases of embryonic development.

METHODS

Eggs from a stock of pure bred Leghorn poultry were received in batches weekly after seven days incubation and were further incubated at 37 to 38° in a HEARSON egg incubator. Embryos were taken for chemical analysis from the eighth day of incubation, until two days following hatching. The common methods of determining embryonic age from the weight of the embryo or from the period of incubation are both subject to errors, but it was decided to adopt the second method and to base the results for ten- to twenty-day embryos on the means of groups of determinations made on successive days. For example the figures for 19.5-day embryos are the means calculated for embryos sampled on the 19th and 20th days of incubation. The investigation was carried on at irregular intervals over a period of two years and the results cover any seasonal variations which may occur in embryonic development.

Immediately after removal of the embryo from the egg, the brain, heart, liver and a portion of skeletal muscle from the leg were removed, chilled in ice and weighed. In the youngest embryos (8 days) the determinations were made on pooled material from twelve embryos; two to six embryos of 10 to 18 days provided sufficient material for analysis, and after 18 days the determinations could be carried out on organs from individual embryos.

The weighed tissues were thoroughly crushed in a mortar or homogenized in a POTTER-ELVEHJEM homogenizer, and were transferred quantitatively to a centrifuge tube in which the proteins and associated materials were precipitated with ice-cold 10% trichloroacetic acid (TCA). The precipitate was washed with ice-cold 10% TCA, washing and extract being combined to form the acid soluble fraction (ASP). Lipids were then removed from the residue by extraction successively with 80% ethanol, 100% ethanol, ethanol-chloroform mixture (3.1) at 70–80°, and ether. These extracts were combined to form the lipid fraction (LP) and the dry residue was incubated over night at 37° in *N* NaOH for determination of the nucleic acids by the SCHMIDT AND THANNHAUSER procedure[29].

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A suitable portion of the alkaline digest was taken for the determination of residual nitrogen (RN) and the remainder was acidified to precipitate DNA and protein, the ribonucleotides from the ribonucleic acid (RNA) remaining in the acid solution. Phosphorus determinations were made on the acid-soluble fraction (ASP), on the lipid fraction (LP), on the RNA fraction (RNAP) and on the DNA fraction (DNAP), in accordance with the usual SCHMIDT AND THANNHAUSER procedure. The figures for RNAP inevitably include a minute amount of phosphorus from the phosphoproteins of the tissue. The phosphorus determinations involved amounts ranging from about 2 to 200 μg. For the range of 2 to 10 μg. P the method of BERENBLUM AND CHAIN[3] as modified by DAVIDSON AND WAYMOUTH[14] was employed. For larger amounts of phosphorus a modification of the method of ALLEN[1] was used. Nitrogen was determined both on the alkaline digest as mentioned above and on combined portions of the acid-soluble and lipid fractions. The micro-Kjeldahl method was employed using perchloric acid as oxidizing agent as described elsewhere[12]. Protein nitrogen (PN) was calculated by subtracting from RN the amount of N corresponding to the total nucleic acid as determined by multiplying nucleic acid P by the factor 1.69.

RESULTS

It is customary to express the results of the chemical analysis of any tissue in terms of the absolute amounts of each component per organ or of the concentrations per unit weight of wet or dry tissue. This has been done in the first two sections of Tables 1–4 for brain, heart and liver, but it has not been possible to give the absolute amounts per organ for skeletal muscle, since irregular samples of this tissue were taken rather than the whole organ. If the amount of DNA remains constant per nucleus throughout embryonic development, it is possible to calculate the amounts per cell of the various cell constituents at each stage of development. Such results are given in the third section of each table, calculated on the basis of $2.35 \cdot 10^{-7}$ μg. DNAP per nucleus in all four tissues. This third section, by relating the wet weight of the tissue to the constant value for DNAP, also shows how the cell mass varies during development.

Comparison of the results expressed as concentrations per unit weight of fresh tissue with the absolute amounts per cell, reveals that there are substantial differences in trend between the two sets of figures during the growth of the brain, liver and muscle. For example, in brain (Table I) the concentration of ASP decreases during development, while at the same time the amount per cell actually doubles. The protein concentration increases by about 40% in brain tissue during the embryonic period, whereas the corresponding increase per cell is in the region of 250%. Similar differences are found in the results for liver (Table III), where there is a consistent fall in the concentration of RNAP in the tissue; in contrast, the amounts per cell remain at the same level over the whole period of development. Although in muscle tissue (Table IV) the figures for concentration and amount per cell show the same general trend, the percentage increases in ASP, LP, and PN are much greater when expressed as amounts per cell. Only in heart tissue, in which the cells show little or no change in composition, are the changes in concentration and in amount per cell materially the same (Table II).

Since the DNAP remains constant in amount per cell, the total amounts of DNAP per organ provide us with a relative measure of cell number throughout embryonic development. This has enabled us to follow changes in the rate of cell multiplication in brain, heart and liver, where whole organs were taken for analysis. The daily increments of DNAP in the three organs were obtained from large scale graphs, on which DNAP per organ was plotted against incubation age, and from these were derived the average or mid-increments for the 24 hour intervals covering the 11th to 20th days. By expressing these mid-increments as percentages of the corresponding amounts of DNAP per organ, (as was proposed and developed by MINOT[22] for weight measurements

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TABLE

COMPOSITION OF

ASP = acid soluble phosphorus; LP = lipid phosphorus; RNAP = ribonucleic acid phosphorus;

Age (days)	Number of samples	Mean wt. of brains (mg/organ)	ASP	LP	RNAP	DNAP	NPN	PN	ASP	LP
			μg/organ						μg/100 mg	
8	12	56	60	43	28	16	168	400	107	77
10	20	107	107	80	49	20	231	786	100	75
11.5	16	177	157	129	65	23	371	1190	89	73
13	12	241	207	190	96	30	496	1636	86	79
14.5	16	333	274	286	125	38	620	2115	82	86
17.5	14	539	470	526	195	53	1370	4098	87	98
19.5	14	666	532	734	269	68	1785	4476	80	110
Hatching	3	816	650	1125	371	94	1996	8295	80	138
2 days after Hatching	3	822	630	1220	434	86	2112	9560	77	148

TABLE

COMPOSITION OF

Abbreviations

Age (days)	Number of samples	Mean wt. of hearts (mg/organ)	ASP	LP	RNAP	DNAP	NPN	PN	ASP	LP
			μg/organ						μg/100 mg	
8	12	4	4	3	2	0.7	6	35	100	75
10	20	9	9.5	5.3	4.8	1.7	16	76	105	59
11	8	13	13	8	7	2.5	26	100	100	62
13	18	36	38	23	17	6	113	288	106	64
14.5	16	68	61	41	32	12	223	446	90	60
17.5	14	133	137	94	71	29	367	1350	103	71
19.5	14	174	158	126	83	39	351	1738	91	72
Hatching	3	237	186	165	116	48	570	2610	78	70
2 days after Hatching	3	310	261	236	118	59	1121	4139	84	76

TABLE

COMPOSITION OF

Abbreviations

Age (days)	Number of samples	Mean wt. of livers (mg/organ)	ASP	LP	RNAP	DNAP	NPN	PN	ASP	LP
			μg/organ						μg/100 mg	
8	12	11	15	10	13	3	35	118	136	91
10	20	22	28	22	25	6	70	342	127	100
11.5	16	49	57	47	49	10	140	583	116	96
13.5	10	117	139	122	126	26	421	1855	119	104
15	6	215	253	223	185	51	—	3015	118	104
17.5	10	392	456	418	372	80	1678	6553	116	107
19.5	16	546	505	573	498	122	1888	7743	93	105
Hatching	3	872	660	870	788	163	2720	15040	75	100
2 days after Hatching	3	1278	893	1303	1133	240	4700	24580	70	102

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I

CHICK EMBRYO BRAIN

DNAP = deoxyribonucleic acid phosphorus; PN = protein nitrogen.

RNAP	DNAP	NPN	PN	Cell mass mg·10⁻⁷	ASP	LP	RNAP	DNAP	NPN	PN
fresh tissue					μg·10⁻⁷/cell					
50	29	300	715	8.2	8.8	6.3	4.1	2.35	24.6	59
46	19	216	735	12.6	12.6	9.4	5.8	2.35	27.1	93
37	13	210	672	18.1	16.0	13.2	6.6	2.35	37.9	121
40	12	206	680	18.9	16.2	14.9	7.5	2.35	38.8	128
38	11	186	635	20.6	16.9	17.7	7.7	2.35	38.3	131
36	10	254	760	23.9	20.8	23.4	8.6	2.35	60.5	182
40	10	268	672	23.0	18.4	25.4	9.3	2.35	61.5	155
45	12	245	1015	20.4	16.2	28.1	9.3	2.35	50.0	207
53	10	257	1160	22.4	17.2	33.3	11.8	2.35	57.5	261

II

CHICK EMBRYO HEART

as in Table I

RNAP	DNAP	NPN	PN	Cell mass mg·10⁻⁷	ASP	LP	RNAP	DNAP	NPN	PN
fresh tissue					μg·10⁻⁷/cell					
50	17	150	870	13.4	13.4	10.1	6.7	2.35	20.2	117
53	19	178	845	12.4	13.1	7.3	6.6	2.35	22.1	105
54	19	200	769	12.2	12.2	7.5	6.6	2.35	24.4	94
47	17	314	800	14.1	14.9	9.0	6.7	2.35	44	113
47	18	328	655	13.3	11.9	8.0	6.3	2.35	44	87
53	22	276	1015	10.8	11.1	7.6	5.7	2.35	30	109
48	22	202	1000	10.5	9.5	7.6	5.0	2.35	21	105
49	20	240	1100	11.6	9.1	8.1	5.7	2.35	28	128
38	19	362	1336	12.3	10.4	9.4	4.7	2.35	45	165

III

CHICK EMBRYO LIVER

as in Table I

RNAP	DNAP	NPN	PN	Cell mass mg·10⁻⁷	ASP	LP	RNAP	DNAP	NPN	PN
fresh tissue					μg·10⁻⁷/cell					
118	27	318	1070	8.6	11.7	7.8	10.2	2.35	27.4	92
114	27	318	1556	8.6	11.0	8.6	9.8	2.35	27.4	134
100	20	286	1190	11.5	13.4	11.0	11.5	2.35	32.9	137
108	22	360	1584	10.6	12.5	11.0	11.4	2.35	38.0	167
86	24	—	1430	9.9	11.7	10.3	8.5	2.35	—	139
95	20	428	1674	11.5	13.4	12.3	10.9	2.35	49.3	193
91	22	346	1420	10.5	9.7	11.0	9.6	2.35	36.4	149
90	19	312	1720	12.6	9.5	12.5	11.4	2.35	39.2	217
89	19	368	1920	12.5	8.8	12.8	11.1	2.35	46.0	241

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TABLE

COMPOSITION OF

Abbreviations

Age (days)	Number of samples	ASP	LP	RNAP	DNAP	NPN	PN
		μg/100 mg fresh tissue					
11.5	12	84	40	45	21	198	410
13.5	22	105	57	59	25	282	795
15	12	82	39	41	19	206	645
17.5	12	85	47	47	20	235	967
19.5	18	85	49	47	17	270	1156
Hatching	3	98	47	36	12	352	1466
2 days after Hatching	3	107	60	38	14	417	1920

in studies on the growth-rate of an organism) it is possible to obtain a picture of the varying rates of cell multiplication in each tissue throughout embryonic development. The resulting curves are shown in Fig. 1, where it is seen that the rate of cell multiplication is remarkably uniform in the brain tissue of 11 to 20 day embryos. In contrast, both heart and liver cells multiply most rapidly between 10 and 14 days, after which there is a steady decline in multiplication rate until the time of hatching. Figures for the actual amounts of DNAP per organ in Tables I–III show that the cell number in heart increases 70 times, in liver 54 times, and in brain only 6 times between the 8th day and hatching time.

The changes in average cell mass (*i.e.* wet weight relative to DNAP) for brain, heart, liver and muscle are compared in Fig. 2. During embryonic growth, brain, liver and muscle cells all increase in mass, while the heart cells show little change in the average weight. The mass of the brain cells increases steadily from 8 to 18 days, and the final values represent an increase of about 200% over the mass of 8-day brain cells. Muscle cells increase approximately 100% between the 12th day and hatching time largely owing to an abrupt rise in cell mass after 18 days. The change in liver cells is small and gradual by comparison since they increase only by about 50% between the 8th day and hatching time. Comparison of the relative cell weights in the four tissues

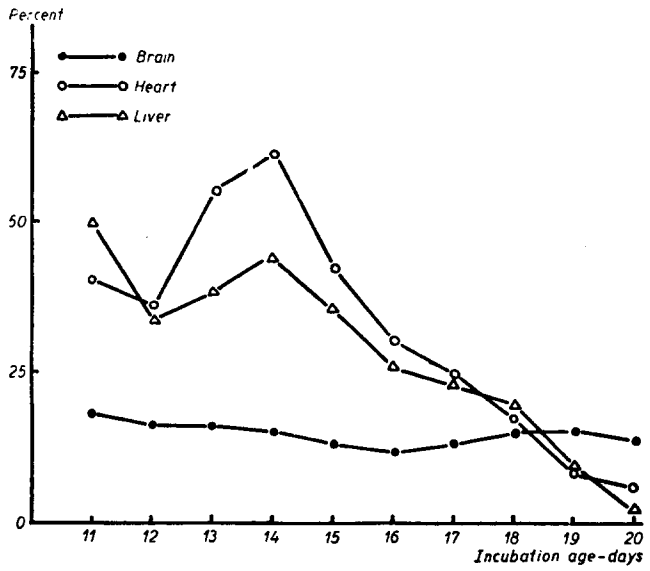

Fig. 1. Percentage increments of deoxyribonucleic acid (DNAP) per organ for 24 hour intervals during embryonic development of chick brain, heart and liver.

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IV

CHICK EMBRYO MUSCLE

as in Table I

Cell mass mg·10^{-7}	ASP	LP	RNAP	DNAP	NPN	PN
	μg·10^{-7}/cell					
11.0	9.2	4.4	4.9	2.35	21.8	45.1
9.5	10.0	5.4	5.6	2.35	26.8	75.5
12.1	9.9	4.7	4.9	2.35	24.9	78
11.8	10.1	5.6	5.6	2.35	27.7	114
14.2	12.0	7.0	6.6	2.35	38.3	164
20.2	19.7	9.4	7.3	2.35	71.0	296
16.9	18.1	10.1	6.4	2.35	70.5	324

of the 2-day old chicks show that heart and liver cells have the same mass, muscle cells are about 35% heavier, and brain cells are 80% heavier (Tables I–IV).

Fig. 3 shows that the acid-soluble phosphorus (ASP) increases in brain and muscle cells in the same way as does cell mass. Again the increase in muscle cells is particularly abrupt in the two days before hatching. Heart and liver cells both show a slight but consistent decline in ASP content, the amounts per cell being highest at the time of rapid cell multiplication in these tissues (Fig. 1).

The increase in the phospholipid (LP) content of the brain cells is very large by comparison with the corresponding increases in liver and muscle cells (Fig. 4). After hatching, the brain cells contain more than five times more phospholipid than do 8-day cells, while muscle cells increase their content twofold after the 12th day. The increase per cell in liver tissue is smaller, and there is no significant change in the phospholipid content of heart cells. The general course of these changes in all four tissues follows closely the pattern found for changes in cell mass (Fig. 2).

Changes in the amounts per cell of RNAP are shown in Fig. 5. Again there is a steady and comparatively large increase in the RNAP content of brain cells, bringing the amount after hatching to three times that in the 8-day cells. Muscle cells increase their RNAP content by about 50% in the period before hatching, while heart cells

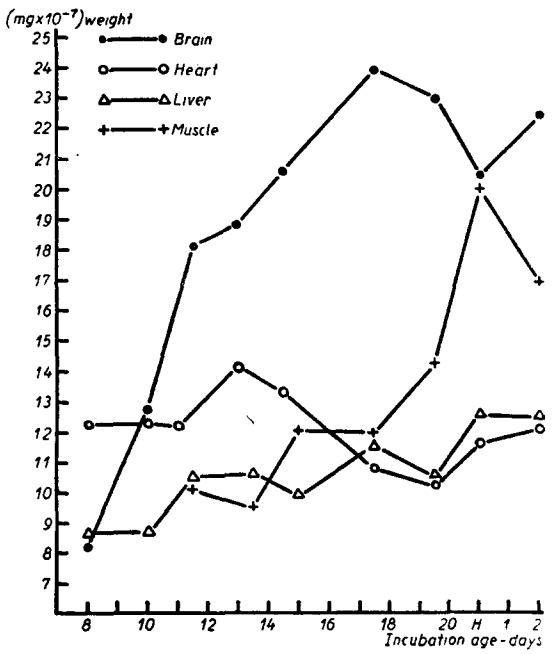

Fig. 2. Variations in cell mass in chick brain, heart, liver and skeletal muscle during embryonic development. Cell mass calculated from fresh weights of organs on the basis that each chick cell contains 2.35 μg DNAP. *H* on time scale for incubation age refers to hatching time.

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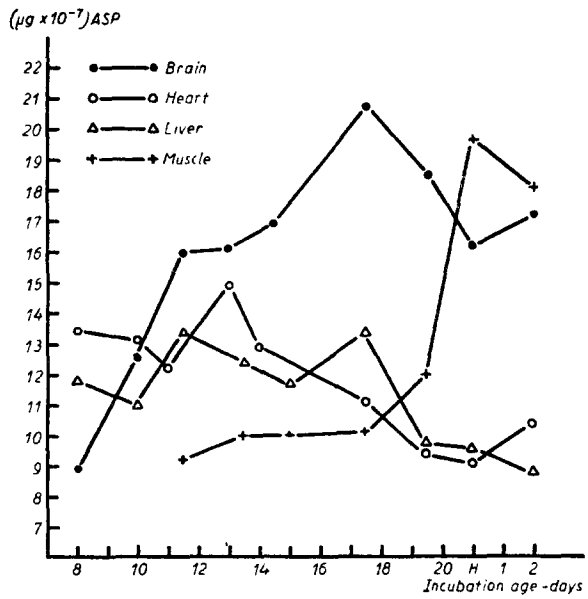

Fig. 3. Variations in amount of acid soluble phosphorus (ASP) per cell in chick brain, heart, liver and skeletal muscle during embryonic development. Otherwise, as for Fig. 2.

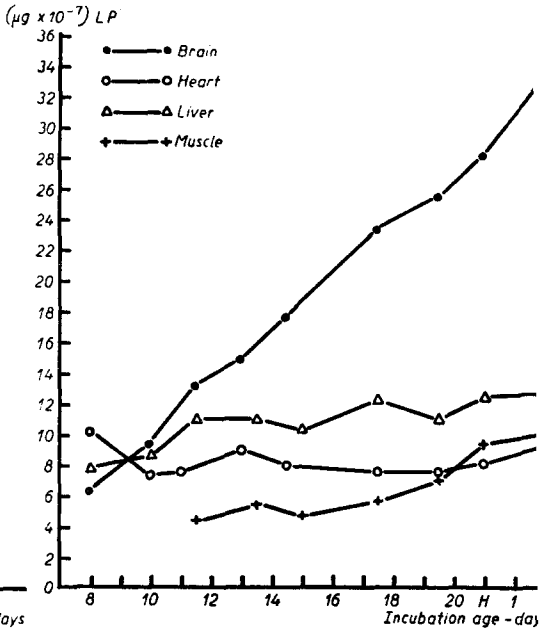

Fig. 4. Variations in amount of lipid phosphorus (LP) per cell in chick brain, heart, liver and skeletal muscle during embryonic development. Otherwise, as for Fig. 2.

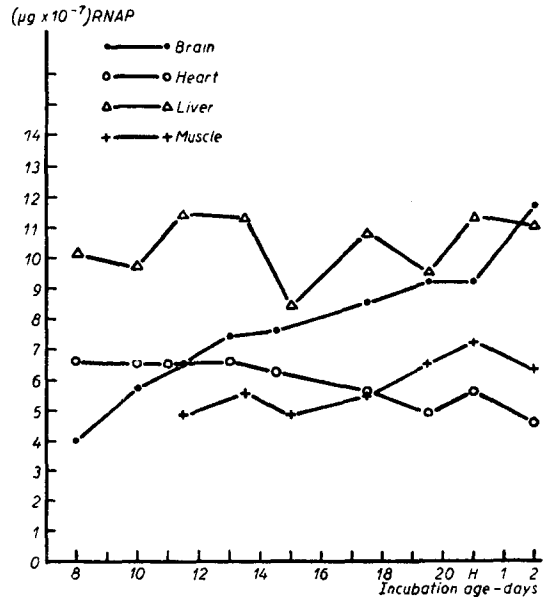

Fig. 5. Variations in amount of ribonucleic acid phosphorus (RNAP) per cell in chick brain, heart, liver and skeletal muscle during embryonic development. Otherwise, as for Fig. 2.

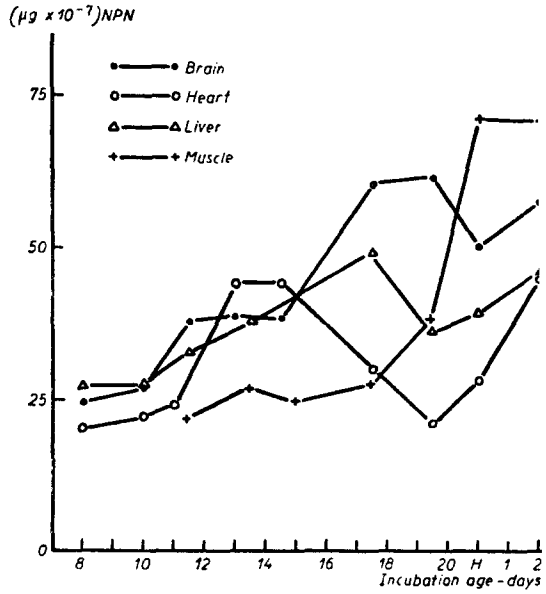

Fig. 6. Variations in amount of non-protein nitrogen (NPN) per cell in chick brain, heart, liver, and skeletal muscle during embryonic development. NPN does not include N present in nucleic acids. Otherwise, as for Fig. 2.

lose about 30% of their RNAP in the course of embryonic development. The amount in the liver cells does not change significantly, and is maintained at a relatively high level in comparison with the amounts per cell in the other tissues.

In the non-protein nitrogen (NPN) fraction are included all the nitrogenous compounds present in the acid-soluble and lipid fractions. Muscle tissue shows an abrupt increase per cell of this rather complex mixture of substances over the three days preceding hatching (Fig. 6). This rise of just over 100% in the NPN per cell coincides with similar increases in cell mass and ASP content (Figs. 2 and 3). The NPN content of brain cells increases from the 8th day and reaches a steady level on the 17th day, while a rather similar, though smaller, increase occurs in the NPN content of liver cells. The fluctuations in the content of the heart cells are large enough to be considered significant, and there is apparently a temporary fall in their NPN before and during hatching.

Changes in the protein N (PN) content of the cells are shown in Fig. 7. The embryonic development of brain, liver, and muscle cells is characterised by large increases in the amounts of PN per cell; in contrast the protein content of heart cells shows no significant change until hatching. The largest rise, over sixfold, occurs in muscle cells, the increase is fivefold in brain cells, and over twofold in liver cells. Such changes should largely account for the increased cell mass found in the same three tissues (Fig. 2). From Tables I–IV it can be seen that the amount of protein per cell in the two day old chick is least in heart tissue, is between 45 and 60% greater in liver and brain, and in muscle cells is twice that in heart cells.

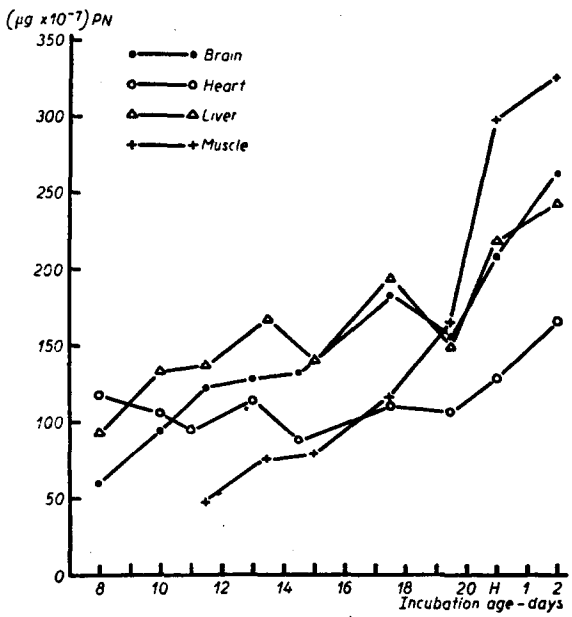

Fig. 7. Variations in amount of protein nitrogen (PN) per cell in chick brain, heart, liver and skeletal muscle during embryonic development. PN does not include N present in nucleic acids. Otherwise as for Fig. 2.

In applying the allometric method to the results from brain, heart and liver, the logarithms of the amounts per organ of the various constituents have been plotted on large scale graphs against the logarithms of the corresponding amounts of DNAP, and the lines have been tested for their 'goodness of fit' by the graphical method proposed by KAVANAGH AND RICHARDS[18]. Figs. 8 and 9 show the contrasting chemical development of the embryonic heart and brain cells. In the heart the growth rate of the components relative to that of the cells remains unchanged for most of the embryonic period between the 8th day and hatching. In brain, where there is a much smaller increase in cell number for the same embryonic period (log DNAP range is half the length of that for the heart), the relative growth rates of the cell constituents are more rapid, and show a very distinct change at a point corresponding to the 14 to 15th day of embryonic development.

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The relative growth rates for the three organs are summarised in Table V. (These rates are represented by $k = \tan\alpha$ in the allometric expression $y = bx^k$). A distinct change in slope occurs in the relative growth rates of components in brain and liver between the 14th and 15th day of development; the only exceptions were the NPN fractions in both tissues and the ASP in liver. The constant values for heart over the whole period are all close to unity as might be expected if the cells are multiplying with

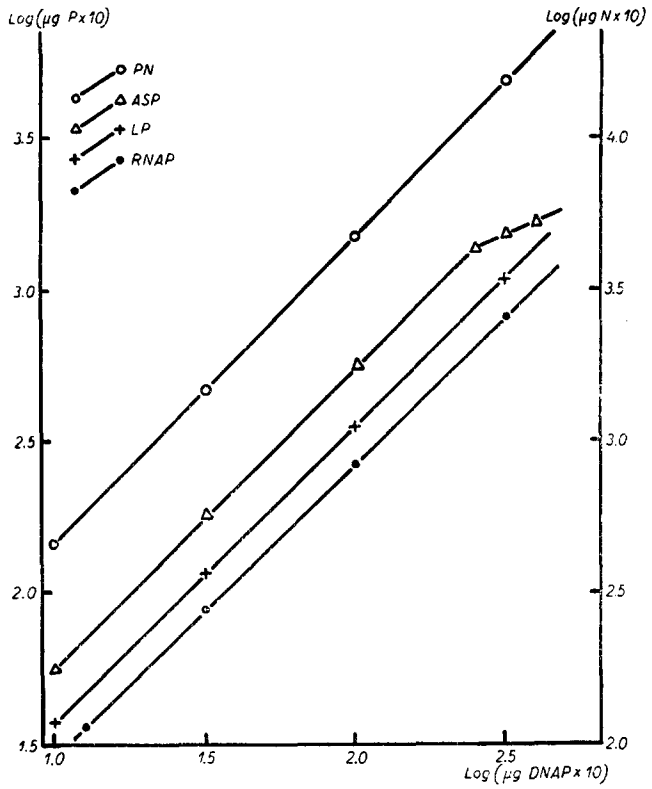

Fig. 8. Growth rates of cell constituents of the chick embryo heart relative to the growth rate of total deoxyribonucleic acid phosphorus (DNAP) per organ. PN-protein nitrogen: ASP- acid soluble phosphorus: LP- lipid phosphorus: RNAP- ribonucleic acid phosphorus. Numerical values of relative growth rates given in Table V.

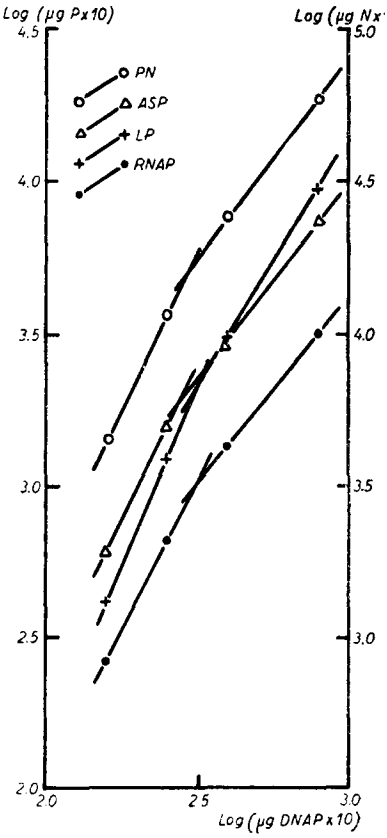

Fig. 9. Growth rates of cell constituents of the chick embryo brain relative to that of the total deoxyribonucleic acid phosphorus (DNAP) per organ. Otherwise, as for Fig. 8.

little or no change in the amounts of cellular constituents. Brain is distinguished by the rapid rate of accumulation of cellular material compared with the rate of increase in cell number; in particular, phospholipids are increasing per cell at a faster rate than the other constituents in both phases of development. In liver, the cells increase their PN and LP content (and hence their weight) more rapidly in the first phase of development than in the second, and in this organ the rates of synthesis of these two components appear to be closely related.

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TABLE V

GROWTH RATES ($k = \tan\alpha$) FOR VARIOUS CELL CONSTITUENTS RELATIVE TO GROWTH RATE OF DEOXYRIBONUCLEIC ACID PHOSPHORUS (DNAP) PER ORGAN

Organ	Incubation period (days)	Weight (mg)	Acid soluble phosphorus (ASP)	Lipid phosphorus (LP)	Ribonucleic acid phosphorus (RNAP)	Protein nitrogen (PN)	Non-protein nitrogen (NPN)
Brain (1)	8–14	3.12	2.07	2.28	1.95	2.04	—
Brain (2)	15–20	1.26	1.28	1.61	1.27	1.31	1.70
Heart	8–20	0.99	0.99	0.99	0.98	1.04	—
Liver (1)	8–14	1.20	1.09	1.26	1.07	1.23	1.15
(2)	15–20	0.95	1.06	1.02	0.94	1.06	—

When the relationships between the components themselves are examined, a close correspondence is found between the relative growth rates of ASP and RNAP in all three tissues (Table V). The relative growth rates of RNAP are consistently lower than those of PN, indicating that the PN increases in proportion to the RNAP in the developing cells. There is, on the other hand, some correlation between the values for LP and PN in liver, whereas, in brain, LP accumulates much more rapidly in the cells than does PN. Finally, it is evident from Table V that the relative growth rate for cell mass follows to some extent the values for LP and PN in all three tissues.

DISCUSSION

In this study we have tried to obtain information on protein synthesis as it is related, on one hand to the other cell constituents, and, on the other, to the physiological development of individual tissues. As there are so many variables in the developing embryonic system some caution is necessary in the form in which results are expressed. When changes in the cell constituents are given as concentrations per unit weight of wet or dry tissue, the results show how the amount of each constituent varies in relation to the tissue substance as a whole. However, they do not necessarily reflect the changes in the composition of the average cell, and they tend to obscure the quantitative relationship between one cell and another. This emerges clearly from the comparison of the results for the four tissues, which were expressed both as concentrations per 100 mg wet weight and as amounts per cell on the basis of the constancy of DNA per nucleus (Tables I–IV). Only in the case of heart tissue, in which cell composition shows little change, is there close correspondence between the two sets of results. In the other three tissues when results are expressed in terms of concentrations or as amounts per cell, either the trend or the extent of the changes is quite different. Interpretations are consequently based on the changes in amount per cell of a constituent since these are independent of other tissue variables; this also applies to the allometric results, which show how the rates of accumulation of cell constituents are quantitatively related to one another, in terms of their common relationship to the DNA of the nucleus.

In embryonic heart and liver the highest average content of ASP per cell occurs when cell multiplication is most rapid, and we have found the same association to hold

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during the growth of chick heart explants *in vitro*[12]. In brain and muscle, however, the increases in ASP per cell coincide with phases during which the amounts of protein per cell are increasing. These increases are particularly rapid in muscle tissue as it develops its full functional activity in the period immediately before hatching. A similar pattern can be seen in the changes of NPN per cell in these two tissues, suggesting that this fraction also is involved in the accumulation of cellular protein. From comparison of the relative growth rates (Table VI), the ASP would seem to be most closely related to the RNAP in all three tissues, and to show quite distinct deviations from both the NPN and phospholipid fractions. This quantitative link between the ASP fraction and the formation of RNAP was also apparent in the tissue culture studies mentioned above.

While the amount of phospholipid corresponds closely to the amount of protein per cell throughout the whole embryonic development of the liver (as reflected in the similarity of their relative growth rates in Table VI), the ratio lipid P: protein N decreases in heart and muscle, and increases in brain. A constant relationship between phospholipid and protein has also been shown to exist in rat liver, where the ratio (lipid P: protein) remains constant during growth and starvation, irrespective of sex[5,19]. It may be of some interest that the liver plays a special part in other aspects of phospholipid metabolism. In the adult organ, at least, it is the only source of plasma phospholipids[16], and recently, POPJAK AND BEECKMANS have shown[28] that the uptake of deuterium rapidly reaches equilibrium in the phospholipids and glyceride fatty acids in the rabbit liver, but not in the lung and intestine. Phospholipid accumulation in the chick embryo brain has been investigated by BIETH AND MANDEL[2], who find that the rising phospholipid content of brain tissue involves three distinct growth phases, during which cephalin, lecithin, and sphingomyelin respectively accumulate in the cells. They report a considerable increase in phosphagen during the rapid accumulation of sphingomyelin, and this phase seems to coincide with the comparative high growth rate of phospholipid relative to DNAP, which we find in the 15–20 day embryo (Fig. 9, Table VI).

CASPERSSON[6] and CASPERSSON AND THORELL[9] in their microspectrophotometric studies have shown that the nucleotide concentration is much higher in the cytoplasm of the early embryonic blood and liver cells than in the corresponding adult cells of the fowl. Their method, employing the ultra-violet absorption of purines and pyrimidines at 260 mμ, determines both the RNA (or polynucleotide) and the individual nucleotides present in the cytoplasm, but does not distinguish between the two. In apparent contrast to their results, we find that the average RNAP content of the chick liver cell is as high in the 2 day-old chick as in the 8-day embryo (Table III). This difference cannot be explained on the grounds that the lower total nucleotide concentration of the adult cell is due to the decreasing concentration of the individual (or acid-soluble) nucleotides, since chemical analyses of fowl tissues have shown that their concentration is actually lower in the embryonic than in the adult chick tissue[15]. It is more probable that the decrease in total nucleotide concentration is, in part, the result of the nearly threefold increase in the amount of protein per cell in the 2 day-old chick liver as compared with amount per cell in the 8-day embryo liver. Thus the lower concentrations in the adult cell may be due to the diluting effect of increased protein and not to a decrease in the absolute amount of total nucleotide per cell.

The threefold increase in the amount of RNAP per cell, which we find in brain tissue during embryonic development, and which MANDEL AND BIETH[21] also report, is

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a particular characteristic of brain tissue. As HYDEN[17] has shown, this persistently high RNA or nucleotide content of nerve cells is related to the intense protein synthesis which normally accompanies nervous activity. The high values for the RNAP content of heart and muscle cells coincide with phases of rapid cell multiplication and protein synthesis.

In an earlier paper[10] we have suggested that the rising protein content of embryonic cells is characteristic of their differentiation towards their final adult form. From the 8th day until hatching the protein per cell increases fourfold in brain, about twofold in liver, and over sixfold in muscle; in contrast, there is no change in heart cells, and it is perhaps relevant that the chick heart has reached its adult morphological form by the 8th day of development. However, there is a slight rise in protein per cell in heart tissue after hatching, and, as the amount per cell at hatching is much lower in the heart than in the other tissues (Tables II–V), it is possible that further differentiation in this organ is delayed over the embryonic period. Again, a factor which might obscure a slight rise in the protein content of the cells is the temporary increase in protein per cell which we find in chick heart explants growing *in vitro*, and which seems to be related to the increase in cell size preceding the onset of mitosis[12]. Such increases would have the effect of raising the average cell composition in tissues in which a high proportion of cells are actively dividing, as is the case in the 12–13 day embryonic heart.

There is no doubt, however, that a permanent increase in protein content occurs in the cells of embryonic tissues while they are growing and assuming their adult functions. The same process is evident during cell development in the salivary gland of *Drosophila*, where the proportion of cell protein to nucleic acid is much larger in the fully developed cells than in the young, rapidly multiplying cells[8]. THORELL[31], too, has found that differentiation during haemopoiesis involves an increase in cytoplasmic protein at the stage between myeloblast and promyelocyte. On the other hand, in cancerous tissue there is a change in cell type to a less differentiated form, and we have recently collected evidence to show that this is accompanied by a considerable decrease in the average amount of protein and RNA per cell[11]. Here, again, the high nucleic acid concentrations, which CASPERSSON AND SANTESSON[7] find in the active cells of malignant tumours, may not be the result of increased amounts of RNA per cell, but may possibly follow from a reduction in the protein content.

As has already been mentioned, the allometric data (Table V) show a close correlation between the growth ratios of ASP and RNAP in all three tissues, and between LP and PN in liver. When RNAP and PN are compared, it is seen that the latter always has the higher relative growth rate. If this difference is to be reconciled with the generally accepted view that RNA is involved in protein synthesis, it must be assumed that an increasing proportion of the protein accumulating in the cells is not immediately related to the metabolism of RNA. In such circumstances, the ratio of PN to RNAP should increase during growth, as is indeed found in heart, liver, muscle, and even in brain, where the RNAP itself is increasing significantly in the cells. It is also of interest that a very distinct decrease should occur in the relative growth rates of protein and its associated constituents in liver and brain tissue at a point corresponding to the 15th day of embryonic development. This offers some confirmation of earlier observations that a critical phase in embryonic development is reached at about the 14th to 15th day of incubation [4].

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SUMMARY

1. The chemical development of the cells in chick brain, heart, liver and muscle has been studied from the 8th day of incubation until the second day after hatching. Using the constant amount of deoxyribonucleic acid (DNA) in the nuclei of fowl tissues as a standard of reference, changes in cell number and in the amounts of acid-soluble phosphorus (ASP), lipid phosphorus (LP), ribonucleic acid phosphorus (RNAP), non-protein nitrogen (NPN), and protein nitrogen (PN) have been followed during growth.

The rate of cell multiplication was at its maximum in heart and liver between the 13th and 15th day of incubation, after which it steadily decreased in both organs. Brain cells, in contrast, showed a fairly uniform multiplication rate over the embryonic period. Average cell mass (wet weight relative to DNA) increased greatly in brain and muscle during development, increased only slightly in liver, and remained constant in heart. High amounts of ASP per cell were found during rapid cell multiplication in heart and liver; in brain and muscle increases in ASP per cell coincided with the rising content of protein in the cells. From the allometric data it is evident that the relative growth rates of ASP and RNAP correspond closely in brain, heart and liver.

Phospholipid (LP) increased greatly in brain cells, and the relative growth rates for this component were higher than for any other in the tissue. In liver, but not in any of the other tissues, the relative proportions of LP and PN remained constant throughout development. Brain cells were also distinguished by their threefold increase in RNAP per cell during development; the liver cells maintained throughout their relatively high RNAP content, and in heart and muscle cells the high values coincided with phases of rapid cell multiplication and protein synthesis.

During differentiation in the embryonic period, the average amount of PN per cell increased twofold in liver, fourfold in brain, and over sixfold in muscle; there was no significant increase in the protein content of the heart cells. It is suggested that changes in the amounts of protein per cell may be an important factor in producing changes in nucleotide concentration. During the development of the embryonic chick liver the protein content per cell increased more than twofold, but, since the absolute amount per cell of RNAP remained constant, its concentration declined.

RÉSUMÉ

On a étudié l'aspect chimique du développement des cellules du cerveau, du coeur, du foie et du muscle du poussin entre le 8ème jour d'incubation jusqu'au 2ème jour après éclosion. Le nombre de cellules et les quantités de phosphore soluble dans l'acide (ASP), de phosphore des lipides (LP), de phosphore de l'acide ribonucleic (RNAP), d'azote non-protéique (NPN) et d'azote protéique (PN) ont été estimées, et leur valeurs comparées à celle de l'acide déoxyribonucleic (DNA) de l'étalon — notamment du nucleus des tissues de la poule.

La rapidité de multiplication des cellules du coeur et du foie a atteint son maximum entre le 13ème et le 15ème jour d'incubation pour décroitre dans la suite progressivement dans les deux organes. Par contre, la multiplication des cellules du cerveau s'est montrée plutôt uniforme pendant toute la période embryonnaire. La masse moyenne des cellules (poids humide divisé par le DNA) s'accrue au cours du développement, considérablement dans le cerveau et le muscle, faiblement dans le foie et est restée constante dans le coeur. Des quantités considérables de ASP per cellule furent trouvées dans la phase de multiplication intense dans le coeur et le foie; dans le cerveau et le muscle l'augmentation du taux de ASP coincidait avec l'accroissement de la teneur des cellules en protéine. Les données allometriques mettent en evidence le fait que les accroissements relatifs du ASP et du RNAP se correspondent étroitement dans le coeur le cerveau et le foie.

Le phospholipid (LP) s'est accru considérablement dans les cellules du cerveau et l'accroissement relatif de cette substance est plus marquée que pour n'importe quelle autre substance dans le tissue. Dans le foie, mais non pas dans les autres tissues, la proportion de LP au PN est restée constante dans la période de développement. Les cellules du cerveau se différenciaient des autres en ce que leur teneur en RNAP par cellule s'est accrue dans la periode de développement de trois fois, tandis que les cellules du foie maintenaient leur valeur, relativement haute en RNAP, et que dans les cellules

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du coeur et du muscle les phases de haute valeur de RNAP coïncidaient avec les phases de multiplication rapide et de synthèse des protéines.

Au cours de la différentiation dans la période embryonnaire la teneur moyenne en PN des cellules s'est accrue de deux fois dans le foie, de quatre fois dans le cerveau et plus de six fois dans le muscle; il n'y a pas eu d'accroissement notable de protéine dans les cellules du coeur. Il parait probable, aux auteurs, que les changements de la teneur en protéines des cellules représentent un facteur responsable des changements de la concentration des nucleotides. Pendant le developpement embryonnaire du foie du poussin la teneur en protéine de la cellule a plus que doublé mais comme la valeur absolue du RNAP est restée constante sa concentration relative a diminué.

ZUSAMMENFASSUNG

Die chemische Entwicklung der Zellen des Hühnergehirns, Herzens, Leber und Muskel ist vom achten Bruttage bis zum zweiten Tage nach der Ausbrütung studiert worden. Änderungen in der Zellenanzahl und im Gehalt von säurelösbaren Phosphor (ASP), lipid Phosphor (LP), ribonukleinsäure Phosphor (RNAP), Proteinfreiem Stickstoff (NPN), und Proteinstickstoff (PN), wurden während des Wachstums beobachtet und mit dem Betrag von deoxyribonukleinsäure des Zellnukleus des Huhnes verglichen.

Die Zellvermehrung im Herzen und in der Leber war während des dreizehnten und fünfzehnten Tages am höchsten; danach verminderte sie sich ständig in beiden Organen. Im Gegensatz dazu war die Vermehrung der Gehirnzellen während der ganzen embryonischen Periode ziemlich gleichmässig. Die durchschnittliche Zellmasse (Nassgewicht im Vergleich zu DNA) hat sich während der Entwicklung in Gehirn und Muskel sehr vergrössert, in der Leber nur wenig, und im Herzen ist sie fast gleichgeblieben. In Herz und Leber wurde ein höher Gehalt von ASP per Zelle während der raschen Zellvermehrung gefunden; in Gehirn ind Muskel ging die Erhöhung in ASP per Zelle mit dem wachsenden Proteingehalt parallel. Von allometrischen Daten ist anzunehmen, dass die verhältnismässige Zunahme von ASP und RNAP in Gehirn, Herz und Muskel nahe übereinstimmen. In den Gehirnzellen hat Phospholipid sehr zugenommen, mehr als irgend eine andere Substanz in diesem Gewebe. In der Leber, aber nicht in den anderen Geweben blieb die Proportion von LP und PN während der Entwicklung gleich. Die Gehirnzellen waren auch dadurch zu unterscheiden dass sich der RNAP während der Entwicklung dreimal vermehrt hat; die Leberzellen haben während der ganzen Zeit ihren verhältnismässig hohen RNAP Gehalt beibehalten; in Herz- und Muskelzellen trafen die hohen Werte mit den Phasen der raschen Zellvermehrung und der Proteinsynthese zusammen.

Während der embryonischen Differentiation vermehrte sich der durchschnittliche PN-Gehalt der Zelle zweimal in der Leber, viermal im Gehirn und mehr als sechsmal in dem Muskel. In den Herzzellen gab es keine bedeutende Erhöhung des Proteingehaltes. Nach den Verfassern konnte der Proteingehalt der Zelle ein wichtiger Faktor in der Verursachung von Änderungen in der Konzentration von Nukleotiden sein. Bei der Entwicklung der embryonischen Hühnerleber hat sich der Proteingehalt per Zelle mehr als zweimal vermehrt, aber, da doch die absolute Menge von RNAP gleichgeblieben ist, hat sich die Konzentration dieser Substanz vermindert.

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Some of our results support earlier observations made by MOOG[34] during her study of apyrase activity in the same four tissues of the chick embryo. In particular, she found a peak in apyrase activity (units/mg N) in skeletal muscle at 18–21 days, and noted a fall in the nitrogen concentration of heart tissue before hatching, similar to the fall in protein per cell, which we find in brain and liver at the same stage.