

THE RELATION BETWEEN THE INTRACELLULAR RIBONUCLEIC ACID DISTRIBUTION AND AMINO ACID INCORPORATION IN THE LIVER OF THE DEVELOPING CHICK EMBRYO

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ABSTRACT

The RNA-P and DNA-P content of the nucleus and the RNA-P content of the whole cell of the livers of 8- to 20-day chick embryos and of adult fowls have been determined. The DNA-P content of the liver nuclei was slightly higher in the 8- and 10-day embryo than in all the other stages examined. A significant decrease in the RNA content of the cell occurred during embryonic development. The RNA content of the adult cell was the same as that of the 14- to 16-day embryo. The proportion of the cellular RNA contributed by the nucleus also decreased during development. In respect to both nuclear RNA content and distribution of RNA between nucleus and cytoplasm, the adult resembled the 8- to 12-day embryo. Examination of the fine structure of the cell showed that, as development progressed, free ribosomes decreased in number and the rough membranes increased. Slices of 8-, 14-, and 20-day embryonic livers and of adult livers were incubated with ^{14}C -leucine, and the amount of labeled amino acid incorporated into whole tissue protein and into the proteins of the subcellular fractions was measured. Embryonic liver incorporated ^{14}C -leucine 15 to 30 times more rapidly than adult liver. The microsomal protein was always more highly labelled than the protein in any other subcellular fraction; however, in the 8-day embryonic and the adult liver the proportion of total counts found in the nuclear fraction was considerably higher than in the 14- or 20-day embryonic liver. The significance of an apparent correlation between the proportion of the cell's RNA contributed by the nucleus and the proportion of total counts in the nuclear fraction is discussed.

INTRODUCTION

In a previous investigation it was demonstrated that embryonic rat liver slices incorporated amino acids at a significantly greater rate than adult liver slices (6). On subsequent cell fractionation the nuclear fractions of the embryonic liver showed consistently high specific activities relative to the other subcellular fractions (6). Because of the

difficulty of obtaining sufficient amounts of liver from the early embryonic stages of the rat, it was decided to use livers of the 8- to 20-day-old chick in subsequent experiments. In this investigation, the distribution of ribonucleic acid (RNA) between the nucleus and the cytoplasm of liver cells of 8- to 20-day chick embryo and of adult hens

was determined. The organization of ribosomes within the cytoplasm during development was studied by electron microscopy, and the radioactivity of the proteins of the various cell fractions was determined after incubation of liver slices with ^{14}C -leucine, so that the distributions of RNA could be compared at various stages.

It has been demonstrated that significant changes take place in the intracellular distribution and organization of RNA during development, and that a correlation exists between the proportion of the cellular RNA present within the nucleus and the proportion of incorporated amino acid found in the proteins of the nuclear fraction.

MATERIALS AND METHODS

The fertile eggs and the hen fowls, all of an inbred White Leghorn strain, were obtained from the New South Wales Government Poultry Experimental Station, Seven Hills, New South Wales. The eggs were incubated at 38–39°C and the stage of development of the embryos after incubation for a given time was checked by reference to the illustrations and measurements of the Hamburger stages (14). The hens were killed and bled by incising into the brain and through the jugular vein and carotid artery.

Preparation of Nuclei

The layering technique used was similar to that of Hogeboom *et al.* (15). Livers were removed and washed in ice-cold 0.9 per cent saline; all subsequent operations were carried out at 0–2°C. Approximately 2 gm of tissue were homogenized with 5 volumes of 0.25 M sucrose–0.0015 M CaCl_2 for 60 seconds in a Teflon-glass homogenizer and then diluted to 20 ml with sucrose– CaCl_2 solution; at this stage, 3 ml were removed for the determination of RNA and deoxyribonucleic acid (DNA) in the whole homogenate. 5-ml samples of the remaining homogenate were placed in 15-ml centrifuge tubes and 2 ml of 0.44 M sucrose–0.00075 M CaCl_2 were introduced slowly into each tube so as to form a layer beneath the homogenate. Nuclei were collected by centrifugation at 270 *g* for 10 minutes. The pellets were resuspended, by gentle homogenization manually, in a total of 10 ml of 0.44 M sucrose–0.00075 M CaCl_2 , and 5-ml portions were underlaid with 0.88 M sucrose–0.00075 M CaCl_2 and again centrifuged at 270 *g* for 10 minutes. The pellets were resuspended and centrifuged as before, using 0.44 M sucrose–0.0003 M CaCl_2 and underlaying with 0.88 M sucrose–0.0003 M CaCl_2 . To remove red blood cells, which at this stage contaminated the nuclear preparation to the extent of approximately 5 per cent, the pellets were suspended in 10 ml of 1 per cent gum

acacia and centrifuged at 134 *g* for 10 minutes after underlaying 5-ml portions with 2 ml of 0.88 M sucrose–0.0003 M CaCl_2 . The resulting pellets were finally suspended in 0.44 M sucrose–0.0003 M CaCl_2 . The preparations were examined at all stages by phase contrast microscopy. The nuclear preparations were almost completely devoid of mitochondria and whole cells. Nuclear counts were carried out with a Spencer Bright Line Haemocytometer; the samples were diluted, as required, with 0.44 M sucrose–0.0003 M CaCl_2 .

Nucleic Acid Determinations

DNA and RNA were determined in whole homogenates and isolated nuclei from the livers of 8-, 10-, 12-, 14-, 16-, 18- and 20-day-old chick embryos and of adult fowls. DNA and RNA fractions were obtained from the sample by a modified Schmidt-Thannhauser method (31). Both the phosphate-P content and the ultraviolet absorption of the extracts were determined. Phosphate-P was determined by the method of Allen (1). In order to relate the ultraviolet absorption measurements to the nucleic acid-P values, small samples of purified RNA and DNA were prepared from embryonic chick liver (20-day) according to the method of Tsuboi and Stowell (30). The extinction coefficient per mole P/l was found to be 9970 for RNA and 9150 for DNA after subjecting them to the same treatment as the tissue samples. All samples to be determined by ultraviolet spectrophotometry were neutralized and then maintained at pH 7.2 with 0.1 M Na_2HPO_4 – NaH_2PO_4 buffer. The optical densities at 260 $m\mu$ and 280 $m\mu$ were measured with a Beckman DU spectrophotometer and approximate corrections were made for the presence of protein breakdown products by calculations similar to those of Drury (11) for pentose and hexose in the one sample. Agreement between results obtained by both methods was consistently well within 5 per cent of the mean for both whole homogenates and isolated nuclei. The mean of the two results (phosphate-P determination and UV determination) for a given sample was taken and expressed as the nucleic acid-P content. Since the number of nuclei present in the sample used for nucleic acid determinations was known, the RNA-P and DNA-P content of the nucleus could be calculated. The mean of the results from 2 to 3 experiments for the DNA-P content of the nucleus at a given growth stage was used to calculate the RNA-P content of the whole cell at that stage. Cell numbers were equated to numbers of nuclei counted in whole homogenates.

Incorporation of ^{14}C -Leucine into Proteins

A. ADULT HEN LIVER: The liver was placed in ice-cold Krebs-Ringer solution, thin slices were cut,

and the incubation was carried out as described previously (6), using an incubation mixture consisting of 2.7 ml Krebs-Ringer-bicarbonate solution, 0.1 ml 0.3 M glucose, and 0.2 ml containing $2 \mu\text{C}$ ^{14}C -leucine (0.32 μmoles). The reaction was stopped after 0, 15, or 60 minutes.

B. EMBRYONIC CHICK LIVER: Sufficient livers to give approximately 1 gm tissue (wet weight) were removed from 8-day, 14-day, and 20-day-old embryos and placed in ice-cold Krebs-Ringer solution. Since the tissue was too soft to slice, the livers from 14- and 20-day-old embryos were chopped into small pieces (1 to 2 mm³) with scissors. The livers from 8-day-old embryos were small enough to be used intact. The incubation was carried out as described for the adult

Tissue Fractionation and Counting of Radioactive Proteins

TISSUE FRACTIONATION: The method used was a modification of that described by Kafer and Pollak (17) for rat liver.

(a) The liver was ground in a mortar with 2.2 ml M sucrose-0.002 M citric acid and then homogenized in a Teflon-glass homogenizer. The homogenization time varied according to the tissue, being 2 minutes for adult liver, 60 seconds for 20-day, 45 seconds for 14-day and 30 seconds for 8-day embryonic liver. 2.8 ml 0.002 M citric acid (pH previously adjusted to 6.4 with NaOH) was used to rinse the mortar and was added to the homogenate, which was then homogenized for a further 30 seconds.

(b) The homogenate was filtered through a layer of flannel which had been soaked in 0.44 M sucrose. Gentle suction was applied if necessary, and the flannel was washed with 1.0 ml 0.44 M sucrose-0.002 M citric acid, pH 6.4.

(c) The pH of the filtrate was checked with bromthymol-blue. It should be between 6.0 and 6.5. The volume of the filtered homogenate was noted and 0.5 ml was set aside.

(d) The homogenate was centrifuged at 700 g for 10 minutes. Since embryonic chick liver has a high lipid content, especially at 20 days, it was usually necessary after centrifugation to remove the lipid from the top of the tube with a small piece of cotton wool. Very little supernatant was lost during this operation. The supernatant (S1) was pipetted off and retained for procedure (g). The nuclear pellet was resuspended in 5 ml 0.44 M sucrose-citric acid, pH 6.4, by gently rotating the tube.

(e) The nuclear suspension was centrifuged as above and the supernatant (S2) was added to that from procedure (d). The nuclear pellet was resuspended in 3 ml 1 per cent gum acacia, and 2 ml 0.44 M sucrose-citric acid, pH 6.4, was placed in a layer at the bottom of the tube before centrifuging at 500 g for 10 minutes.

(f) The supernatant was discarded and the final nuclear pellet was resuspended in 3 ml 0.44 M sucrose.

(g) The combined supernatants S1 and S2 were centrifuged at 13,000 g for 10 minutes. The supernatant S3 was retained for procedure (h). The pellet was resuspended in 10 ml 0.44 M sucrose by gentle manual homogenization and centrifuged as before. The mitochondrial pellet was resuspended in 5 ml 0.44 M sucrose.

(h) S3 was centrifuged at 105,000 g for 60 minutes in the Spinco Ultracentrifuge (Rotor 40). The microsomal pellet was resuspended in 5 ml 0.44 M sucrose.

(i) The supernatant from procedure (h) was retained as the supernatant fraction.

The proteins of the various fractions were collected and washed as described previously (17).

The sucrose-citrate method was used since the fractionation of the homogenate into all its constituent subcellular fractions was desired for a balance sheet study. The sucrose-calcium method gives a cleaner nuclear preparation, but does not enable one to fractionate the various cytoplasmic fractions.

COUNTING: The washed proteins obtained from each fraction and the whole homogenates were dissolved in 0.1 N NaOH to an estimated concentration of 5 mg per ml. The exact protein concentration was determined by the Biuret method (18) so that the concentration could be adjusted to give exactly 1 mg protein per 0.2 ml 0.1 N NaOH for plating onto brass planchets as a thin film. The radioactivity was then determined with a windowless gas-flow counter with a counting efficiency of 17 per cent.

DETERMINATION OF FREE LEUCINE: After dissection in the cold, 1 gm of tissue was homogenized in the cold with 8 ml 75 per cent ethanol, made up to 10 ml, and left to extract at 2°C overnight. The suspension was then centrifuged and the sediment washed two times with 3 ml 75 per cent ethanol. The combined supernatants were extracted with 3 volumes of chloroform, the aqueous layer separated, and its volume reduced. Leucine was separated by paper chromatography, using Whatman No. 1 paper for ascending-descending chromatography and butanol: acetic acid:water (4:1:1) as solvent. Under these conditions, leucine followed by *iso*-leucine were well separated from all the other ninhydrin-positive spots of the tissue extract. The amount of *iso*-leucine was negligible when compared to the amount of leucine present, and leucine could, in fact, be resolved free of *iso*-leucine. Leucine determinations were carried out with a cadmium acetate-ninhydrin reagent (2).

Cytological Techniques

For electron microscopy, pieces of liver (approximately 1 mm³) from the different embryonic stages and from adult liver were fixed for 2 hours in 1 per cent unbuffered OsO₄ made hypertonic with sucrose

(8). Washing and dehydration were carried out in the conventional manner, and Araldite was used for embedding. The embedding into Araldite was carried out at 48°C. Silver and gold sections were cut on a Porter-Blum microtome and collected onto carbon-coated copper grids. Electron micrographs were taken with a Siemens Elmiskop I with a 30 μ objective aperture.

nuclear DNA content has been found in regenerating rat liver (29) and in regenerating guinea pig liver (24). Other workers have found the same amount of DNA in the nucleus of embryonic chick liver from 8 days to hatching (19).

A considerable decrease in the RNA content of the liver nucleus was found during embryonic

TABLE I
The Nucleic Acid Content of the Nucleus and the Distribution of RNA between the Nucleus and Cytoplasm of Chick Liver Cells during Development

Mean values are shown, and individual values are given in parentheses.

Age Chick liver	Nucleic acid-P/Nucleus (pg)		RNA-P/Cell (pg)	Total RNA in the nucleus <i>per cent</i>
	DNA-P	RNA-P		
8-day embryonic	0.34 (0.39, 0.31, 0.31)	0.22 (0.23, 0.21, 0.21)	1.25 (1.16, 1.34, 1.24)	18
10-day embryonic	0.34 (0.38, 0.30)	0.20 (0.21, 0.18)	1.12 (1.10, 1.13)	18
12-day embryonic	0.30 (0.30, 0.30)	0.17 (0.17, 0.16)	1.07 (1.11, 1.02)	16
14-day embryonic	0.29 (0.33, 0.26, 0.29)	0.15 (0.17, 0.16, 0.13)	1.02 (1.03, 1.07, 0.97)	15
16-day embryonic	0.29 (0.30, 0.27)	0.14 (0.13, 0.15)	0.90 (0.97, 0.83)	16
18-day embryonic	0.27 (0.28, 0.25)	0.12 (0.12, 0.12)	0.84 (0.89, 0.79)	14
20-day embryonic	0.29 (0.29, 0.29)	0.12 (0.11, 0.13)	0.88 (0.84, 0.91)	14
Adult	0.27 (0.28, 0.26)	0.17 (0.17, 0.17)	0.97 (0.93, 1.00)	18

RESULTS

The results of the nucleic acid determinations are shown in Table I. The value of 0.27 microgram (pg) DNA-P per nucleus for adult fowl liver agrees quite well with the results of other workers (19). However, there is a tendency for the DNA content of the nucleus to be greater than this in embryonic tissue, especially in 8- to 10-day embryonic liver (Table I). The higher DNA value could be due to the fact that during rapid growth a greater proportion of nuclei are tetraploid than in more slowly proliferating tissue. A similar high

development, from 0.21 pg at 8 days to 0.12 pg in 18- to 20-day embryonic liver (Table I). Thomson *et al.* (29) suggested that the RNA content of the nucleus may be quite dependent on the cytoplasmic RNA content. In the present investigation the decrease in the RNA content of the nucleus is not merely a reflection of the decrease in the amount of RNA in the whole cell, since the percentage of total RNA found in the nucleus also decreased (Table I). Thus a definite change in the distribution of RNA existed between the nucleus and cytoplasm during embryonic development.

The liver cell of the adult hen contained as much RNA as that of the 16-day-old embryo, and the distribution between the nucleus and cytoplasm was similar to that of the 8- to 10-day-old embryo (Table I).

The incorporation of ^{14}C -leucine into proteins of the liver of the chick at various stages of development is shown in Table II. Embryonic liver incorporated leucine about 15 to 30 times more rapidly than the adult liver during the first 15

a higher specific activity than the protein of any of the other fractions, indicating that the microsomes were the sites at which the most rapid incorporation of amino acids into proteins occurred. This is in accord with most published work (9). The percentage of the total counts incorporated into the microsomal fraction of embryonic chick liver was lower after 60 minutes' than after 15 minutes' incubation; this could be due to a redistribution of proteins among cellular organelles,

TABLE II
Incorporation of ^{14}C -Leucine into Total Protein of Chick Liver Slices
For description of techniques and incubation conditions, see Methods section.

Age Chick liver	$\mu\text{moles endogenous}$ leucine/gm tissue		Incubation time	*Specific activity of total protein
	(Mean value of 4 deter- minations)	Range		
8-day embryonic	0.24	0.22-0.25	<i>min.</i>	
			15	1260
14-day embryonic	0.41	0.37-0.45	60	2150
			15	980
20-day embryonic	0.33	0.30-0.37	60	2580
			15	608
Adult	0.16	0.15-0.17	60	1420
			15	32
			60	83

* Specific activity = $\mu\mu\text{moles leucine incorporated/mg protein}$.

N.B. The percentage of added leucine incorporated does not include incorporation into any protein which became dispersed in the medium during incubation and was lost during the filtration procedure. At 8 days this was probably quite significant.

minutes of incubation, although the adult liver was sliced thinly whereas the embryonic liver was chopped with scissors. The difference in the rate of incorporation into embryonic and adult liver was even more marked than that found for rat liver (6). In the embryonic chick liver the rate of incorporation was most rapid at 8 days and slowed down as development proceeded. This is in agreement with the rate at which the protein content of the liver increased during development (Duck-Chong, unpublished results).

Table III shows the specific activities of the proteins of the nuclei, mitochondria, microsomes, and supernatant obtained by fractionating the liver slices which had been incubated with ^{14}C -leucine. In each case, the microsomal protein had

or to the presence of separate precursor pools for different organelles, or to differences in the ability of organelles to maintain amino acid incorporation under *in vitro* conditions. In the 8-day embryonic and the adult liver, the proportion of total counts found in the nuclear fraction was considerably higher than that of the 14-day and 20-day embryonic liver (Table III).

In Table IV the contribution of the nuclear fraction to the total cell protein is shown. In both 8-day embryonic liver and adult liver relatively more protein was found within the nuclear fraction than in 14-day or 20-day embryonic liver. This increase in the percentage of total radioactivity in the nuclear fraction coincides with an increase in the percentage of protein within the nucleus. The

relative specific activities of the nuclear proteins show that the extra protein in the nuclei of 8-day embryonic liver and adult liver does not entirely account for the increase in labelling (Table IV). The relative specific activities of the nuclear fractions were obtained by correcting the specific activities for differences in the over-all rate of incorporation between different embryonic stages

change in the RNA content and metabolism of liver nuclei of old rats. It is interesting to note that in a quite unrelated organism, a change in nuclear RNA similar to that found in embryonic chick liver occurs during development. In the developing starfish oocyte, growth of the single cell is accompanied by a decrease in the relative contribution of nuclear RNA to total cell RNA. A

TABLE III
Specific Activities of Subcellular Fractions of Adult and Embryonic Liver after Incubation with ¹⁴C-Leucine

For description of techniques and incubation conditions, see Methods section.

Incubation time	Embryo						Adult	
	8 days		14 days		20 days		15	60
	15	60	15	60	15	60		
<i>min.</i>								
Nuclei	560 (10)	1,110 (14)	210 (4)	580 (7)	150 (4)	330 (7)	16 (14)	36 (11)
Mitochondria	390 (13)	830 (19)	310 (24)	750 (23)	150 (14)	350 (19)	10 (14)	13 (10)
Microsomes	1,050 (41)	1,310 (29)	680 (39)	1,110 (23)	510 (48)	990 (39)	29 (34)	73 (37)
Supernatant	570 (36)	1,140 (38)	280 (33)	1,020 (47)	230 (34)	520 (35)	23 (38)	60 (42)
Per cent recovery of counts	84	84	70	71	84	70	82	69
Per cent recovery of protein	93	94	98	88	91	89	88	87

Specific activity = $\mu\text{moles leucine incorporated/mg protein}$. Figures in brackets represent the percentage of total counts recovered that were found in a particular fraction. The percentage recovery of counts and protein was calculated by adding the counts or amount of protein for each of the fractions and comparing the total with the results obtained with the homogenate in the absence of subfractionation.

and adult liver. This correction was carried out by expressing the incorporation into nuclei in terms of a percentage of the total cellular incorporation and dividing this by the percentage of the cellular protein found within the nucleus. In the 8-day embryonic liver and in the adult liver, the relative specific activity of the nuclear protein was greater than in the 14-day or 20-day embryonic liver (Table IV).

OBSERVATIONS AND DISCUSSION

Little is known of the RNA content of cell nuclei and the factors affecting it. The work of Detwiler and Draper (10) suggests a possible age-related

decrease in concentration of total RNA also occurs during growth (13).

The higher nuclear RNA content of the liver of early stage embryos and of the adult is difficult to interpret, especially since it represents a change in the distribution of RNA between the nucleus and the cytoplasm. It is known that nuclear RNA is quite heterogeneous (9, 28). Even before interest in the passage of RNA from the nucleus to cytoplasm was aroused by the "messenger" theory (5), considerable evidence indicated that the nucleus is the site of synthesis of much of the cell's RNA (21, 27). In more recent work, with rat

liver, the *in vitro* transfer of RNA from the nucleus to the cytoplasm has been demonstrated (26).

It is, therefore, possible that the RNA content of the nucleus is not related to the role of the nucleus in protein synthesis, since only a small portion of the nuclear RNA need be concerned with nuclear protein synthesis. RNA synthesis may be stepped up at stages of rapid growth, or under other conditions requiring rapid production of cytoplasmic RNA. A high nuclear RNA content could then be the result of a lag in the passage of RNA from nucleus to cytoplasm.

The decrease in total cell RNA from 1.25 pg at 8 days to 0.88 pg at 20 days (Table I) and the

plasmic reticulum is visible (Fig. 1). If, as suggested by a number of workers (3, 16, 22), the free ribosomes are involved in the synthesis of cellular protein for purposes of growth or turnover, then the liver cell of the 8-day embryo can be regarded as a typical retaining cell. On the other hand, ribosomes attached to cytomembranes are thought to be responsible for the production of secretory proteins. In the liver cell of the 14-day embryo, rough membranes, *i.e.* membranes with attached ribosomes, begin to appear (Fig. 2). As development proceeds, these rough membranes become more abundant, the liver cell of the 20-day-old chick embryo being a typical secreting

TABLE IV
Comparison of RNA Distribution and Incorporation of ¹⁴C-Leucine
(Incubation time, 15 minutes)

	Embryonic chick liver			
	8-day	14-day	20-day	Adult fowl liver
Per cent of total RNA in nucleus	18	15	14	18
Per cent of incorporated ¹⁴ C-leucine in nucleus (A)	10	4	4	14
Per cent of total protein in nucleus (B)	12	7	7	18
Relative specific* activity of nuclear protein (A/B)	0.8	0.6	0.6	0.8

* Relative specific activity of nuclear protein is defined in the Results section.

decrease in the rate of amino acid incorporation observed during development (Table II) are consistent with the now classical concept that there is a correlation between the RNA content of a tissue and its capacity for synthesizing protein (4). In the liver the rate of amino acid incorporation is not simply an indication of the growth rate with respect to protein, because at some stage the liver begins to produce protein for secretion. Serum albumin is synthesized by slices of liver from chicks 6 weeks after hatching (23), and some evidence has been obtained for the presence of albumin in the microsome fraction of 8-day-old chick embryos (12). It is, therefore, highly probable that at some stage during development the liver begins making and secreting serum albumin. Figs. 1 to 4 are electron micrographs of chick liver at various stages of development. At 8 days the ribosomes are scattered throughout the cytoplasm and no endo-

cell (Fig. 3), differing very little from the liver cell of the adult fowl in this respect (Fig. 4). Thus, the cytological data also suggest that the embryonic chick liver cell may form serum proteins some time between the 8th day and 14th day of development.

The incorporation of amino acids into embryonic chick liver (Table III) did not show the same distribution among subcellular fractions as was reported for rat liver (6). In the liver of 17- to 18-day-old embryonic rats, the ratio of specific activities of nuclear and microsomal protein was 1.1 compared with 0.6 in the adult (6). In embryonic chick liver the specific activity of the nuclear protein was never higher than that of the microsomal protein. It has been pointed out that the liver of 17- to 18-day-old embryonic rats contains many haemopoietic cells, in which the nucleus makes up most of the cell (20). These cells would in-

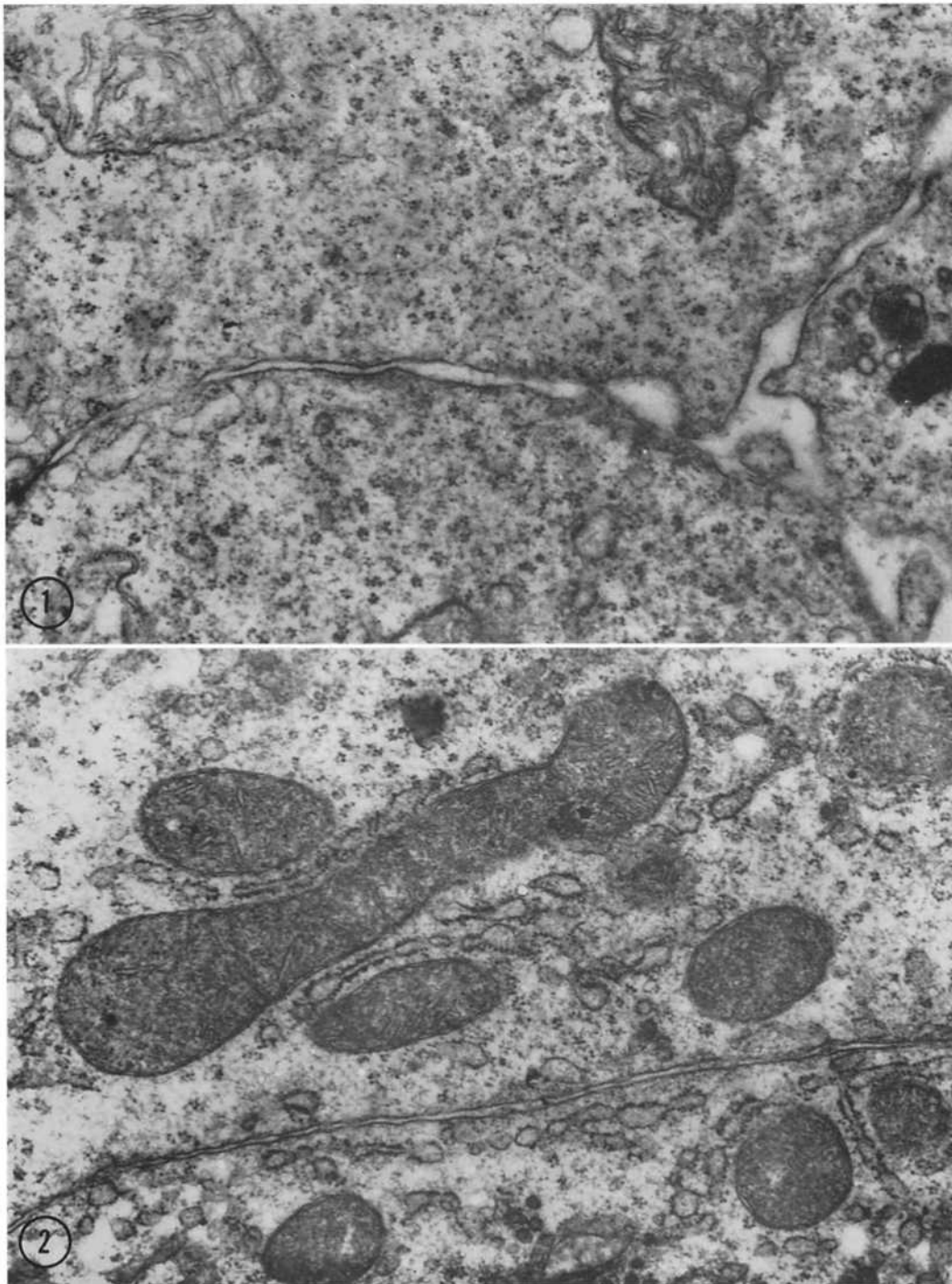


FIGURE 1 Electron micrograph of 8-day embryonic chick liver. Ribosomes are scattered throughout the cytoplasm and no endoplasmic reticulum is visible. $\times 33,500$.

FIGURE 2 Electron micrograph of 14-day embryonic chick liver. Free ribosomes are still plentiful, but some ribosomes are attached to cytomembranes. $\times 33,500$.

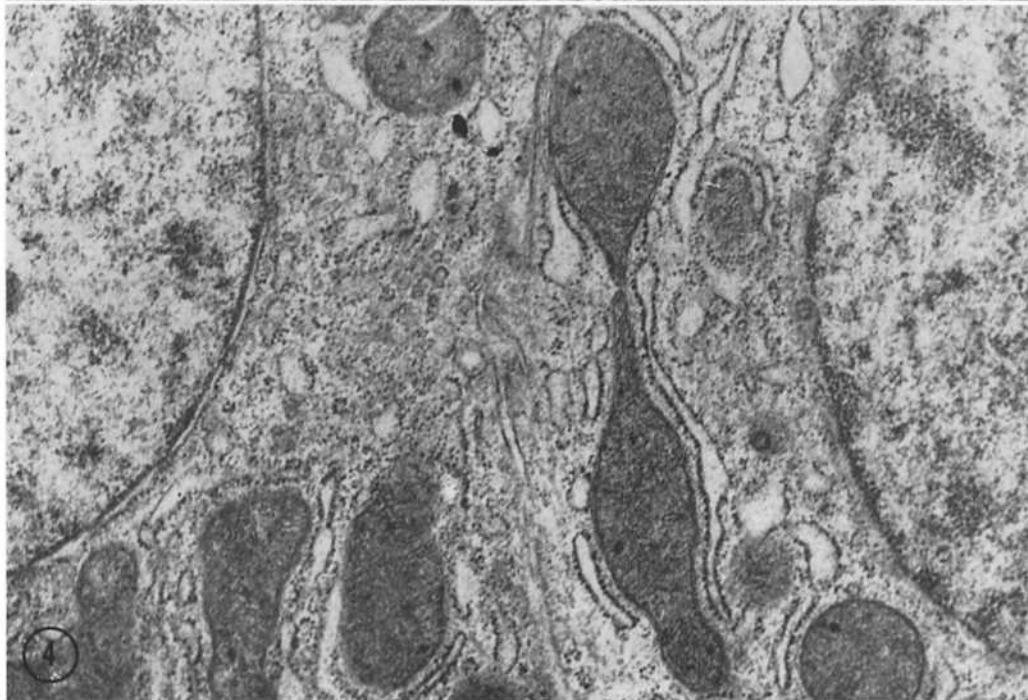
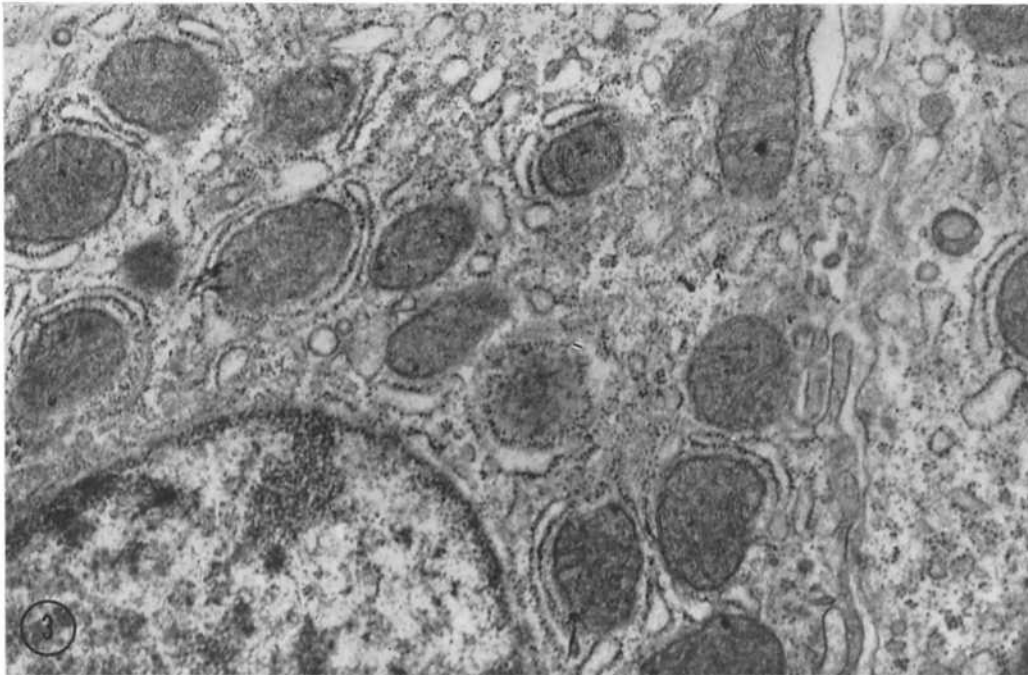


FIGURE 3 Electron micrograph of 20-day embryonic chick liver. This cell with its rough cytomembranes differs very little from the adult fowl liver cell. $\times 33,000$.

FIGURE 4 Electron micrograph of adult fowl liver. This picture shows a typical secreting cell with its large number of rough cytomembranes. $\times 33,000$.

corporate amino acids rapidly, and it is quite possible that the high specific activities of protein from embryonic rat liver nuclei reported earlier were actually due to contamination of the nuclear preparation with haemopoietic cells or their nuclei. However, the avian embryonic liver seems to be far less concerned in haemopoiesis than the mammalian embryonic liver (25).

An examination of Table IV will show that an apparent correlation exists between the percentage of the RNA found in the nucleus and the relative specific activity of the nuclear protein after incubation for 15 minutes. In the 8-day embryonic liver and adult liver, both the percentage of the RNA in the nucleus and the relative specific activity of the nuclear protein were higher than in the 14-day and 20-day embryonic liver (Table IV). The interpretation of the incorporation data is complicated by the fact that radioactive protein appears to have been transferred from one subcellular fraction to another between 15 and 60 minutes of incubation (Table III). It is most likely that some transfer also occurred during the first 15 minutes, at least in embryonic tissue. In the 8-day embryo in which the incorporation was very rapid, this may be an important factor contributing to the high specific activity of the nuclear protein. Further investigation is required to clarify this point. In the adult, however, incorporation was very slow, and Table III shows that if there was any redistribution of proteins it must have occurred slowly. Therefore, the higher relative specific activity of the protein of the nucleus of adult liver cells is probably a true indication of the rate of incorporation in the nucleus itself.

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The results shown in Table IV are not an illustration of the classical concept of a correlation between the amount of RNA and the rate of protein synthesis. The RNA contents of the liver cells at different embryonic stages and of adult liver cells, while differing from each other, remain of the same order; on the other hand, the rate of amino acid incorporation into adult liver slices is only a fraction of that of either 8-day, 14-day, or 20-day embryonic liver.

Previous reports have demonstrated an increased incorporation of amino acids into nuclear protein compared with cytoplasmic protein in rapidly growing tissues, such as liver tumour and ascites tumour cells (7). In regenerating rat liver the nuclei do not show any increased incorporation of amino acids in relation to the cytoplasm (7). While the amino acid incorporation into embryonic chick liver was considerably greater than into adult liver (Table II), the contribution of the nuclei to the total cellular incorporation rate was actually smaller in embryonic than in adult tissue.

The results of the present study suggest that relatively simple concepts, such as a direct relation between RNA and protein synthesis, or the predominance of nuclear protein synthesis during rapid growth, will have to be modified considerably. Further work on the distribution and activity of transfer-RNA and ribosomal RNA in developing chick liver has been commenced and will be reported at a later date.

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