LOUIS GLUCK*
MARIE V. KULOVICH**

*Department of Pediatrics, Yale University School of Medicine*

# NUCLEIC ACID AND PROTEIN CHANGES DURING EMBRYONIC ORGAN DEVELOPMENT IN THE CHICK†

The rapid acceleration of an embryo into an increasingly heterogeneous organism begins early in development with a flood of biochemical changes that accompanies the appearance and growth of organs. The nucleic acids, particularly the ribonucleic acids (RNA), are involved in these profuse changes although the manner of their involvement is only partly understood. We are attempting to define further the role of RNA in embryonic differentiation and organogenesis by several methods. These include among others the histochemical localization of RNA during development and the determination of nucleotide composition and analysis of amino acid incorporation in relation to different species of RNA in the developing embryo. This paper is a report of some baseline studies of the changing levels of nucleic acids (and protein) during the development and growth of seven organs in the chick embryo.

A number of reported studies of changes in nucleic acids deal almost entirely either with particular stages of the early total embryo or longitudinally with the total embryo through its development into the newborn animal.[1-8] These are concerned only with the aggregate of changes in the total embryo, in effect considering the embryo a homogeneous tissue. Few reported investigations deal with changes in nucleic acids in individual organs during embryonic development.[9-14] Measurements of nucleic acid phosphorous in several developing organs and tissues of the chick embryo were published by Leslie and Davidson[10] (brain, heart, liver, skeletal muscle) and by Szepsenwol, Mason, and Shontz[14] (brain, heart, liver, skeletal muscle, digestive tract muscle, chorioallantoic membrane), but the results obtained by the two groups of authors are not comparable. Studies

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* Assistant Professor of Pediatrics.
** Associate in Research.
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such as these have not been reported employing more recent techniques for measurement of nucleic acids nor has adequate consideration been given to finding optimal methods of expressing results of analyses in developing embryos.

The changing levels of RNA and their relation to DNA and protein in seven developing organs of the chick embryo—heart, liver, kidney, brain, intestine, lung, and skeletal muscle—are reported here. Although this report emphasizes the fallacy of considering the developing embryo sufficiently homogeneous to warrant measurements on the total embryo, some of the studies done of the first six days of development are presented here on the total embryo for comparison with later organ development.

## MATERIALS

All embryonic and adult hen tissues studied were from a pure strain of chicken, the sex-linked Hallcross.* Studies on the first six days of embryonic development were done on entire embryos, numbering from 50 six-day embryos to 288 blastoderms. Daily studies were done on heart, liver, kidney (metanephros and mesonephros), digestive tube ("gut"), brain, lung, and skeletal muscle (breast), beginning at the sixth day of development when these organs are clearly discrete. From 24 to 72 of each of the individual organs were dissected daily for determinations, depending upon the stage.

### *Preparation of tissues*

Embryonic tissues and organs were dissected directly into cold 10 per cent trichloroacetic acid (TCA) and immediately homogenized at 2° C. The homogenate was washed twice with cold (2° C.) 10 per cent TCA and twice or more with a 3:1 diethyl ether-ethanol mixture until the supernatant was clear.

For the multiple determinations done, the washed homogenized tissue was divided among pre-weighed test tubes and then dried to constant weight (1 to 3 hours) under high vacuum. The dried tissue, free of lipids and acid-soluble compounds, was weighed and determinations done on this exact amount of dry tissue directly in the pre-weighed test tubes.

### *Determinations*

All nucleic acids determinations were done by two methods as a check and safeguard against errors in technique. Nucleic acids were extracted from the dried weighed tissue with hot TCA (90° C.) and pentose analy-

* Obtained from Hall Brothers Hatcheries, Wallingford, Connecticut.

TABLE 1. ORGAN WEIGHTS AND RESIDUE DRY WEIGHTS AFTER EVAPORATION OF WATER; CHANGES WITH DEVELOPMENT IN CHICK EMBRYO*

| Day of development | No. of samples | Organ weights, wet and dry, in mg. per total organ (average weights) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lung | | Heart | | Gut | | Brain | | Kidney | | Liver | |
| | | Wet weight | Dry weight | Wet weight | Dry weight | Wet weight | Dry weight | Wet weight | Dry weight | Wet weight | Dry weight | Wet weight | Dry weight |
| 6 | 18 | 3.0 | 0.3 | 3.8 | 0.4 | 2.2 | 0.2 | 123.6 | 6.9 | — | — | 2.8 | 0.5 |
| 7 | 12 | 3.5 | 0.4 | 7.7 | 0.7 | 6.4 | 0.6 | 147.1 | 10.6 | — | — | 3.5 | 0.7 |
| 8 | 12 | 7.0 | 0.8 | 10.0 | 0.7 | 15.5 | 1.5 | 155.1 | 12.3 | 10.5 | 1.0 | 6.6 | 1.1 |
| 9 | 12 | 19.6 | 1.1 | 16.5 | 2.0 | 39.5 | 3.9 | 170.6 | 14.3 | 23.1 | 5.3 | 13.5 | 2.3 |
| 10 | 12 | 25.2 | 2.2 | 19.0 | 2.1 | 36.8 | 2.4 | 186.7 | 14.5 | 16.5 | 1.8 | 24.0 | 4.9 |
| 11 | 8 | 37.7 | 2.5 | 30.4 | 3.0 | 108.9 | 9.8 | 197.8 | 20.0 | 23.5 | 2.8 | 57.1 | 11.4 |
| 12 | 8 | 71.5 | 5.3 | 43.5 | 4.7 | 154.7 | 14.8 | 295.8 | 29.4 | 39.1 | 4.8 | 84.1 | 16.2 |
| 13 | 8 | 121.2 | 9.9 | 74.7 | 8.8 | 411.0 | 37.7 | 361.4 | 40.9 | 55.5 | 9.0 | 133.2 | 25.8 |
| 14 | 6 | 141.4 | 18.6 | 116.2 | 14.5 | 833.0 | 140.3 | 413.1 | 45.6 | 64.7 | 9.7 | 230.2 | 57.0 |
| 15 | 6 | 155.3 | 19.8 | 140.1 | 18.9 | 929.6 | 149.6 | 615.6 | 69.8 | 114.4 | 17.6 | 313.5 | 75.2 |
| 16 | 6 | 157.8 | 23.2 | 158.8 | 19.6 | 1230.3 | 202.4 | 677.8 | 77.7 | 163.3 | 19.9 | 386.0 | 112.3 |
| 17 | 6 | 178.2 | 25.7 | 182.4 | 23.7 | 1610.3 | 283.3 | 691.0 | 84.3 | 216.7 | 32.5 | 463.5 | 116.3 |
| 18 | 4 | 212.9 | 27.0 | 192.0 | 25.1 | 1684.4 | 271.7 | 768.8 | 115.9 | 102.2 | 17.0 | 501.8 | 160.4 |
| 19 | 4 | 237.6 | 28.9 | 239.6 | 34.4 | 2073.3 | 366.1 | 834.3 | 105.1 | 170.4 | 23.5 | 601.1 | 209.5 |
| 20 | 4 | 254.8 | 33.5 | 249.6 | 36.8 | 2895.1 | 434.9 | 852.4 | 127.3 | 176.2 | 30.4 | 687.7 | 236.3 |
| Newborn | 4 | 281.2 | 41.1 | 252.7 | 39.6 | 4406.6 | 754.6 | 877.3 | 141.3 | 234.8 | 41.7 | 958.2 | 330.0 |

* Organs were dried slowly for 12 hours at 80° C. and then to constant weight at 100° C.

ses[15-17] done for DNA with diphenylamine[15, 18] and for RNA with orcinol[15, 19] reagents. Hot perchloric acid (PCA) extraction[20] was also done for total ultraviolet absorption of the nucleic acids at 260 mμ and analysis of DNA with diphenylamine reagent.[15, 18] Values for RNA were obtained by the difference between the values for the total nucleic acids and DNA. Cor-

TABLE 2. LOSSES OF ACID-SOLUBLE COMPOUNDS (TCA SOLUBLE) IN TISSUE PREPARATION

(Values are percentages of residue per wet weight of tissue. Residue prepared by drying washings to constant weight at 100° C.)

| *Day of development* | *No. of samples* | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
|---|---|---|---|---|---|---|---|---|
| 8 | 60 | 1.8 | 3.1 | 1.9 | 2.1 | 2.0 | 1.6 | 2.8 |
| 9 | 60 | 1.8 | 2.6 | 2.2 | 2.1 | 2.5 | 1.9 | 2.7 |
| 10 | 48 | 2.9 | 3.9 | 2.1 | 2.4 | 3.2 | 5.1 | 3.0 |
| 11 | 48 | 1.8 | 3.2 | 2.8 | 2.4 | 1.5 | 2.2 | 3.3 |
| 12 | 48 | 1.9 | 3.2 | 2.3 | 2.3 | 2.1 | 2.1 | 3.0 |
| 13 | 36 | 1.8 | 4.6 | 2.1 | 2.2 | 2.3 | 3.8 | 4.1 |
| 14 | 36 | 4.2 | 6.2 | 5.2 | 6.1 | 4.0 | 5.5 | 8.9 |
| 15 | 36 | 2.6 | 5.9 | 2.1 | 3.7 | 2.7 | 2.5 | 8.5 |
| 16 | 36 | 1.7 | 1.6 | 1.4 | 2.2 | 2.5 | 1.5 | 4.3 |
| 17 | 24 | 2.4 | 3.1 | 2.4 | 2.2 | 2.6 | 2.3 | 3.5 |
| 18 | 24 | 3.2 | 3.7 | 2.4 | 2.2 | 2.3 | 2.3 | 6.9 |
| 19 | 24 | 2.2 | 2.7 | 2.8 | 2.1 | 2.5 | 2.5 | 3.1 |
| 20 | 24 | 2.3 | 3.0 | 2.0 | 2.8 | 2.5 | 3.3 | 2.5 |
| Newborn | 24 | 4.1 | 2.5 | 6.7 | 9.6 | 5.6 | 5.2 | 8.0 |
| Adult | 8 | 2.6 | 3.0 | 2.2 | 3.2 | 2.0 | 1.8 | 4.6 |

respondence between the TCA and PCA methods was within three per cent. Total protein was determined by the biuret method.[21] Total nitrogen was determined by the Kjeldahl method.[22, 23] All absorption readings were made on a Beckman Model DU Spectrophotometer. RNA and DNA used to prepare standard curves were purchased from Calbiochem, Los Angeles, California.

*Dry weights*

It is very difficult to relate the value of a chemical analysis in the embryo to a suitable single reference point. Expressing a determination per total organ is limited both because of the rapid growth of embryonic organs and because it introduces the additional variable of changing water content, as is also the case if wet weight is used. Table 1 illustrates these changes, show-

ing organ weights in the embryo during development and their actual dry weights after water and volatile substances are evaporated. This suggests that results expressed per dry weight—that is, eliminating the changing variable of water content—are more accurate measures of concentration than those expressed per wet weight. However, analytic measurements of

TABLE 3. LOSSES OF LIPIDS IN TISSUE PREPARATION (ETHER AND ETHANOL WASHINGS)

(Values expressed as percentages of residue per wet weight of tissue. Residue prepared by drying washings to constant weight at 40° C. in a water bath.)

| *Day of development* | *No. of samples* | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
|---|---|---|---|---|---|---|---|---|
| 8 | 60 | 1.9 | 2.9 | 1.8 | 1.7 | 2.3 | 1.5 | 4.9 |
| 9 | 60 | 1.0 | 2.8 | 1.4 | 1.5 | 2.0 | 1.1 | 4.9 |
| 10 | 48 | 1.9 | 2.1 | 1.8 | 1.9 | 2.9 | 1.6 | 3.7 |
| 11 | 48 | 1.2 | 2.3 | 2.2 | 1.9 | 1.2 | 3.1 | 3.3 |
| 12 | 48 | 1.1 | 2.9 | 7.2 | 1.6 | 2.2 | 0.9 | 2.9 |
| 13 | 36 | 1.0 | 1.4 | 1.6 | 1.3 | 1.3 | 0.8 | 3.0 |
| 14 | 36 | 4.2 | 6.2 | 5.2 | 6.1 | 0.4 | 5.5 | 1.9 |
| 15 | 36 | 2.3 | 2.2 | 1.6 | 2.3 | 2.8 | 2.7 | 0.9 |
| 16 | 36 | 1.7 | 2.2 | 2.0 | 2.1 | 2.0 | 2.1 | 0.6 |
| 17 | 24 | 2.8 | 2.3 | 1.8 | 1.7 | 5.1 | 1.6 | 4.5 |
| 18 | 24 | 3.3 | 2.6 | 2.3 | 2.6 | 3.9 | 2.4 | 7.3 |
| 19 | 24 | 2.2 | 2.1 | 1.7 | 2.5 | 2.6 | 1.4 | 10.6 |
| 20 | 24 | 2.3 | 4.4 | 1.1 | 2.8 | 3.0 | 3.3 | 19.6 |
| Newborn | 24 | 0.4 | 2.4 | 6.7 | 6.8 | 2.6 | 5.2 | 8.3 |
| Adult | 8 | 5.0 | 2.5 | 2.8 | 5.5 | 1.7 | 0.5 | 2.7 |

microgram quantities, as in the present studies, require means of determining accurate and reliable reference dry weights. In our experience taking aliquot fractions of homogenate separately for dry weights and separately for determinations can introduce errors as great as 30 per cent when measuring very small amounts of tissue as in very young embryos. Eliminating this major source of error can be done easily by making measurements both of dry weights and chemical determinations directly on weighed dried tissue without removing it from pre-weighed test tubes.

Ideally, the most nearly accurate results are obtainable with tissues from which *only* water has been removed without other manipulation. These can be only quick-frozen (liquid nitrogen, e.g.) tissues dried (5 to 7 days) under high vacuum at low temperatures (ca—30° C.), weighed, homogenized, and then extracted of their nucleic acids. The formidability of this method,

designed only for minute amounts of tissue, renders it almost totally impracticable. Attempts to dry homogenates under various modified conditions without first washing out the acid soluble substances and lipids gave results that were not reproducible. The method described above under preparation of tissues yielded nucleic acid determinations reproducible to within five per cent.

TABLE 4. TCA-PRECIPITATED VACUUM-DRIED TISSUE, FREE OF ACID-SOLUBLE COMPOUNDS AND FREE OF LIPIDS

(Values expressed as percentages per wet weight of tissue.)

| *Day of development* | *No. of samples* | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
|---|---|---|---|---|---|---|---|---|
| 8 | 60 | 5.2 | 6.4 | 7.0 | 6.2 | 8.4 | 4.8 | 11.6 |
| 9 | 60 | 5.4 | 8.1 | 8.9 | 9.1 | 10.0 | 5.9 | 14.5 |
| 10 | 48 | 4.7 | 7.8 | 7.5 | 6.1 | 10.7 | 6.5 | 15.8 |
| 11 | 48 | 4.5 | 9.0 | 6.5 | 6.7 | 3.9 | 5.6 | 14.4 |
| 12 | 48 | 6.1 | 5.8 | 8.9 | 7.8 | 7.5 | 5.7 | 15.1 |
| 13 | 36 | 6.5 | 9.6 | 5.6 | 8.3 | 13.3 | 6.5 | 18.3 |
| 14 | 36 | 8.5 | 9.3 | 8.9 | 12.3 | 7.3 | 6.8 | 17.8 |
| 15 | 36 | 11.0 | 9.9 | 11.6 | 5.25 | 13.2 | 10.8 | 16.0 |
| 16 | 36 | 10.8 | 10.8 | 11.4 | 9.3 | 11.8 | 8.5 | 18.1 |
| 17 | 24 | 10.8 | 12.7 | 11.7 | 8.7 | 12.5 | 9.3 | 17.8 |
| 18 | 24 | 11.0 | 9.6 | 9.9 | 9.6 | 11.8 | 9.7 | 14.8 |
| 19 | 24 | 12.3 | 11.6 | 14.0 | 6.1 | 14.8 | 10.5 | 20.5 |
| 20 | 24 | 12.0 | 11.3 | 18.8 | 11.3 | 15.0 | 12.2 | 23.0 |
| Newborn | 24 | 13.0 | 10.8 | 18.4 | 11.7 | 15.0 | 4.4 | 19.5 |
| Adult | 8 | 10.0 | 17.0 | 12.5 | 10.0 | 18.0 | 7.8 | 25.0 |

In Table 2, below, are shown the tissue losses in the combined TCA washings, comprising acid-soluble substances, expressed as the percentage relationships of the dried TCA extracts to the total wet weights of tissue used. The dry weights of the TCA extracts were obtained by heating the extracts at 100° C. until dry, which causes decomposition and disappearance of the TCA.

In Table 3 are shown the losses of tissue, primarily lipids, in the ether-ethanol washings. These successive washings were pooled for each tissue and evaporated to dryness in a water bath at 40° C. Again, losses are expressed as the percentages of extracted material of the total wet weights of tissue used.

In Table 4 are listed the actual "dry weights" of tissue extracted for nucleic acids. The "dry weights" are expressed as percentages of the total

wet weights of tissue used for determinations. Fluctuations and disparities between the total percentages of the TCA-extractable compounds plus the lipid fractions plus the extracted "dry weights" compared to the percentages of dry weights of organs shown in Table 1 reflect errors inherent in the preparation of tissues. These include residual tissue on the homogenizing pestle, tissue adherent to test tubes after transfer, etc. These are additional sources of error which do not affect the final determinations under the

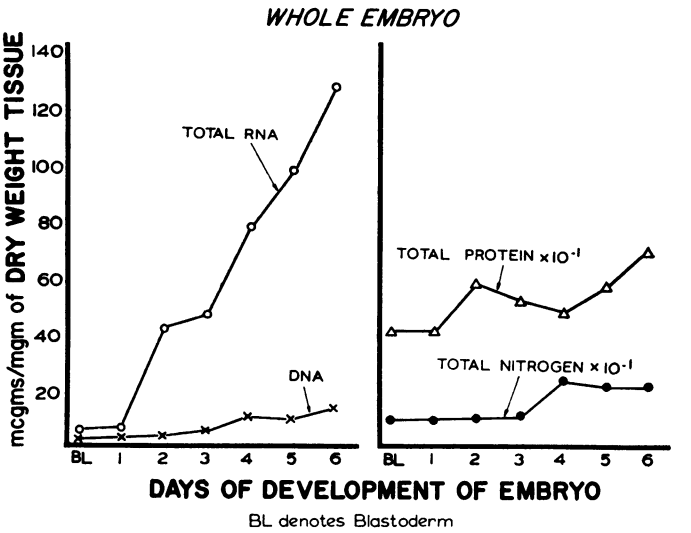


FIG. 1. Changes in RNA, DNA, nitrogen, and protein in the early *whole* embryo.

presently described methods but they do introduce significant errors when determinations made are based on total organ or on wet weight of tissue, especially when there are only small amounts of tissue.

Thus, the "dry weights" used throughout this report represent TCA-precipitable, vacuum-dried tissue, free of acid-soluble compounds and free of lipids. These will henceforth be referred to as "dry weight" tissue.

## RESULTS

Changes in concentration of RNA, DNA, total nitrogen and protein per milligram of "dry weight" tissue in the whole chick embryo from the blastoderm stage through the first six days of development are shown in Figure 1. About 48 hours after fertilization, or approximately 24 hours of development beyond the blastoderm, a sharp rise in synthesis of RNA is found.

These data support the findings in *Rana pipiens*[8] of an initial lag period which precedes a sharp rise in synthesis of RNA. This is primarily ribosomal RNA, nearly all the measurable RNA recoverable in the pH 7.6 fraction by phenol extraction.[24] The steep increase in concentration of RNA in the total embryo is seen during these first six days of development, the sharpest rise occurring between days 1 and 2. Only a slight, gradual increase in concentration of DNA in the total embryo occurs during this time.

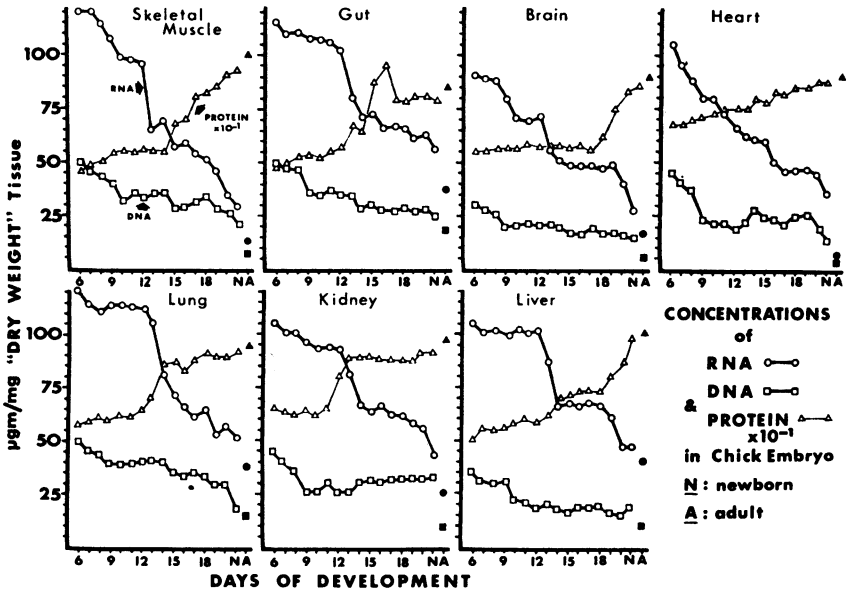


FIG. 2. Concentrations of RNA, DNA, and protein in seven organs in developing chick embryo.

From the sixth day of development, concentrations of RNA, DNA and protein per unit "dry weight" tissue were determined daily in seven different organ systems as shown in Tables 5, 6, and 7 and in Figure 2. In all of these data, standard deviations did not vary more than 10 per cent from the average values cited. The most consistent pattern seen in these tissues is the marked decrease in concentration of RNA occurring between days 12 and 15 in nearly all tissues. All tissues studied have high concentrations of RNA early in development which decrease to the relatively low concentrations of total RNA found in the newborn and adult. Adult lung and gut show a decrease of 69 per cent in levels of RNA from those in the organs of the six-day embryo, while adult cardiac and skeletal muscle show the

TABLE 5. CONCENTRATIONS OF RNA IN DEVELOPING ORGANS OF THE CHICK EMBRYO

| *Day of development* | *No. of samples* | *Concentration of RNA in μgm/mg "dry weight" tissue* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
| 6 | 72 | 120 | 105 | 115 | 92 | 105 | 120 | 105 |
| 7 | 60 | 115 | 95 | 110 | 90 | 100 | 120 | 100 |
| 8 | 60 | 110 | 89 | 110 | 87 | 100 | 115 | 102 |
| 9 | 60 | 115 | 79 | 107 | 79 | 95 | 107 | 98 |
| 10 | 48 | 115 | 78 | 106 | 71 | 90 | 99 | 101 |
| 11 | 48 | 113 | 71 | 105 | 70 | 89 | 99 | 100 |
| 12 | 48 | 112 | 65 | 102 | 72 | 91 | 96 | 100 |
| 13 | 36 | 106 | 62 | 81 | 55 | 81 | 65 | 86 |
| 14 | 36 | 80 | 61 | 71 | 51 | 65 | 69 | 65 |
| 15 | 36 | 70 | 59 | 72 | 49 | 62 | 56 | 66 |
| 16 | 36 | 65 | 50 | 65 | 49 | 65 | 58 | 65 |
| 17 | 24 | 65 | 45 | 66 | 50 | 61 | 55 | 66 |
| 18 | 24 | 65 | 45 | 65 | 49 | 61 | 51 | 65 |
| 19 | 24 | 52 | 46 | 61 | 50 | 58 | 46 | 60 |
| 20 | 24 | 57 | 43 | 62 | 41 | 56 | 35 | 48 |
| Newborn | 24 | 50 | 35 | 58 | 26 | 42 | 28 | 48 |
| Adult | 8 | 37 | 6 | 36 | 16 | 25 | 12 | 40 |

TABLE 6. CONCENTRATIONS OF DNA IN DEVELOPING ORGANS OF THE CHICK EMBRYO

| *Day of development* | *No. of samples* | *Concentration of DNA in μgm/mg "dry weight" tissue* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
| 6 | 72 | 50 | 45 | 50 | 30 | 45 | 50 | 35 |
| 7 | 60 | 45 | 40 | 48 | 28 | 40 | 45 | 30 |
| 8 | 60 | 42 | 37 | 48 | 26 | 35 | 42 | 30 |
| 9 | 60 | 39 | 24 | 36 | 19 | 25 | 38 | 30 |
| 10 | 48 | 39 | 22 | 35 | 19 | 25 | 30 | 22 |
| 11 | 48 | 40 | 22 | 38 | 22 | 29 | 35 | 20 |
| 12 | 48 | 40 | 19 | 35 | 20 | 26 | 31 | 18 |
| 13 | 36 | 41 | 24 | 35 | 21 | 25 | 35 | 20 |
| 14 | 36 | 40 | 29 | 27 | 20 | 29 | 35 | 18 |
| 15 | 36 | 36 | 24 | 31 | 16 | 29 | 27 | 17 |
| 16 | 36 | 34 | 24 | 28 | 15 | 29 | 26 | 18 |
| 17 | 24 | 36 | 22 | 27 | 19 | 29 | 31 | 18 |
| 18 | 24 | 34 | 25 | 28 | 16 | 30 | 33 | 18 |
| 19 | 24 | 29 | 24 | 27 | 15 | 28 | 27 | 14 |
| 20 | 24 | 29 | 20 | 27 | 14 | 30 | 25 | 12 |
| Newborn | 24 | 17 | 13 | 25 | 14 | 29 | 21 | 15 |
| Adult | 8 | 15 | 5 | 18 | 5 | 9 | 8 | 10 |

sharpest declines of all tissues to only 6 and 10 per cent respectively. Brain and kidney during development show decreases in concentrations of RNA of 82 and 76 per cent. Liver shows the least decline (62 per cent) during development of the tissues studied and in the adult has the highest concentrations.

TABLE 7. CONCENTRATIONS OF PROTEIN IN DEVELOPING ORGANS OF THE CHICK EMBRYO

| *Day of development* | *No. of samples* | *Concentration of protein in μgm/mg "dry weight" tissue* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Lung* | *Heart* | *Gut* | *Brain* | *Kidney* | *Muscle* | *Liver* |
| 6 | 72 | 575 | 675 | 475 | 550 | 650 | 450 | 500 |
| 7 | 60 | 580 | 675 | 490 | 550 | 640 | 475 | 525 |
| 8 | 60 | 600 | 700 | 525 | 560 | 625 | 500 | 550 |
| 9 | 60 | 590 | 710 | 510 | 560 | 640 | 540 | 550 |
| 10 | 48 | 620 | 725 | 525 | 560 | 630 | 550 | 560 |
| 11 | 48 | 610 | 730 | 550 | 570 | 650 | 540 | 600 |
| 12 | 48 | 640 | 750 | 575 | 560 | 800 | 560 | 590 |
| 13 | 36 | 700 | 750 | 675 | 560 | 875 | 550 | 625 |
| 14 | 36 | 850 | 800 | 650 | 570 | 880 | 550 | 675 |
| 15 | 36 | 860 | 775 | 875 | 550 | 880 | 675 | 725 |
| 16 | 36 | 830 | 825 | 950 | 570 | 875 | 700 | 750 |
| 17 | 24 | 875 | 820 | 800 | 550 | 875 | 800 | 750 |
| 18 | 24 | 900 | 850 | 780 | 625 | 875 | 820 | 750 |
| 19 | 24 | 880 | 850 | 820 | 750 | 900 | 850 | 800 |
| 20 | 24 | 880 | 875 | 820 | 825 | 875 | 900 | 860 |
| Newborn | 24 | 900 | 875 | 780 | 850 | 900 | 920 | 950 |
| Adult | 8 | 920 | 900 | 850 | 875 | 950 | 980 | 1000 |

Concentrations of DNA per "dry weight" tissue are comparatively stable throughout development in kidney and brain. Gradual decreases in concentrations are seen in the other tissues. Lung, gut, and muscle contain unusually high early concentrations of DNA.

Concentrations of total protein per unit "dry weight" tissue, shown in Table 7 and Figure 2, differ markedly during development from tissue to tissue. In heart and liver there are gradual increases in concentration during development. In the other tissues there are abrupt increases occurring between days 13 and 15 in muscle, gut, and lung. In kidney a somewhat earlier sharp rise at day 12 is seen. Brain shows a rise in protein concentration late in development, at day 18, while gut is unusual in showing a peak concentration and then a fall around day 17.

The marked structural differences between the mucosa and muscularis of the gut provides an ideal model to demonstrate heterogeneity of developing organs. Careful separations from a number of digestive tubes with a dissecting microscope were made of mucosa and muscularis and the RNA concentrations determined for each at various stages as shown in Table 8. As can be seen from the data, muscularis and mucosa show marked differences in concentrations of RNA during development of the gut.

Figures 3, 4, and 5 present the ratios of RNA: DNA, protein: DNA, and RNA: protein during development of organs. The species constancy in

TABLE 8. COMPARISON OF CONCENTRATIONS OF RNA IN MUCOSA AND MUSCULARIS OF DEVELOPING GUT

| *Day of development* | *No. of samples* | *Concentrations of RNA in μgm per mg. "dry weight" tissue* | |
|---|---|---|---|
| | | *Mucosa* | *Muscularis* |
| 8 | 36 | 102 | 118 |
| 15 | 24 | 88 | 52 |
| Newborn | 18 | 70 | 30 |
| Adult | 2 | 46 | 8 |

amount of DNA per somatic cell invites the use of DNA as divisor to indicate concentrations of RNA or protein per cell.[9, 10] Although this is useful in helping to interpret data, it offers at best only a rough approximation for any given organ since organs are composed of multiple cell types in varying proportions. Those cell types differ in size and with progressing differentiation may vary considerably in their content both of DNA and of RNA.

In their ratios of RNA: DNA (Fig. 3) all organs studied exhibited a rise around days 9 and 10. This is a reflection of the decreasing DNA concentrations occurring at this time, as shown in Figure 2. The variations in concentration of RNA with development have been shown above. The impressive decreases in concentration of RNA that occurs in the tissues around days 12 to 15, coupled with an earlier drop in concentration of DNA, leads to the peaking increases in RNA: DNA ratios seen in Figure 3.

The ratios of RNA: protein in Figure 4 parallel closely the concentrations of total RNA shown in Figure 2. This is best explained by the fact that the bulk of the "dry weight" tissue is protein. The RNA: protein ratio is very useful in interpretation since it represents an inverse of the actual rate of protein synthesis during development.

The protein: DNA ratios represent the average cellular concentration of protein, as shown in Figure 5. Late rises in intracellular protein mass are found in skeletal muscle, brain, heart, and lung, while kidney and gut show surprising constancy.

## DISCUSSION

In describing their studies on the chemical composition of the chick embryonic cell, Leslie and Davidson[10] considered the dilemma of the inter-

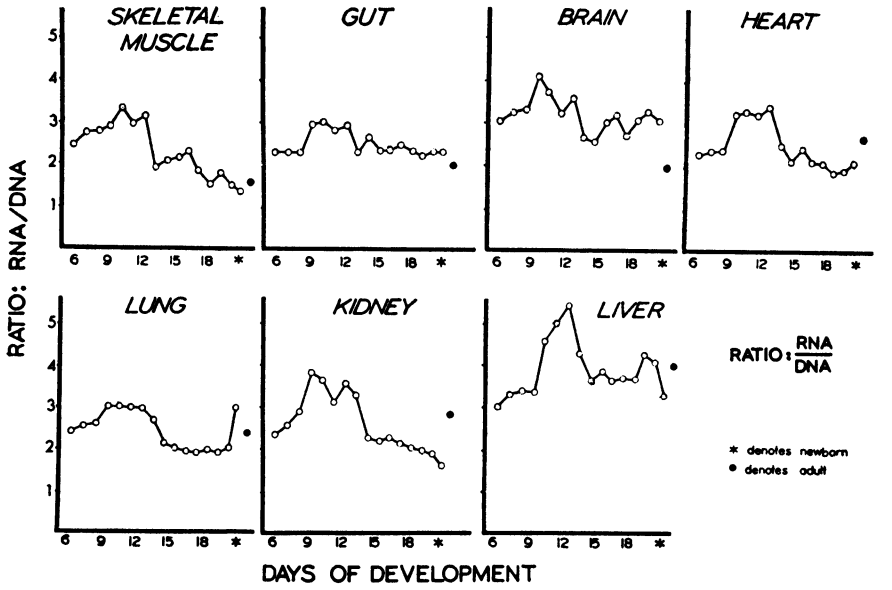


FIG. 3. Ratios of concentrations of RNA: DNA in seven organs in developing chick embryo.

pretation of analytical figures in the embryo. They follow the chemical development of embryonic tissues by the content of various constituents "per cell," relating these to the changes in "cell number" in organs with growth. This is based on observations that the DNA content of the cell nucleus is constant in amount in the somatic tissues of a given species. The number of cells in an organ is calculated by dividing the total amount of DNA in that organ by the amount of DNA per nucleus (taken by them to be "the approximate mean figure of $2.35 \cdot 10^{-7}$ $\mu$g DNA phosphorous"[10] per nucleus). The average cell content of a particular constituent is then calculated by determining the total content of that substance in an organ and

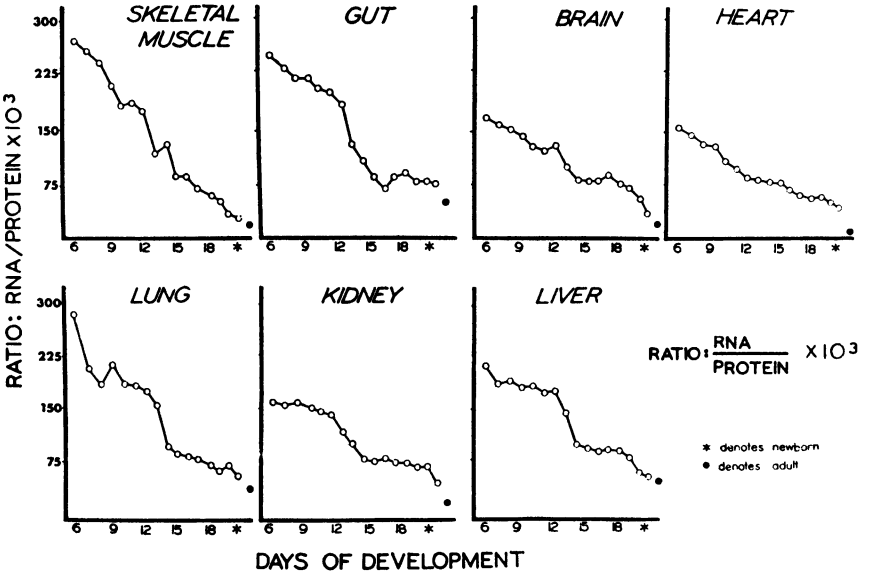


FIG. 4. Ratios of concentrations of RNA: protein in seven organs in developing chick embryo.

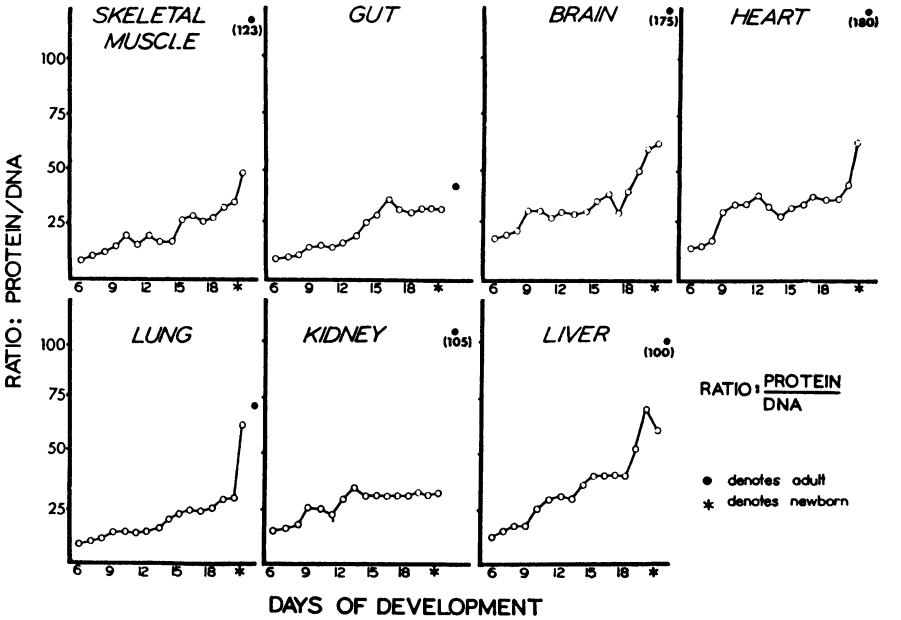


FIG. 5. Ratios of concentrations of protein: DNA in seven organs in developing chick embryo.

expressing it in relation to the amount of DNA phosphorous per nucleus. Although this is a useful way to express analytical values, the method proposed by the authors has important disadvantages and inherent errors. All organs are composed of many types of cells which vary in size and differ during development in concentrations of RNA. In the very young embryo an appreciable proportion of cells may contain up to 100 per cent more DNA than others since they are in various stages of cell division and a significant error might be incurred by dependence on one particular value of DNA per nucleus. As the embryo becomes older this variation among nuclei decreases. However, in a serious attempt to avoid what they termed "a constantly changing complex of variables" by using a fixed, but only approximate, value for DNA per nucleus Leslie and Davidson[10] actually may have introduced one. Knowledge of the relative changes among nucleic acids and protein is valuable in assessing growth. It is felt, however, that ratios of accurately determined concentrations as prescribed in this report are perhaps more meaningful than data expressed as "concentration per cell," determined by less accurate methods and based on an approximation of DNA per cell. The nucleic acid phosphorous determinations reported by Leslie and Davidson[10] were done on aliquot portions of lipid-free homogenate, which introduces errors of measurement magnified by their sampling of relatively very few embryos (and, therefore, very little tissue), as well as errors from losses in preparing tissues which have been mentioned earlier in this report.

Nucleic acid determinations in embryonic tissues of the chick reported by Szepsenwol, Mason, and Shontz[14] were determinations of DNA and RNA phosphorous expressed as milligrams of phosphorous per 100 grams of wet weight tissue. Their studies of nucleic acid concentrations showed but few changes during development and those changes that did occur were slight and gradual. In all tissues studied by their calculations there was a *rise* with development in RNA except in liver, which showed a drop. In general, their method of expressing analytical results per 100 grams of wet weight tissue is very limited in representing changes that take place in concentration of RNA in tissues during development, since these tissues contain as their chief constituent a constantly changing water content.

The prime advantages of reporting concentrations of nucleic acids per unit "dry weight" tissue as described here are the considerable technical advantages over other methods mentioned, particularly in assessing *changes* in nucleic acids in tissues during development. For example, when comparing the developing lung as shown in Table 9, which weighs an average of 7 milligrams at 8 days and 281 milligrams in the newborn, the concentra-

tions of RNA are 110 and 50 micrograms per milligram "dry weight" tissue respectively. The total amounts of RNA in the lungs, however, are 40 micrograms at 8 days and 1,685 micrograms in the newborn, while the concentrations of RNA per milligram of wet weight of tissue are 5.7 micrograms in the 8-day embryo and 5.9 micrograms in the newborn.

Thus, data expressed per total organ or on the basis of a given weight of wet tissue are not informative in reflecting actual concentrations of nucleic acids in developing tissues.

The values expressed per "dry weight" tissue correlate closely with the changes in RNA seen in histochemical studies of RNA during development of the embryo. There is little correlation with histochemical findings[26] in the trends of the values expressed by Leslie and Davidson, and none with those of Szepsenwol, *et al.*[14]

TABLE 9. COMPARISON OF RNA IN LUNG AT TWO DIFFERENT STAGES

| | *Lung* | |
|---|---|---|
| | *8-day embryo* | *Newborn chick* |
| Total weight, mg. | 7 | 281 |
| Concentration RNA per "dry weight," μgm | 110 | 50 |
| Total content of RNA in lungs, μgm | 40 | 1685 |
| Concentration RNA per mg. wet weight lung, μgm | 5.7 | 5.9 |

The quantitative studies reported here reveal no invariably consistent relationships between concentrations of total RNA (or DNA) and protein in the embryo during organ development and growth, nor are there any absolute relationships between levels of DNA and RNA. Developing organs are unique, however. Each shows its own characteristic changes in DNA and RNA levels and their relations to protein formation, and no two embryonic organs are identical in their chemical development. As general principles, concentrations of RNA and DNA are higher per unit of "dry weight" tissue in the embryo than in the adult, and the younger the embryo, the higher are these levels. Nearly all organs studied show their most precipitous drops in RNA concentration around days 12 to 15 of development; brain, heart, and kidney in addition show a sudden decrement around the time of term. The variability among tissues during development is well seen when comparing muscle and liver, for example. In skeletal muscle the level of RNA decreases 90 per cent from that in the six-day embryo to the adult level; adult liver, on the other hand, shows a much smaller decrement to about 38 per cent the levels of RNA of the six-day embryo.

Studies on *total embryo* may present confusing pictures of chemical development, shown for example, by levels of RNA in the six-day whole embryo of 130 micrograms per milligram "dry weight" tissue, as compared with those in individual organs at six days varying from 90 to 120 micrograms per milligram "dry weight" tissue.

Although the quantitative data are reported here as concentrations in tissues and organs it is felt that this is not a complete picture of the quantitative changes in RNA levels without additional data about changes among the various parts of organs. Thus, diversification of organs during development implies not only the chemical uniqueness of each total organ during development but also variation and diversification as well in the cell types of the organ. Except perhaps for skeletal muscle (where there are relatively few cell types and a high concentration of a relatively small number of different proteins), there is enormous heterogeneity of any developing organ, as illustrated by notable differences in RNA content between mucosa and musculature in the developing gut (see Table 8).

## SUMMARY

As with other non-definitive descriptive studies of biochemical phenomena of differentiation, the information presented here provides a baseline for further studies but contributes little toward understanding the basic mechanisms of differentiation. It does, however, point up several fundamental problems concerning the study of chemical development in the embryo. Studying almost any substance in the "total embryo" is misleading because of the false assumption in any such study that the "total embryo" is homogeneous. It is necessary that biochemical measurements of substances in the developing embryo be standardized in a meaningful way. Values expressed per wet weight or per total organ must be corrected for the continuously changing water contents and organ weights associated with development. The use of the enticing species-constant value of DNA per somatic cell leads to the employment of simplistic "concentration per cell" terminology for the description of highly heterogeneous organisms and tissues.

Perhaps the most acceptable standardization for nucleic acids in embryos combines the methods described in this report. Absolute values are expressed as concentrations per "dry weight" of tissue. Relative changes are shown by ratio relationships of the concentrations of RNA: DNA, RNA: protein, and protein: DNA. Use of concentration per "dry weight," as described above, has technical accuracy and reliability. It eliminates con-

siderations of water and weight changes in tissues (as well as storage of foodstuffs in tissues such as glycogen and lipids).

Further value of studies of nucleic acids such as described here would be in assessing changes that occur in different areas of the same tissue and in structural components of organs, such as shown above for gut. More intensive studies are needed to help define further the role of nucleic acids in differentiation, including histochemical studies[25, 26] to show the distribution of RNA, studies of the base compositions of the RNA species with development, and studies of the role of the species of RNA in the incorporation of various amino acids as possible specific events in differentiation.

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