The role of synthesis and of degradation in regulation of malic enzyme concentration in the liver of growing chicks has been studied. Malic enzyme was assayed by enzymic activity and by immunological analysis with immunoglobulin containing specific anti-malic enzyme. Quantitative immunoprecipitin curves and equivalence point determinations indicated that there was a constant relationship between immunologically precipitable protein and units of enzyme activity in livers of neonatal and growing chicks during a time period in which total enzyme activity increased 60-fold. Hence, changes in malic enzyme activity were due to changes in enzyme content of the liver rather than to activation or inhibition of preformed enzyme.

The relative rate of malic enzyme synthesis was determined by measuring incorporation of counts into malic enzyme protein after pulse labeling total protein with L-[4,5-3H]leucine. Malic enzyme protein was purified by quantitative precipitation of the enzyme by antibody followed by disc gel electrophoresis in the presence of sodium dodecyl sulfate. Radioactivity in the band corresponding to malic enzyme was estimated after electrophoresis of the immunoprecipitate.

Relative to synthesis of total protein, the rate of synthesis of malic enzyme increased more than 50-fold when neonatal chicks were fed. Starvation of week-old chicks for 2 days caused a 60 to 70% decrease in synthesis of malic enzyme.

Degradation of malic enzyme was very slow in unfed, neonatal chicks ($t_{1/2} = 350$ hours). Feeding, which increased the synthesis and concentration of malic enzyme also caused an increase in the rate of degradation. In 8- and 11-day-old chicks degradation of malic enzyme was found to be first order with a $t_{1/2}$ of 55 hours. In fasting chicks a $t_{1/2}$ of 28 hours was observed, but there was also an increase in the rate of degradation of total liver protein suggesting that part of the increased rate of degradation of malic enzyme was due to a general increase in protein breakdown.

The temporal relationship between synthesis of malic enzyme and synthesis of fatty acids was studied in unfed neonatal chicks given a single glucose meal. Fatty acid synthesis from [1-14C]acetate was increased 7-fold at 13 hours and 20-fold at 3 hours after the glucose meal. Neither the activity nor the synthesis of malic enzyme were increased at 3 hours but both were significantly increased at 6 hours (100% and 160%, respectively). These results suggest that the increased synthesis of malic enzyme may have been initiated by an increased flux through the pathway for fatty acid synthesis.

The activity of malic enzyme (L-malate-NADP oxidoreductase (decarboxylating) (EC 1.1.1.40)) and the rate of fatty acid synthesis are highly correlated in several animal systems (1-7). This correlation between fatty acid synthesis and the activity of malic enzyme, whose function appears to be to provide NADPH for de novo fatty acid synthesis (1, 2), suggests a possible causal relationship. This paper reports the rates of synthesis and degradation of malic enzyme in the liver of neonatal and growing chicks. In the embryonic and unfed neonatal chick, malic enzyme and fatty acid synthesis are very low; both are increased manyfold when neonatal chicks are fed but show no change if the chicks are not fed (4, 9). In older chicks, fasting reduces and refedding increases both activities (6, 8).

With the use of immunological techniques, we have investigated the following questions. (a) Are changes in the activity of malic enzyme the consequence of changes in the concentration of malic enzyme protein? (b) What are the roles of synthesis and degradation in the regulation of malic enzyme concentration? (c) What is the temporal relationship between an increased rate of fatty acid synthesis and an increase rate of malic enzyme synthesis?

**Experimental Procedure**

*Animal Care*—Unincubated embryonated eggs from white Leghorn chickens were obtained from a commercial supplier and incubated in an electric forced draft incubator at 35.5 ± 0.5°C and 60% relative humidity. One-day-old chicks1 were removed from the incubator and placed in battery brooders which had thermostatically controlled heaters and wire mesh floors. Commercial chicken mash (Master Starter Krumbs, Maple Leaf Mills Ltd., Toronto, Canada) and water were available *ad libitum*. For the *in vivo* isotope incorporation experiments, the chicks were placed in an incubator (31 ± 0.5°C) which was vented into a fume hood. In the forced feeding experiments 20% glucose (2 ml) was administered directly into the cardiac stomach of unanesthetized chicks.

1 The term neonatal is used to mean 1-day-old chicks. Birds fed for 2 days are 3 days old; birds fed for 7 days are 8 days old, etc.

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**SYNOPSIS**

The increased synthesis of malic enzyme may have been initiated by an increased flux through the pathway for fatty acid synthesis.

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1 The term neonatal is used to mean 1-day-old chicks. Birds fed for 2 days are 3 days old; birds fed for 7 days are 8 days old, etc.
**Chemicals**—NADP, L-malic acid (sodium salt), dithiothreitol, \( \beta \)-mercaptoethanol, phenazine methosulfate, nitro blue tetrazolium (Sigma); crystalline bovine serum albumin ( Armour); Mannex DEAE-cellulose (Mann); Sephadex G-200, DEAE-Sephadex (Pharmacia); aldolase, glutamic dehydrogenase (Boehringer Mannheim); acrylamide, N,N-methylene bisacrylamide, \( N,N'N'N' \)-tetramethylethylenediamine (Eastman); Freud's complete adjuvant (Difco); \([l-14C]\)acetate (Amersham-Searle); Ringer Mannheim; creatine phosphokinase (Boehringer Mannheim); Sephadex G-200, DEAE-Sephadex (Pharmacia); aldolase, glutamic dehydrogenase (Boehringer Mannheim); acrylamide, N,N-methylene bisacrylamide, \( N,N'N'N' \)-tetramethylethylenediamine (Eastman); Freud's complete adjuvant (Difco); \([1-3C]\)acetate (Amersham-Searle); and L-[4,5-\(^3\)H]leucine (New England Nuclear) were obtained from the designated sources.

**Purification of Malic Enzyme**—Malic enzyme was isolated from the livers of 4- to 6-week-old white Leghorn chicks by the method used by Hsu and Lardy (9) to purify pigeon liver malic enzyme. The following modifications were made: (a) DTT (1 mm) was used instead of \( \beta \)-mercaptoethanol throughout the procedure; (b) the first and second ethanol fractionations were from 21% to 36% and 25% to 40% ethanol, respectively; (c) the zinc acetate step was omitted because it completely inactivated the enzyme; and (d) a Sephadex G-200 chromatography step was added between the ammonium sulfate fractionation and DEAE-cellulose chromatography. A typical recovery sheet is shown in Table I. All subsequent references to protein fractions refer to Table I.

For the Sephadex G-200 chromatography step, Fraction V was dialyzed overnight against 0.03 M Tris-HCl-1 mM DTT, pH 7.4. Dialyzed Fraction V (2 or 3 ml) was applied to a column of Sephadex G-200 (1.5 x 90 cm) which had been equilibrated with 0.03 M Tris-HCl-1 mM DTT, pH 7.4. The enzyme was eluted in the void volume with the same buffer. Fractions containing malic enzyme of high specific activity were pooled and concentrated by ultrafiltration (Amicon Corporation, Cambridge, Massachusetts). Concentrated Fraction VI was dialyzed against 0.03 M Tris-HCl-1 mM DTT, pH 7.7, prior to DEAE-cellulose chromatography.

After DEAE-cellulose chromatography, the fractions containing activity were pooled, concentrated by ultrafiltration (Amicon), and dialyzed against 0.05 M Tris-HCl-1 mM DTT, pH 7.4. The enzyme could be stored in this form (Fraction VII) at -15° for a few months without any detectable loss of activity.

Malic enzyme was assayed at 40° by the method of Wise and Ball (1). All assays were linear with respect to time and protein concentration. A unit of enzyme activity is defined as 1 pmole of malic acid released per minute. Protein was determined by the method of Lowry et al. (10) with crystalline bovine serum albumin as a standard.

**Polyacrylamide Disc Gel Electrophoresis**—Polyacrylamide gels were prepared by the method of Davis (11) in buffer containing 0.05 M Tris-HCl-0.4 M glycine, pH 8.5. The running gels contained 5% acrylamide and 1.0% acrylamide. Electrophoresis was carried out in glass tubes (0.5 x 11 cm) at 2 ma per tube for 45 min at 4°. Protein in the gels was stained with Amido black. Malic enzyme activity in the gels after electrophoresis was determined by the method of Henderson (12). For the latter experiments the gels underwent electrophoresis for 45 min prior to adding the protein sample in order to remove contaminant ions that might inhibit enzyme activity.

Molecular weight determination by SDS polyacrylamide gel electrophoresis was carried out as described by Weber and Osborn (13). The samples were incubated at 37° for 2 hours in 0.01 M sodium phosphate buffer, pH 7.0, 1.0% SDS and 1% \( \beta \)-mercaptoethanol prior to electrophoresis.

**Analytical Ultracentrifugation**—Sedimentation velocity was determined in a Spinco model E analytical ultracentrifuge. Fraction VII was dialyzed against 0.05 M Tris-HCl-1 mM DTT, pH 7.4. Sedimentation equilibrium experiments were carried out in a Beckman model E analytical ultracentrifuge equipped with ultraviolet absorption optics. The apparent molecular weight (14) was calculated according to the relationship

\[
M_{w, app} = \frac{2R \rho t \, \text{dn}c}{(1 - \rho)_{0} \rho c \, \text{dn} \, d^2}
\]

where \( R \) is the gas constant, \( t \) is the absolute temperature, \( \omega \) is the rotor speed in radians per sec, \( v \) is the partial specific volume, \( \rho \) is the density of the buffer solution, and \( c \) is the concentration (milligrams per ml) at radial distance \( r \) from the axis of rotation.

**Immunological Procedures**—Fraction VII was thawed and centrifuged to remove traces of denatured protein. The preparation was diluted to a protein concentration of 1 to 2 mg per ml with 0.05 M Tris-HCl-1 mM DTT, pH 7.4. The diluted enzyme was mixed with an equal volume of Freund's complete adjuvant and was injected from a 5-cc glass syringe forcibly and repeatedly until a thick smooth emulsion was obtained. This emulsion was injected into rabbits at multiple subcutaneous sites. Injections (0.5 to 1.0 mg of malic enzyme per rabbit) were made three times at 7- to 10-day intervals. The rabbits were bled weekly after the last injection. Peak antibody levels were achieved 2 to 4 weeks after the last injection. Sera with high antibody titers were pooled and frozen at -15°. Immunoglobulins were purified from undialyzed serum by a batch DEAE-Sephadex procedure (15). Ouchterlony double diffusion patterns and quantitative precipitin tests were performed as outlined by Ouchterlony (16) and by Kabat and Mayer (17).

**Synthesis and Degradation of Malic Enzyme**—Chicks were injected intraperitoneally with 0.5 mCi of L-[4,5-\(^3\)H]leucine (1 Ci per \( \mu \)mole). At various times after the injection, the birds were killed and their livers removed. The livers were homogenized in 0.25 \( M \) sucrose-0.005 \( M \) Tris-HCl-1 mM DTT, pH 7.4. Total protein was precipitated from a portion of the whole homogenate by adding an equal volume of 10% trichloroacetic acid. The precipitate was collected by centrifugation, washed...
twice with 5% trichloroacetic acid containing 0.5% unlabeled leucine and once with chloroform-methanol (2:1) and then dissolved in 0.5 ml of formic acid (90%). Fifteen ml of scintillation fluid (toluene-ethylene glycol monoethyl ether, 7:3 (v/v); 2,5-
diphenyloxazole, 0.5%, 1,4-bis(5-phenyloxazol-2-yl)benzene, 0.025%) was added and radioactivity measured in a Nuclear-Chicago Unilux II liquid scintillation spectrometer. The remainder of the homogenate was purified to Fraction II (Table I). In samples from all but the unfed, neonatal chicks, Fraction II was diluted with 0.05 M Tris-HCl-0.15 M NaCl-1 mM DTT, pH 7.4, to give a malic enzyme concentration of 0.35 to 0.40 unit per 0.2 ml. In samples from unfed, neonatal chicks, nonradioactive Fraction VII malic enzyme was added to Fraction II to bring the malic enzyme concentration up to 0.35 to 0.40 unit per 0.1 ml, thus maintaining a constant ratio of antibody to antigen in all preparations. Malic enzyme (0.35 to 0.40 unit in 0.1 or 0.2 ml as noted above) was precipitated with 10 mg of immunoglobulin containing specific malic enzyme antibody (an amount sufficient to precipitate 1 unit of malic enzyme). The reaction mixture contained 0.05 M Tris-HCl-0.15 M NaCl-1 mM DTT, pH 7.4, in a total volume of 2 ml and was incubated at 37° for 30 min and washed twice with cold 0.9% NaCl. The washed precipitate was dissolved in 0.2 to 0.5 ml of 1 M acetic acid. After 30 min at room temperature, the sample was lyophilized. The residue was immediately dissolved in 50 µl of 0.01 M sodium phosphate buffer (pH 6.0) containing 1% SDS and 1% β-mercaptoethanol. The entire sample (approximately 100 µg of protein) was subjected to SDS-polyacrylamide gel electrophoresis at 30 ma per tube for 4 hours. After electrophoresis the gels were removed from the tubes and cut into 1.0 to 1.5-mm fractions. Each fraction was incubated in 0.5 ml of NCS solubilizer (Amersham-Searle) at 60° for 2 hours (18). Scintillation fluid (toluene-methanol, 4:1 (v/v); 2,5 diphenyloxazole, 0.4%; 1,4-bis(5-phenyloxazol-2-yl) benzene, 0.1%) was added and the radioactivity measured as noted above.

**Fatty Acid Synthesis in Vivo**—Each bird received 10 µmoles of [1-14C]acetate (10 PCi) by intraperitoneal injection. After 30 min the birds were killed and their livers removed and frozen on solid CO2. Fatty acids were extracted and their radioactivity measured as previously described (4). At 0 and 3 hours after the glucose meal, incorporation into fatty acids increased for at least 30 min. At 6 hours after the glucose meal, incorporation leveled off at 15 min, suggesting an increased output of newly synthesized fatty acids into the blood.

**RESULTS**

**Homogeneity and Molecular Weight**—Fig. 1 shows the sedimentation pattern of purified malic enzyme in the analytical ultracentrifuge. A single symmetrical peak with an S30w value of 9.5 was observed. This indicates the enzyme was homogeneous with respect to size. Fig. 2 shows the results of polyacrylamide gel electrophoresis of the purified enzyme. Protein was stained on one gel (Fig. 2a), enzyme activity on the other (Fig. 2b). Three protein bands, each staining for malic enzyme activity, are visible. Thus, chick liver may contain three or more isozymes of malic enzyme. Isozymes of malic enzyme have been described in mouse tissues (12). The multiple bands also could represent subunits or polymers of malic enzyme.

The molecular weight of undissociated chicken liver malic enzyme was determined by the sedimentation equilibrium method (14). By assuming a partial specific volume of 0.74 (9), an apparent molecular weight of 2.6 × 104 was calculated from the slope of the plot of ln O.D. versus r². After dissociation by
RELATIVE MOBILITY

FIG. 3. The relation between electrophoretic migration in SDS-polyacrylamide gels and subunit molecular weight. Malic enzyme (†), crystalline bovine serum albumin (●), glutamic dehydrogenase (X), and aldolase (△) were incubated in 0.01 M sodium phosphate (pH 7.0) containing SDS (1%) and β-mercaptoethanol (1%) for 2 hours at 37°C and subjected to polyacrylamide gel electrophoresis in the presence of 0.1% SDS as described by Weber and Osborn (13). About 50 μg of each protein were used.

FIG. 4. Ouchterlony double diffusion analysis of malic enzyme antibody and liver extracts. The center well contained 0.1 ml of malic enzyme antiserum from rabbit. Well 1 contained 0.10 units of malic enzyme purified to Fraction VII (specific activity, 0.01 units per mg of protein); □—□; Well 2 contained 0.08 units of malic enzyme (Fraction II) from neonatal chicks (specific activity, 0.3 units per mg of protein); △—△; Well 3 contained 0.11 units of malic enzyme (Fraction II) from chicks fed for 2 days (specific activity, 0.34 units per mg of protein); O—O; Well 4 contained 0.12 units of malic enzyme (Fraction II) from chicks fed for 7 days (specific activity, 0.66 units per mg of protein).

SDS and mercaptoethanol, SDS electrophoresis of the enzyme indicated a molecular weight of about 0.56 × 10^{6} (Fig. 3). SDS electrophoresis of the purified enzyme revealed a single sharp band of protein. The undissociated enzyme, therefore, may contain four or five subunits of similar molecular weight.

Characterization of Antibody to Malic Enzyme—Ouchterlony double diffusion analysis revealed a single precipitin band when antiserum was allowed to react with purified malic enzyme (Fraction VII) or malic enzyme from Fraction II of unfed, neonatal chicks and 3- and 8-day-old chicks (Fig. 4). The results of quantitative precipitin reactions between anti-malic enzyme and Fraction II preparations from chicks of different ages are shown in Fig. 5. By measuring enzyme activity in the supernatants after removal of the antibody-antigen complexes, equivalence points were established. The same equivalence point, namely 0.1 unit per mg of immunoglobulin, was observed for the Fraction II preparations from (a) unfed, neonatal chicks, (b) chicks fed for 2, 7, or 10 days, and (c) 10-day-old chicks which had been fasted for 2 days. The same equivalence point also was observed for purified malic enzyme (Fraction VII). The amount of protein precipitated by 2 mg of antibody also is shown in Fig. 5. Similar curves were observed for Fraction II preparations from all the kinds of chicks. In sum, these results indicate that the antibody precipitated a protein immunologically identical with purified malic enzyme no matter what kind of chick liver was the source of enzyme. In addition, the precipitin reactions show that the changes in enzyme activity which followed the feeding of neonatal chicks or the fasting of older chicks (Table II) were paralleled by exactly proportional changes in the concentration of immunoprecipitable malic enzyme.

FIG. 5. Quantitative precipitin reactions of malic enzyme in liver extracts (Fraction II). Malic enzyme was from five sources: (a) neonatal chicks (specific activity, 0.01 units per mg of protein), □—□; (b) 3-day-old chicks (specific activity, 0.3 units per mg of protein), △—△; (c) 8-day-old chicks (specific activity, 0.70 units per mg of protein), O—O; (d) 11-day-old chicks (specific activity, 0.75 units per mg of protein), □—□; (e) 10-day-old chicks which had been starved for 2 days (specific activity, 0.4 units per mg of protein), △—△. The upper curve is protein; the lower curve is activity. Precipitation reaction mixtures contained 2 mg of anti-malic enzyme immunoglobulin and various amounts of Fraction II extracts in 2 ml of 0.05 M Tris-HCl-0.15 M NaCl-1 mM DTT, pH 7.4. This mixture was incubated at 37°C for 30 min and 4°C overnight. The precipitates were collected by centrifugation and washed twice with ice-cold 0.9% NaCl. When immunoglobulin from nonimmunized rabbits was used, there was no precipitate.

Isolation and Identification of Radioactive Malic Enzyme in Antibody-Antigen Precipitate—Previous immunochemical stud-
Each chick was given an intraperitoneal injection of 500 μCi of \([^3H]\)leucine 60 min before killing the birds. The results are expressed as means ± S.E. For neonatal chicks three livers were pooled for each experiment. For all other chicks one liver was used per experiment. Synthesis is expressed as disintegrations per min incorporated into malic enzyme per 10,000 dpm incorporated into total liver protein. In all experiments the immunoprecipitate was subjected to SDS-polyacrylamide electrophoresis as outlined under "Experimental Procedure," and only the malic enzyme peak was counted.

### Table II

| Age | Number of experiments | Enzyme activity (units per mg Fraction II protein) | Relative synthesis (dpm in malic enzyme per 10,000 dpm in total liver protein) |
|-----|-----------------------|-------------------------------------------------|---------------------------------------------------------------------------|
| days |                       |                                                 |                                                                           |
| 1   | 6                     | 0.042 ± 0.001                                   | 0.20 ± 0.01                                                               |
| 3   | 4                     | 0.28 ± 0.02                                    | 7.5 ± 0.24                                                                |
| 8   | 4                     | 0.70 ± 0.03                                    | 10.6 ± 0.37                                                               |
| 11  | 2                     | 0.75                                            | 13.4                                                                      |
| 10, fasted 2 | 3                 | 0.41 ± 0.06                                    | 4.4 ± 0.02                                                               |

Fig. 6. Polyacrylamide gel electrophoresis of the radioactive immunoprecipitate. Migration of the proteins proceeded from left to right. A partially purified liver extract (Fraction II) was prepared from 3-day-old chicks which had been injected with \([^3H]\)leucine 3 hours prior to sacrifice. One milliliter of diluted extract, containing 0.4 units of malic enzyme (specific activity, 0.35 units per mg of protein), was mixed with 5 mg of anti-malic enzyme immunoglobulin and the immunoprecipitate (a) was collected. One milliliter of the same diluted extract was supplemented with 1.2 units of nonradioactive purified malic enzyme (Fraction VII, specific activity, 25 units per mg of protein) and was reacted with 5 mg of anti-malic enzyme immunoglobulin to give immunoprecipitate (b). The quantity of precipitate formed was approximately the same in both cases. The immunoprecipitates were collected, washed, dissociated with acetic acid, and subjected to polyacrylamide gel electrophoresis in the presence of SDS as described under "Experimental Procedure." The inset represents a separate gel on which purified malic enzyme (Fraction VII) had been electrophoresed after treatment with 1 M acetic acid, SDS, and β-mercaptoethanol.

### Notes

- In which nonradioactive enzyme is added to the sample after the original antibody-antigen precipitate has been removed. The nonradioactive enzyme is then precipitated by an amount of antibody equivalent to that used in the first precipitation. A second correction for nonspecific precipitation is determined by counting the precipitate formed by adding nonimmune serum to a duplicate of each sample. The sum of counts in the second precipitate and counts in the nonimmune precipitate is subtracted from the value for the first precipitate. The difference is taken to represent incorporation into the specific antigen being studied.

In order to increase our confidence that the radioactive precipitated by immunoglobulin was pure malic enzyme, we dissociated the antibody-antigen precipitate in 1 \text{M} \text{acetic acid} and separated the component proteins by polyacrylamide gel electrophoresis in the presence of SDS. Four major radioactive peaks were present (Fig. 6a). Pure malic enzyme which underwent electrophoresis on a separate gel migrated to the same position as the peak at Fraction 20 (Fig. 6a). When excess unlabeled enzyme was added prior to immunoprecipitation, only the peak at Fraction 20 was displaced, thus identifying it as malic enzyme (Fig. 6b). The possibility remained that the other peaks represented proteins which could be cleaved to form active malic enzyme. In the above experiments 3-day-old birds were killed 3 hours after administration of the \([^3H]\)leucine. The relative distribution of radioactivity among the major peaks remained the same in another series of experiments in which the birds were killed 6 hours after administration of the leucine. This suggested that the nonmalic enzyme peaks were not precursors of malic enzyme. The size and distribution of the nonmalic enzyme peaks varied somewhat from experiment to experiment suggesting that they were nonspecific in nature.

Measurements of radioactivity in immunoprecipitable malic enzyme by the SDS electrophoresis method and the nonspecific adsorption method described above are compared in Table III. Recovery of radioactivity from the gel was 98% or more (compare Columns a and d, counting efficiency varied by less than 5%). In 3-day-old birds, actual incorporation into malic enzyme (Column e) was 71% of that calculated from the nonspecific adsorption method. In our experiments, we could not detect any precipitate when immunoglobulin prepared from unimmunized rabbits was added to the samples. Hence, that correction was not applied. The difference between the nonspecific adsorption and electrophoresis methods was even greater when Fraction II prepared from unfed, neonatal chicks was used. In this case incorporation into malic enzyme was only 25% of the value predicted by the nonspecific adsorption method. Attempts to reduce nonspecific adsorption by further purification of the malic enzyme in liver extracts from neonatal chicks were unsuccessful. In view of these results, in all experiments described below, we isolated radioactive malic enzyme from the antibody-antigen precipitate by SDS electrophoresis and counted only the peak corresponding to malic enzyme. Identity of the enzyme peak was frequently checked by competition with purified nonradioactive enzyme (as in Fig. 6).

### L-[4,5-3H]Leucine Incorporation Studies

Incorporation of leucine into total protein reached a maximum at about 1 hour after the isotope injection (Fig. 7). Radioactivity remaining in the free amino acid pool (counts soluble in trichloroacetic acid) decreased to a minimum at 1 hour (Fig. 7). The quantity of radioactivity actually in leucine was determined by subjecting...
secretion of protein is an important function of the liver, we did not appear to be a first order reaction (Fig. 9). Since radiation of total liver protein was difficult to estimate because of total protein, we eliminated this factor in the interpretation of the measurements of malic enzyme synthesis. Degradation increased leucine pool size of the fed birds. By expressing the results for synthesis of malic enzyme relative to synthesis of total protein (2s22), we estimated to free leucine was barely detectable (Fig. 7). The time course shown in Fig. 7 was obtained with chicks which had been fed for 2 days. Similar patterns were obtained with unfed, neonatal chicks. Therefore, we selected a 1-hour incorporation period to estimate synthesis of malic enzyme and 1-, 24, and 48-hour periods to estimate degradation.

When neonatal chicks were fed there was a rapid increase in the rate of synthesis of malic enzyme relative to the synthesis of total protein (Table II). The 54-fold increase in the rate of synthesis was comparable to the 63-fold increase in total activity of the enzyme, suggesting that increased synthesis played a major role in increasing the concentration of malic enzyme. This finding was confirmed when the degradation of malic enzyme was investigated (Fig. 8). Degradation of malic enzyme was barely detectable in unfed, neonatal chicks. After the chicks had been fed for 7 or 10 days the rate of degradation increased, a phenomenon which would tend to oppose the observed increase in activity. The $t_1$ for degradation of malic enzyme in neonatal chicks was estimated to be 350 hours. In 8- and 11-day-old chicks, the $t_1$ was about 55 hours.

Incorporation of [3H]leucine into total liver protein (per bird) decreased by about 40% during the feeding experiment (Fig. 8). This decrease was probably due to the increased body size and hence increased leucine pool size of the fed birds. By expressing the results for synthesis of malic enzyme relative to synthesis of total protein (2s22), we eliminated this factor in the interpretation of the measurements of malic enzyme synthesis. Degradation of total liver protein was difficult to estimate because it did not appear to be a first order reaction (Fig. 9). Since secretion of protein is an important function of the liver, we estimated to free leucine was barely detectable (Fig. 7). The time course shown in Fig. 7 was obtained with chicks which had been fed for 2 days. Similar patterns were obtained with unfed, neonatal chicks. Therefore, we selected a 1-hour incorporation period to estimate synthesis of malic enzyme and 1-, 24, and 48-hour periods to estimate degradation.

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Incorporation of [3H]leucine into total liver protein (per bird) decreased by about 40% during the feeding experiment (Fig. 8). This decrease was probably due to the increased body size and hence increased leucine pool size of the fed birds. By expressing the results for synthesis of malic enzyme relative to synthesis of total protein (2s22), we eliminated this factor in the interpretation of the measurements of malic enzyme synthesis. Degradation of total liver protein was difficult to estimate because it did not appear to be a first order reaction (Fig. 9). Since secretion of protein is an important function of the liver, we estimated to free leucine was barely detectable (Fig. 7). The time course shown in Fig. 7 was obtained with chicks which had been fed for 2 days. Similar patterns were obtained with unfed, neonatal chicks. Therefore, we selected a 1-hour incorporation period to estimate synthesis of malic enzyme and 1-, 24, and 48-hour periods to estimate degradation.

When neonatal chicks were fed there was a rapid increase in the rate of synthesis of malic enzyme relative to the synthesis of total protein (Table II). The 54-fold increase in the rate of synthesis was comparable to the 63-fold increase in total activity of the enzyme, suggesting that increased synthesis played a major role in increasing the concentration of malic enzyme. This finding was confirmed when the degradation of malic enzyme was investigated (Fig. 8). Degradation of malic enzyme was barely detectable in unfed, neonatal chicks. After the chicks had been fed for 7 or 10 days the rate of degradation increased, a phenomenon which would tend to oppose the observed increase in activity. The $t_1$ for degradation of malic enzyme in neonatal chicks was estimated to be 350 hours. In 8- and 11-day-old chicks, the $t_1$ was about 55 hours.
killed 30 min after the injection of acetate. Livers were removed and frozen in solid CO₂. Total fatty acids were extracted and determined as described under "Experimental Procedure." The radioactivity measured as previously described (4). In the malic enzyme synthesis experiments, the birds were killed 60 min after the leucine injection. The livers were removed and homogenized and incorporation into total protein and malic enzyme de-

In the fatty acid synthesis experiments the chicks were fed for 7 to 10 days, suggesting that the increased rate of degradation of malic enzyme which occurred with feeding was not an artifact due to isotope reutilization in unfed, neonatal chicks nor was it simply a reflection of changes occurring over of total liver protein was very similar in unfed, neonatal chicks and in the g-day-old chicks. By means of the enzyme activities actually measured (0.28 and 25 units per mg fat-free dry wt) suggest that the initial high rate of degradation of total protein may represent secretion of blood proteins. In any event, turnover of total liver protein was very similar in unfed, neonatal chicks or chicks fed for 7 to 10 days, suggesting that the increased rate of degradation of malic enzyme which occurred with feeding was not an artifact due to isotope reutilization in unfed, neonatal chicks nor was it simply a reflection of changes occurring in all liver proteins.

Synthesis also played a major role in the regulation of malic enzyme concentration during starvation. Chicks (10 days old) which had been fasted for 2 days had an enzyme activity which was about one-half that of normally fed controls (Table II). Relative synthesis of malic enzyme was inhibited by about 60% by fasting (Table II). The rate of degradation of enzyme was increased by starvation (Fig. 8), but the increased rate of degradation was accompanied by an increase in the degradation of all liver protein (Fig. 9). Part of the increased degradation of malic enzyme, therefore, was due to a general increase in the breakdown of liver protein.

**TABLE IV**

| Hours after glucose meal | Fatty acid synthesis (10⁻² × %[¹⁴C]acetate incorporated per mg fat-free dry wt) | Malic enzyme |
|-------------------------|--------------------------------------------------------------------------------|--------------|
|                         | Enzyme activity (units/mg Fraction II protein) | Relative synthesis (dpm in malic enzyme per 10,000 dpm in total protein) |
| 0                       | 0.06 ± 0.02 (7) | 0.012 ± 0.001 | 0.25 ± 0.01 (6) |
| 0.5                     | 0.10 ± 0.02 (7) | 0.014 ± 0.002 | 0.27 ± 0.04 (4) |
| 3                       | 0.42 ± 0.16 (8) | 0.024 ± 0.002 | 0.05 ± 0.06 (4) |
| 6                       | 0.21 ± 0.03 (3) | 0.014 ± 0.001 | 0.27 ± 0.04 (4) |

suggest that the initial high rate of degradation of total protein may represent secretion of blood proteins. In any event, turnover of total liver protein was very similar in unfed, neonatal chicks or chicks fed for 7 to 10 days, suggesting that the increased rate of degradation of malic enzyme which occurred with feeding was not an artifact due to isotope reutilization in unfed, neonatal chicks nor was it simply a reflection of changes occurring in all liver proteins.

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**DISCUSSION**

Total activity of malic enzyme in the livers of neonatal and growing chicks was regulated almost exclusively by regulating the concentration of the enzyme. Concentration, in turn, was regulated by varying the rate of synthesis. Activation or inhibition of existing malic enzyme has been virtually eliminated as a possible explanation for the observed changes in total enzyme activity. However, this conclusion is subject to the reservation that neonatal chick liver does not contain a form of malic enzyme which is enzymatically inactive and immunologically unreactive. To our knowledge, no such form of malic enzyme has been described or postulated.

Degradation of malic enzyme appeared to play a minor role in controlling the concentration of malic enzyme. The rate of degradation (τₙ) for liver malic enzyme was 55 hours in normally fed birds. This rate of degradation is much slower than degradation rates of enzymes such as tryptophan pyrrolase (τₙ = 24 hours) or tyrosine aminotransferase (τₙ = 2 hours) (20, 24) but about the same as that reported for acetyl-CoA carboxylase in fed rats (τₙ = 48 to 59 hours) (22, 25). The degradation of acetyl-CoA carboxylase is increased by starvation (τₙ = 18 to 30 hours) (22, 25) but neither Majorus and Killburn nor Nakanishi and Numa (22, 25) indicated whether or not this was specific for malic enzyme or general for all liver proteins. Malic enzyme in starved chicks was degraded with a τₙ of 28 hours but much of the increased breakdown was due to the increased rate of breakdown of all liver proteins. The extremely long half-life of malic enzyme found in the liver of unfed, neonatal chicks (τₙ = 330 hours) is grossly similar to the finding that arginase is not degraded in liver from fasted rats (10). However, the biological significance of such a low turnover rate for malic enzyme is difficult to assess. It may represent a means of conserving energy during embryonic life, as the potential for adaptive regulation of this enzyme would appear to be of little functional value to the embryo.

Under steady state conditions it is possible to calculate a rate constant for enzyme synthesis (26). In our experiments, steady state conditions were approximated in the unfed, neonatal chicks and in the 8-day-old chicks. By means of the enzyme activities actually measured (0.28 and 25 units per liver for neo-
natal and 8-day-old chicks, respectively) it is possible to calculate the synthetic rates for the two nutritional states from the expression \( k_s = k_d E \), where \( E \) is the content of enzyme per chick, \( k_d \) is the first order rate constant for enzyme degradation as time\(^{-1}\), and \( k_s \) is the rate of enzyme synthesis per chick. Since, as shown in Fig. 5, the enzyme activity measured accurately reflects the enzyme content, the activities obtained were converted to amount of enzyme, assuming a specific activity of 28 units per mg of homogeneous enzyme (Table I). In the unfed, neonatal chick the absolute rate of synthesis was 0.014 \( \mu \)g of malic enzyme per hour per chick. In the 8-day-old chicks, the calculated rate was 8.2 \( \mu \)g per hour per chick. Hence, the absolute rate of synthesis was increased by about 500-fold. The difference between the increase in synthesis calculated from absolute and relative values was due to an increase in the rate of degradation of malic enzyme and an increase in liver size and to possible differences in the absolute rate of synthesis of total protein in the two types of chicks. As noted previously (25), this model is undoubtedly a highly simplified one; however, it does allow a crude estimation of the rate constant for synthesis. The total activity of malic enzyme is not limiting for fatty acid synthesis in chick liver (5, 6). In unfed, neonatal chicks the maximum velocity of the enzyme exceeds by \( 1 \times 10^4 \) the observed rate of incorporation of acetate or glucose carbon into fatty acids (23). Even 6 hours after a glucose meal, the differential is \( 1 \times 10^9 \) (Table IV) (23). However, the maximum velocity for malic enzyme in neonatal chick liver would not be sufficient to supply all of the NADPH required for fatty acid synthesis in the livers of week-old birds (4). Hence, if malic enzyme provides a large fraction of the NADPH during fatty acid synthesis, an increase in the total activity of the enzyme must occur. We postulate that the increased synthesis of malic enzyme was initiated by the increased flux of glucose to fatty acids which accompanied normal ad libitum feeding (4, 5) or glucose feeding (Table IV) (23). Consistent with this idea is the lag of 2 or more hours which occurred between the increase in fatty acid synthesis and the increase in malic enzyme synthesis when unfed chicks were given a glucose meal (Table IV). The concentration of many of the intermediates of carbohydrate and fat metabolism are altered dramatically when the liver changes from a primarily gluconeogenic function in the embryo to glycolytic and lipogenic functions in the fed chick (4–6). One or more of these intermediates may be an inducer or corepressor of malic enzyme synthesis.

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