The Effect of Starvation and Starvation Followed by Feeding on Enzyme Activity and the Metabolism of [U-¹⁴C]Glucose in Liver from Growing Chicks

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1. The conversion of [U-¹⁴C]glucose into carbon dioxide, cholesterol and fatty acids in liver slices and the activities of 'malic' enzyme, citrate-cleavage enzyme, NADP-linked isocitrate dehydrogenase and hexose monophosphate-shunt dehydrogenases in the soluble fraction of homogenates of liver were measured in chicks that were starved or starved then fed. 2. In newly hatched chicks the incorporation of [U-¹⁴C]glucose and the activity of 'malic' enzyme did not increase unless the birds were fed. The response to feeding of [U-¹⁴C]glucose incorporation into fatty acids increased as the starved chicks grew older. 3. Citrate-cleavage enzyme activity increased slowly even when the newly hatched chicks were unfed. On feeding, citrate-cleavage enzyme activity increased at a much faster rate. 4. In normally fed 20-day-old chicks starvation decreased the incorporation of [U-¹⁴C]glucose into all three end products and depressed the activities of 'malic' enzyme and citrate-cleavage enzyme. Re-feeding increased all of these processes to normal or higher-than-normal levels. 5. In both newly hatched and 20-day-old chicks starvation increased the activity of isocitrate dehydrogenase and feeding or re-feeding decreased it. 6. Very little change in hexose monophosphate-shunt dehydrogenase activity was observed during the dietary manipulations. 7. The results indicate that increased substrate delivery to the liver is the principal stimulus to the increased rate of glucose metabolism observed in newly hatched chicks. The results also suggest that changes in the activities of 'malic' enzyme and citrate-cleavage enzyme are secondary to an increased flow of metabolites through the glucose-to-fatty acid pathway and that the dehydrogenases of the hexose monophosphate shunt play a minor role in NADPH production for fatty acid synthesis.

The dietary transition from the high-fat diet of embryonic yolk to the high-carbohydrate diet of mash that occurs when the chick hatches is accompanied by a very large and prompt increase in hepatic lipogenesis and in the activities of certain enzymes concerned in fatty acid synthesis (Goodridge, 1968*a,b*). In the present study the effects of starvation and starvation followed by feeding were studied in newly hatched chicks and normally fed 20-day-old chicks.

MATERIALS AND METHODS

Care and handling of the chicks, procedures for killing the chicks and preparing the tissues, incubation and analytical procedures for the isotope experiments, methods

of enzyme assay, procurement of chemicals and statistical analyses were as described in the preceding papers (Goodridge, 1968*a,b*).

RESULTS

[U-¹⁴C]Glucose incorporation into liver fatty acids

The effects of starvation and feeding on the rate of fatty acid synthesis in liver slices from newly hatched chicks are shown in Table 1. If the chicks were not fed for 3 days after hatching the rate of fatty acid synthesis showed essentially no change. In contrast, a dramatic increase (approx. 1000-fold) occurred by the seventh day if feeding was begun after an initial 1 day of starvation (Goodridge, 1968*a*). If the chicks were initially starved for 3 days, the increase in fatty acid synthesis resulting from the first day of feeding was much greater than in chicks fed for 1 day after 1 day of starvation.

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Table 1. *Incorporation by liver slices of [U-¹⁴C]glucose into total fatty acids*

The techniques used are described by Goodridge (1968a). Results are expressed as means $\pm$ s.e.m. of the numbers of experiments given in parentheses.

		Glucose incorporated (m μ g.atoms of C/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Days fed					
Newly hatched	0	0.64 $\pm$ 0.11 (12)	0.42 $\pm$ 0.04 (6)	0.45 $\pm$ 0.05 (4)	—
	1	—	23 $\pm$ 8.0 (6)	92 $\pm$ 53 (6)	280 $\pm$ 50 (6)
	3	—	270 $\pm$ 60 (6)	480 $\pm$ 160 (6)	—
	7	—	440 $\pm$ 90 (7)	980 $\pm$ 90 (5)	—
	10	—	390 $\pm$ 60 (7)	—	—
20-day-old	0	700 $\pm$ 95 (10)*	45 $\pm$ 14 (8)	1.7 $\pm$ 0.4 (7)	0.49 $\pm$ 0.04 (4)
	1	—	—	1110 $\pm$ 140 (4)	560 $\pm$ 160 (4)
	3	—	—	1810 $\pm$ 140 (6)	2040 $\pm$ 130 (8)
	5	490 $\pm$ 80 (4)†	—	1210 $\pm$ 120 (7)	—

* Includes some values for 21-day-old chicks.

† These birds were actually 28–30 days old.

Table 2. *Incorporation by liver slices of [U-¹⁴C]glucose into carbon dioxide*

The techniques used are described by Goodridge (1968a). Results are expressed as means $\pm$ s.e.m. of the numbers of experiments given in parentheses in Table 1.

		Glucose incorporated (m μ g.atoms of C/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Days fed					
Newly hatched	0	170 $\pm$ 10	110 $\pm$ 6	62 $\pm$ 4	—
	1	—	620 $\pm$ 80	800 $\pm$ 130	830 $\pm$ 50
	3	—	1070 $\pm$ 70	1050 $\pm$ 130	—
	7	—	1310 $\pm$ 60	1430 $\pm$ 90	—
	10	—	1120 $\pm$ 70	—	—
20-day-old	0	1260 $\pm$ 100*	750 $\pm$ 70	220 $\pm$ 30	120 $\pm$ 20
	1	—	—	1400 $\pm$ 150	1170 $\pm$ 170
	3	—	—	2120 $\pm$ 70	2150 $\pm$ 70
	5	1130 $\pm$ 80†	—	1610 $\pm$ 90	—

* Includes some values for 21-day-old chicks.

† These birds were actually 28–30 days old.

After 5 days of starvation the response to a day's feeding was greater still. During succeeding days of feeding the difference between the response of the 1-day-starved and 3-day-starved chicks decreased, but, even after 3 and 7 days of feeding, those initially starved for 3 days had rates of synthesis approximately double those of chicks whose feeding was begun after 1 day of starvation.

The rate of hepatic fatty acid synthesis is very high in normally fed 20-day-old chicks (Goodridge, 1968a). In Table 1 this rate is given as 700 m μ g.atoms of glucose C incorporated/mg. of N/hr. After starvation for 1, 3 and 5 days this rate declined to 45, 1.7 and 0.49 m μ g.atoms of glucose C incorporated/mg. of N/hr. respectively, i.e. about 6,

0.24 and 0.07% of the value for normally fed chicks. One day of feeding after 3 days of starvation raised the rate to 1110 m μ g.atoms of glucose C incorporated/mg. of N/hr., whereas a day of feeding after 5 days of starvation was only half as effective, raising the rate to 560 m μ g.atoms of glucose C incorporated/mg. of N/hr. After 3 days of re-feeding, however, the rates of fatty acid synthesis were similar for birds initially starved 3 or 5 days and about three times the rate in normally fed chicks. After 5 days of re-feeding the rate began to return towards normal.

Unlike the rate of fatty acid synthesis, the rate of glucose oxidation by liver slices from newly hatched chicks (Table 2) decreased significantly and

Table 3. *Incorporation by liver slices of [U-¹⁴C]glucose into cholesterol*

The techniques used are described by Goodridge (1968a). Results are expressed as means $\pm$ S.E.M. of the numbers of experiments given in the parentheses in Table 1.

		Glucose incorporated (mμg.atoms of C/mg. of N/hr.)				
Days starved before feeding	Chicks	...	0	1	3	5
Days fed						
Newly hatched	0	0.10 ± 0.03	0.03 ± 0.01	0.07 ± 0.02	—	—
	1	—	0.17 ± 0.03	3.6 ± 0.5	0.27 ± 0.03	—
	3	—	0.14 ± 0.04	1.4 ± 0.9	—	—
	7	—	8.8 ± 3.6	24 ± 3	—	—
	10	—	11.0 ± 2.3	—	—	—
20-day-old	0	33 ± 5*	7.7 ± 1.0	0.38 ± 0.11	0.05 ± 0.01	—
	1	—	—	14 ± 2	15 ± 4	—
	3	—	—	40 ± 6	31 ± 5	—
	5	33 ± 4†	—	43 ± 3	—	—

* Includes some values for 21-day-old chicks.

† These birds were actually 28–30 days old.

progressively on starvation. Feeding caused a prompt increase in glucose oxidation. The duration of the period of starvation had no significant effect on the rate of glucose oxidation attained after 1 day's feeding. Starvation also inhibited glucose oxidation in birds starved after 19 days of normal feeding. After 1, 3 and 5 days of starvation, the rate decreased to 60, 17 and 9.5% respectively of that in normally fed chicks. Re-feeding for 1 day of birds previously starved for 3 or 5 days increased the rate of glucose oxidation to about 1200–1400 m μ g.atoms of glucose C incorporated/mg. of N/hr. After 3 days of re-feeding the rate of glucose oxidation had increased to almost twice the normal rate in birds that had been starved for 3 or 5 days. A return towards the normal rate occurred in birds starved for 3 days and re-fed for 5 days.

The response of cholesterol synthesis to starvation and feeding (Table 3) was similar to that of fatty acid synthesis in the older chicks but not in the newly hatched chicks. There was only a small increase in the rate of cholesterol synthesis when 1-day-old chicks were fed, the major increase occurring after the chicks were 5 days old (Goodridge, 1968a). When the birds were fed after starving for 3 days, the pattern of change in cholesterol synthesis was similar to that occurring after 1 day of starvation except that the rates were much higher. After 5 days of starvation, however, the response to 1 day's feeding (0.27 $\pm$ 0.03 m μ g.atom of glucose C incorporated/mg. of N/hr.) was similar to that after 1 day of starvation (0.17 $\pm$ 0.03 m μ g.atom of glucose C incorporated/mg. of N/hr.). The data suggest a transient peak in the responsiveness of cholesterol synthesis to feeding.

After 1, 3 and 5 days of starvation the rate of hepatic cholesterol synthesis in 20-day-old chicks

decreased to 23, 1.1 and 0.15% respectively of the rate in normally fed chicks (Table 3). The rate after 1 day's feeding was the same for birds starved 3 or 5 days before re-feeding. After 3 days of re-feeding the rate of cholesterol synthesis was restored to normal. No increase over the values for normally fed birds was noted for any of the starvation–re-feeding regimens.

Liver enzyme activities. The activity of liver 'malic' enzyme [L-malate–NADP oxidoreductase (decarboxylating), EC 1.1.1.40] in newly hatched chicks responded to starvation and feeding (Table 4) in a manner almost identical with that of fatty acid synthesis (Table 1). Starving for 3 or 5 days caused no marked change in activity, but there was a prompt increase in activity when the chicks were fed. The response to feeding for 24 hr. became greater as the length of the prior period of starvation increased, as observed for fatty acid synthesis. The difference between responses to feeding after 1 and 5 days of starvation was significant ($P=0.05$). When the birds had been fed for more than 1 day, the difference in response due to length of the period of starvation disappeared.

When normally fed 20-day-old chicks were starved and re-fed, the changes in 'malic' enzyme activity (Table 4) and the rate of fatty acid synthesis (Table 1) were similar in direction but very different in magnitude. Thus starvation for 1 day caused a 17% decrease in 'malic' enzyme activity ($P=0.05$) and a 94% decrease in fatty acid synthesis. After 3 and 5 days of starvation 'malic' enzyme activity had decreased to 30 and 22% and fatty acid synthesis to 0.2 and 0.07% of normal respectively. After 5 days of starvation, 'malic' enzyme activity was still more than 16 times that in unfed newly hatched chicks, whereas the rate of fatty acid

Table 4. 'Malic' enzyme activity in chick liver

The techniques used are described by Goodridge (1968b). Results are expressed as means $\pm$ s.e.m. of the numbers of experiments in parentheses.

		Enzyme activity (μ moles of NADPH formed/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Newly hatched	0	0.80 $\pm$ 0.04 (4)	0.80 $\pm$ 0.07 (8)	0.77 $\pm$ 0.06 (4)	1.07 $\pm$ 0.05 (6)
	1	—	4.7 $\pm$ 1.0 (11)	7.1 $\pm$ 1.4 (6)	11.4 $\pm$ 2.4 (6)
	3	—	30 $\pm$ 2 (10)	33 $\pm$ 4 (6)	—
	7	—	67 $\pm$ 3 (7)	58 $\pm$ 1 (5)	—
	10	—	63 $\pm$ 8 (3)	—	—
20-day-old	0	64 $\pm$ 3 (10)	53 $\pm$ 2 (4)	19 $\pm$ 2 (4)	14 $\pm$ 1 (4)
	1	—	—	37 $\pm$ 2 (4)	33 $\pm$ 2 (4)
	3	65 $\pm$ 3 (4)	—	68 $\pm$ 5 (6)	84 $\pm$ 5 (8)
	5	67 $\pm$ 4 (7)*	—	71 $\pm$ 4 (7)	—

* These birds were actually 26 days old.

Table 5. Citrate-cleavage enzyme activity in chick liver

The techniques used are described by Goodridge (1968b). Results are expressed as means $\pm$ s.e.m. of the numbers of experiments in parentheses in Table 4.

		Enzyme activity (μ moles of citrate cleaved/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Newly hatched	0	1.4 $\pm$ 0.2	2.6 $\pm$ 0.2	3.9 $\pm$ 0.2	7.8 $\pm$ 1.1
	1	—	11 $\pm$ 2	13 $\pm$ 1	15 $\pm$ 1
	3	—	18 $\pm$ 3	17 $\pm$ 1	—
	7	—	22 $\pm$ 2	22 $\pm$ 2	—
	10	—	23 $\pm$ 6	—	—
20-day-old	0	17 $\pm$ 0.6	11 $\pm$ 2	4.9 $\pm$ 0.2	3.1 $\pm$ 0.2
	1	—	—	16 $\pm$ 2	17 $\pm$ 1
	3	23 $\pm$ 2	—	28 $\pm$ 2	34 $\pm$ 2
	5	19 $\pm$ 2*	—	25 $\pm$ 2	—

* These birds were actually 26 days old.

synthesis was the same in livers from unfed newly hatched chicks and 5-day-starved 25-day-old chicks. Re-feeding resulted in a slightly higher than normal activity for 'malic' enzyme. This was significant only for birds previously starved for 5 days ($P < 0.02$).

In an experiment not shown in the Tables, 3-day-old normally fed chicks were starved for 5 days. Initially 'malic' enzyme activity was $25 \pm 3 \mu$ moles/mg. of N/hr. or less than one-third of the activity attained after a week of normal feeding (Goodridge, 1968b). After starvation the activity was 20% of the value for fed chicks but still more than six times the activity in livers from unfed newly hatched chicks. Fatty acid synthesis showed a decrease to 0.5% of the rate in livers from unstarved chicks. Therefore no matter whether the activity for unstarved chicks was 65 or 25μ moles/

mg. of N/hr. starvation for 5 days caused an 80% decrease in 'malic' enzyme activity.

The effect of starvation and starvation followed by feeding on the activity of citrate-cleavage enzyme [ATP-citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating), EC 4.1.3.8] in newly hatched chicks (Table 5) was somewhat different from that observed with 'malic' enzyme and fatty acid synthesis. Starvation of newly hatched chicks caused an increase in citrate-cleavage enzyme activity that amounted to more than fivefold after 5 days of starvation. Feeding after starvation always caused a further significant increase in activity. Though the activity of citrate-cleavage enzyme after 1 day of feeding was slightly, but not significantly, greater as the length of the prior period of starvation was increased, the increment of increase in activity after 1 day's feeding actually

Table 6. *HMP-shunt dehydrogenase activity in chick liver*

The techniques used are described by Goodridge (1968*b*). Results are expressed as means $\pm$ S.E.M. of the number of experiments in parentheses in Table 4 except as noted in parentheses in this Table.

		Enzyme activity (μ moles of NADPH formed/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Chicks	Days fed				
Newly hatched	0	6.0 $\pm$ 0.4	4.9 $\pm$ 0.3	4.2 $\pm$ 0.3	3.9 $\pm$ 0.1
	1	—	6.1 $\pm$ 0.2 (8)	7.1 $\pm$ 1.4	5.8 $\pm$ 0.5
	3	—	7.2 $\pm$ 0.2 (4)	5.9 $\pm$ 0.3	—
	7	—	4.6 $\pm$ 0.2 (4)	5.2 $\pm$ 0.1	—
	11	—	6.7 $\pm$ 0.3 (4)	—	—
20-day-old	0	4.8 $\pm$ 0.1	4.9 $\pm$ 0.3	3.9 $\pm$ 0.3	3.7 $\pm$ 0.1
	1	—	—	4.7 $\pm$ 0.3	5.0 $\pm$ 0.3
	3	5.6 $\pm$ 0.2	—	5.1 $\pm$ 0.3	6.3 $\pm$ 0.2
	5	5.3 $\pm$ 0.2*	—	5.9 $\pm$ 0.2	—

* These birds were actually 26 days old.

Table 7. *Isocitrate dehydrogenase activity in chick liver*

The techniques used are described by Goodridge (1968*b*). Results are expressed as means $\pm$ S.E.M. of the numbers of experiments in parentheses in Table 4 except as noted in parentheses in this Table.

		Enzyme activity (μ moles of NADPH formed/mg. of N/hr.)			
Days starved before feeding ...	Chicks	0	1	3	5
Chicks	Days fed				
Newly hatched	0	170 $\pm$ 7	160 $\pm$ 10	190 $\pm$ 10	250 $\pm$ 20
	1	—	150 $\pm$ 8 (8)	160 $\pm$ 3	170 $\pm$ 8
	3	—	120 $\pm$ 5 (4)	120 $\pm$ 7	—
	7	—	83 $\pm$ 2 (4)	89 $\pm$ 4	—
	11	—	62 $\pm$ 3 (4)	—	—
20-day-old	0	75 $\pm$ 3	99 $\pm$ 6	110 $\pm$ 5	130 $\pm$ 8
	1	—	—	100 $\pm$ 10	140 $\pm$ 5
	3	63 $\pm$ 6	—	110 $\pm$ 7	100 $\pm$ 3
	5	76 $\pm$ 5*	—	85 $\pm$ 5	—

* These birds were actually 26 days old.

decreased as the chicks grew older. Re-feeding for more than 1 day had the same effect in birds previously starved 1 or 3 days.

In 20-day-old chicks the effects of starvation and starvation followed by feeding on citrate-cleavage enzyme activity were similar to those on 'malic' enzyme activity. The activities of both enzymes were decreased almost identically on starvation (65, 29 and 18% of normal after 1, 3 and 5 days of starvation for citrate-cleavage enzyme) and re-feeding resulted in higher than normal activities. On re-feeding the difference with respect to the normal activity in 20-day-old chicks was significant for birds previously starved both 3 and 5 days and re-fed for 3 days ($P < 0.002$).

Hepatic HMP-shunt* dehydrogenase [D-glucose 6-phosphate-NADP oxidoreductase (EC 1.1.1.49)

* Abbreviation: HMP shunt, hexose monophosphate shunt.

plus 6-phospho-D-gluconate-NADP oxidoreductase (decarboxylating) (EC 1.1.1.44)] activity was little affected by starvation or starvation followed by feeding (Table 6). When newly hatched chicks were starved for 5 days, activity decreased by only 35% ($P = 0.01$). Feeding for 1 day caused a small increase in activity, which was significant after 1 or 5 days of starvation ($P = 0.004$ and $P = 0.002$ respectively) but not after 3 days of starvation ($P > 0.05$). Continued feeding of birds previously starved from hatching caused small and variable changes in activity. Starvation also caused a small decrease in HMP-shunt dehydrogenase activity in chicks that had been previously fed for 19 days (20 days old). The activity after 5 days of starving was 77% of that in normally fed chicks ($P = 0.002$). Re-feeding caused small but significant increases in activity ($P < 0.002$).

Changes in the activity of soluble NADP-linked

Table 8. *Liver nitrogen content*Results expressed as means $\pm$ s.e.m. of the numbers of experiments given in parentheses.

		Liver N content (mg. of N/100mg. wet wt.)				
Days starved before feeding	Chicks	Days fed	... 0	1	3	5
Newly hatched		0	2.58±0.04 (16)	2.95±0.05 (14)	3.14±0.10 (4)	2.81±0.10 (6)
		1	—	2.44±0.06 (17)	2.55±0.05 (6)	2.09±0.06 (6)
		3	—	2.48±0.07 (10)	2.68±0.08 (6)	—
		7	—	2.55±0.08 (7)	2.90±0.03 (5)	—
		10	—	2.92±0.10 (7)	—	—
20-day-old		0	2.87±0.05 (17)*	3.18±0.09 (8)	3.33±0.06 (7)	3.35±0.08 (4)
		1	—	—	2.25±0.13 (4)	2.37±0.14 (4)
		3	2.89±0.06 (4)	—	2.58±0.10 (6)	2.49±0.12 (8)
		5	2.93±0.04 (4)†	—	2.88±0.05 (7)	—

* Includes some values for 21-day-old chicks.

† These birds were actually 28–30 days old.

isocitrate dehydrogenase [*threo*-D₈-isocitrate–NADP oxidoreductase (decarboxylating) (EC 1.1.1.42)] activity (Table 7) in chick liver caused by starvation and feeding are considerably different from those observed for 'malic' enzyme or citrate-cleavage enzyme. When newly hatched chicks were starved for 5 days the activity of isocitrate dehydrogenase increased by almost 50% ($P=0.01$). Feeding the chicks caused a decrease in activity. The length of the previous period of starvation had no significant effect on this decrease. The effects of starvation and starvation followed by re-feeding in 20-day-old chicks were similar to those observed in newly hatched chicks. One day of starvation raised the activity by slightly more than 30% ($P<0.02$); after 5 days of starvation it was increased by almost 75% ($P<0.002$). Though re-feeding for 1 day had no effect, re-feeding 3-day-starved birds for 5 days significantly decreased ($P<0.02$) the isocitrate dehydrogenase activity, though it was still higher than in normally fed birds ($P<0.002$). A significant decrease in activity occurred in birds re-fed for 3 days after starvation for 5 days ($P<0.02$).

Effect of liver nitrogen concentration. The general pattern of the results described here would be the same if the data were expressed on a liver-wet-weight or a body-weight basis. The nitrogen content of the liver increased from about 2.4 to 2.9 mg./100 mg. wet wt. as the normally fed chicks grew older (Table 8). In general, starvation caused an increase in liver nitrogen content. After 1 day's feeding the nitrogen content decreased to lower than normal values; continued re-feeding resulted in a return to normal values.

DISCUSSION

The increased rate of hepatic fatty acid synthesis that accompanies re-feeding of a starved animal is

clearly related to the restoration of normal body weight, and one might therefore expect that the response would increase as the length of the period of starvation increased. The response should be considered in terms of absolute rates attained because it is this that will determine how fast new fat is made. The increased responsiveness with increasing length of the period of starvation could be achieved by progressively higher rates of fatty acid synthesis or by prolongation of the elevated rate of synthesis or both. Since food consumption (hence the stimulus to fatty acid synthesis) is probably maximal immediately after starvation, the latter seems more likely. In any event, one would not expect the response to re-feeding to decrease as the period of starvation increased. Such was the case, however, with 20-day-old chicks that had been starved 3 or 5 days (Table 1). This suggests (1) the presence of inhibitors that modify the lipogenic response to re-feeding or (2) decreased amounts of an enzyme(s) that become rate-limiting after the rate of fatty acid synthesis has been stimulated by feeding. An enzyme that responded secondarily to changes in the flow of metabolites through the pathway would be a good candidate.

The increased responsiveness of hepatic fatty acid synthesis on re-feeding in newly hatched chicks that had been starved for increasingly longer periods could have several explanations. It may be related to the decrease in liver lipid content that occurs during the first week after hatching (Entenman, Lorenz & Chaikoff, 1940). Another possibility is that birds starved for longer periods eat more during the first day of feeding, though this was not reflected in an increased response of glucose oxidation. It is also possible that the capacity of the liver to respond to the stimulus of feeding develops during this period. In any event, the older starved chicks responded to 1 day's

feeding with a far greater increase in lipogenesis. Perhaps the response to feeding in newly hatched chicks represents the synthesis of new protein whereas in older starved chicks it represents activation of pre-existing protein (cf. Swanson, Curry & Anker, 1967; Fritz & Hsu, 1967).

The enzyme results provide further evidence that citrate-cleavage enzyme and 'malic' enzyme play a role in the synthesis of fatty acids from carbohydrate precursors (cf. Kornacker & Ball, 1965; Ball, 1966). Activity of these enzymes usually increased or decreased in liver at the same time as fatty acid synthesis was increasing or decreasing. The enzyme activity changes were probably secondary to changes in the rate of fatty acid synthesis, however, because they were much smaller in magnitude, particularly in the older chicks. Further, citrate-cleavage enzyme activity and fatty acid synthesis exhibited non-parallel behaviour in starved newly hatched chicks, fatty acid synthesis being unchanged or slightly decreased whereas citrate-cleavage enzyme activity increased very significantly. In addition, incorporation of [U-¹⁴C]-glucose into fatty acids in liver slices is significantly increased 3 hr. after feeding 1-day-old chicks, whereas 'malic' enzyme and citrate-cleavage enzyme activities are not increased even after 6 hr. of feeding (A. G. Goodridge, unpublished work).

The observed effects of starvation and feeding on the activity of isocitrate dehydrogenase in chick liver were unexpected. In rat liver, isocitrate dehydrogenase activity is not affected by starvation and re-feeding (Pande, Kahn & Venkatasubramanian, 1964; Young, Shrago & Lardy, 1964). 'Malic' enzyme, the other major soluble NADP-linked dehydrogenase in chick liver, was markedly affected by starvation and feeding, but the activity changes were opposite in direction to those of isocitrate dehydrogenase. Perhaps isocitrate dehydrogenase has a role in the synthesis of glucose from non-carbohydrate precursors.

The effects of starvation and starvation followed by re-feeding are very similar in 20-day-old chicks and rats. Starvation markedly inhibits fatty acid and cholesterol synthesis, whereas re-feeding increases fatty acid synthesis to greater than normal values and restores cholesterol synthesis to near normal values (Masoro, Chaikoff, Chernick & Felts, 1950; Wyshak & Chaikoff, 1953; Medes, Thomas & Weinhouse, 1952; Hutchens, Van Bruggen, Cockburn & West, 1954; Tepperman & Tepperman, 1958). One significant difference may be the greater inhibition of glucose oxidation caused by starvation in the chick (cf. Masoro *et al.* 1950; Wyshak & Chaikoff, 1953). Citrate-cleavage enzyme and 'malic' enzyme also show similar responses to starvation and re-feeding in the chick

and rat, and in the pigeon also. In the avian liver, however, citrate-cleavage and 'malic' enzyme activities return to near normal values when the birds are re-fed (Goodridge & Ball, 1966), whereas a very large 'overshoot' has been observed in rat liver after re-feeding (Kornacker & Lowenstein, 1965; Wise & Ball, 1964; Young *et al.* 1964; Pande *et al.* 1964). The less pronounced activity changes in chick and pigeon liver may be related to the much higher activities of these enzymes found in the avian livers (Goodridge & Ball, 1966; Goodridge, 1968b). In contrast with the situation in rat liver (Pande *et al.* 1964; Young *et al.* 1964), the HMP-shunt dehydrogenase activities of chick and pigeon liver are relatively unaffected by starvation and re-feeding (Goodridge & Ball, 1966), providing further evidence that these enzymes are not important suppliers of NADPH for fatty acid synthesis in avian liver (cf. Goodridge, 1968b).

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