with antibody ("precipitating") to IF. These studies did not resolve the precise location (outside or inside the cell) of the B12-^{57}Co in the ileal extracts.

Summary. Sequential incubation studies using everted sacs of guinea pig small intestine and vitamin B12 labeled with two different isotopes of cobalt, ^{60}Co and ^{57}Co, showed that IF-saturated with B12, could effect specific attachment of the complex to distal sacs. In a subsequent incubation IF could: (i) mediate in specific fashion (not exchange) the uptake of additional B12, (ii) interact with IF-"blocking" antibody, and (iii) be partially dissociated by EDTA from the sac (in a form biologically active for this system and retaining specificity of activity) leaving its B12 attached to the mucosa. These results were interpreted to indicate that, in this experimental system, IF is not absorbed into the intestinal cell during the B12 uptake that is mediated by intrinsic factor.

We wish to express our gratitude to Miss Barbara Peart for expert technical assistance and to Mrs. Elizabeth White for assistance with this manuscript.

1. Gulberg, R. and Kistner, S., Acta Med. Scand. **174**, 573 (1963).

2. Ardeman, S., Chanarin, I., and Berry V., Brit. J. Haematol. **11**, 11 (1965).

3. Cooper, B. A. and White, J. J., Brit. J. Haematol. **14**, 73 (1968).

4. Bernier, G. M. and Hines, J. D., New Engl. J. Med. **277**, 1968 (1967).

5. Gottlieb, C., Lau, K. S., Wasserman, L. R., and Herbert, V., Blood **25**, 875 (1965).

6. Anderson, B. B., J. Clin. Pathol. **17**, 14 (1964).

7. Abels, J., Veqter, J. J., Woldring, M. G., Jans, J. H., and Niewig, H. O., Acta Med. Scand. **165**, 105 (1959).

8. Strauss, E. W. and Wilson, T. H., Am. J. Physiol. **198**, 103 (1960).

9. Strauss, E. W. and Wilson, T. H., Proc. Soc. Exptl. Biol. Med. **99**, 224 (1958).

10. Herbert, V., J. Clin. Invest. **38**, 102 (1959).

11. Sullivan, L. W., Herbert, V., and Castle, W. B., J. Clin. Invest. **42**, 1943 (1963).

12. Cooper, B. A., Medicine **43**, 689 (1964).

13. Cooper, B. A. and Castle, W. B., J. Clin. Invest. **39**, 199 (1960).

14. Donaldson, R. M., Mackenzie, I. L., and Trier, J. S., J. Clin. Invest. **46**, 1215 (1967).

15. Rothenberg, S., J. Clin. Invest. **47**, 913 (1968).

16. Highley, D. R., Davies, M. C., and Ellenbogen, L., J. Biol. Chem. **242**, 1010 (1967).

17. Cooper, B. A., Am. J. Physiol. **214**, 832 (1968).

Received May 23, 1968. P.S.E.B.M., 1968, Vol. 129.

The Metabolism of Acetate-1-^{14}C in the Day-Old Chicken (33391)

JOHN B. ALLRED[1] AND F. H. KRATZER

Department of Poultry Husbandry, University of California, Davis 95616

It has been repeatedly demonstrated that a large variety of animals develop ketosis when their diet contains a high proportion of lipid relative to carbohydrate (1). However, the chick embryo (2) as well as growing chicks (3, 4) can utilize diets essentially devoid of carbohydrates without developing ketosis. Since the enzymes necessary for acetoacetate biosynthesis are demonstrable in chick liver (5) and ketosis can be produced if fatty acids furnish all nonprotein calories (4), the lack of ketosis in the chicken can not be attributed to an inability to synthesize ketone bodies.

Ketosis could be avoided, however, if the chick could perform a net synthesis of carbohydrate from acetate via some pathway which is not physiologically important in other animals (6) but may become important in the developing chick where lipid is the only available energy source. Three possible pathways which could account for a net synthesis of carbohydrate from acetate are: (a) initial

[1] Recipient of Fellowship GF-12995, Division of General Medical Science, Public Health Service. Present address: Institute of Nutrition and Food Technology, The Ohio State University, Columbus, Ohio.

TABLE I. Isotopes Injected and Percentage Oxidized to Carbon Dioxide in 2.5 hr.

Compounds injected[a]	Amount injected (mCi/g of body wt.)[b]	Recovered in CO_2 (%)
Sodium acetate-1-^{14}C	6.20	36.3
Sodium acetate-1-^{14}C	0.71	40.5
Glucose-U-^{14}C	0.11	26.2
Acetone-2-^{14}C	3.95	0.9
Glyoxylic acid-1,2-^{14}C	0.28	7.8

[a] Purchased from Volk Chemical Co., Los Angeles, except glyoxylic acid-1,2-^{14}C was from California Corporation for Biochemical Research, Los Angeles.

[b] Determined by wet combustion (15) of a sample of solution injected, except that manufacturers data was used for acetone-2-^{14}C. Sequential analysis (12) showed that 99.9% of the label in acetate was in the carboxyl carbon.

synthesis of acetoacetate, which then is converted to a glycolytic intermediate via either acetone (7) or pyruvaldehyde (8), (b) synthesis of butyrate which is converted to succinate by ω-oxidation (9), or (c) synthesis of malate via the glyoxalate bypass (10, 11). The first of these would not only decrease ketone body synthesis by making carbohydrate available, but would also reduce circulating ketone bodies by utilization.

Utilization of acetate by any of these pathways can be distinguished from its oxidation by the classical citric acid cycle by injecting acetate-1-^{14}C and measuring incorporation of radioactivity into amino acids (12). Using this technique, it was found that acetate is metabolized primarily via the citric acid cycle and that, if any of these special pathways are present in the chick, they are of limited importance.

Materials and Methods. Day-old, unfed chickens were injected intraperitoneally with the radioactive compounds shown in Table I. The chicks were placed in a glass metabolism cage that was slowly swept with carbon dioxide-free air. Respiratory carbon dioxide was trapped in 1.0 *N* sodium hydroxide, precipitated as barium carbonate and assayed for radioactivity using a thin-window, gas flow detector.

After the chicks injected with the larger amount of acetate-1-^{14}C were in the metabolism cage for 2.5 hr, they were decapitated. Livers were removed, homogenized in distilled water and liver protein was precipitated with a 20% trichloroacetic acid solution. The precipitated protein was thoroughly washed with the trichloroacetic acid solution and hydrolyzed with 6 *N* hydrochloric acid for 2.5 hr under reflux conditions. The hydrolyzate was decolorized with activated charcoal, and alanine, aspartic acid, glutamic acid, glycine, and serine were separated with ion-exchange chromatography (13). Further purification of these amino acids was accomplished with paper chromatography using phenol–ammonium hydroxide–water (200:1:20) and butanol–acetic acid–water (60:25:15) as developing solvents. Radioactivity of the purified amino acids were measured by counting an infinitely thin sample. Amino acid concentration was measured by the ninhydrin method (14) and specific activity was calculated.

Carrier alanine was added to a solution of radioactive alanine obtained by the isolation technique described above. The solution was evaporated to dryness at room temperature under reduced pressure and redissolved in a minimum amount of 1.0 *N* hydrochloric acid. Propylene oxide was added to destroy the excess acid. Alanine was crystallized by adding a mixture of acetone–ethanol (1:1). After recrystallization, the alanine was degraded sequentially (12) and the carbon dioxide from each carbon was recovered as barium carbonate and assayed for radioactivity on infinitely thick planchets using a gas-flow detector. A sample of alanine was also completely oxidized to carbon dioxide by wet combustion (15) to check recovery of radioactivity obtained from the stepwise degradation. The same procedures were used to isolate amino acids from carcass protein and measure their specific activities as well as determine the distribution of radioactivity in alanine.

Results. The chicks readily metabolized the injected acetate as shown by the percentage of isotope expired as carbon dioxide (Table I). Another 15% of the injected iso-

tope was associated with carcass and liver protein at the time of killing. Oxidation of glucose, glyoxylate, and acetone to carbon dioxide is also shown in Table I for comparison. Injected glucose was utilized readily but glyoxylate was oxidized much slower, and acetone was not metabolized to CO_2.

Distribution of radioactivity in alanine, isolated from hydrolyzates of carcass and liver protein, was obtained by sequential degradation (Table II). Recovery of radioactivity using this technique agreed very closely with values obtained by total combustion and showed that alanine was labeled predominantly in the carboxyl carbon when acetate-1-^{14}C was injected.

The relative specific activity of glutamate, aspartate, alanine, glycine, and serine after injection of acetate-1-^{14}C is shown in Table III. Glutamate had the highest specific activity, followed by aspartic acid. Of these amino acids, glycine had the lowest specific activity.

Discussion. Of the pathways proposed which could account for a net synthesis of carbohydrate from acetate, conversion of acetate to pyruvate via acetoacetate seems most plausible since it has been established unequivocally that such a pathway is present in animals (7, 8). Whether such a conversion occurs via acetone (7) or directly from acetoacetate (8), acetate-1-^{14}C would produce alanine, the transamination product of pyruvate, labeled exclusively in the alpha position. The fact that a relatively small percentage of isotope was found in this position in alanine (Table II) indicates that if such a synthesis occurs, it must be of minor importance. The small amount of label in the alpha and beta position of alanine can readily be explained by activity of the hexose monophosphate shunt (16). Additional evidence that acetate is probably not converted to pyruvate via acetone is the demonstration that injected acetone was oxidized to carbon dioxide to a very limited extent (Table I). In comparison, over 30% of the radioactivity was recovered as CO_2 in 3 hr when acetone-2-^{14}C was injected into rats (7). The rat is known to be able to convert acetone to a three-carbon glycolytic intermediate but in amounts insufficient to be physiologically important (7).

TABLE II. Intramolecular Distribution of Radioactivity in Alanine from Chicks Injected with Acetate-1-^{14}C.

Item	Carcass (sp act.[a])	(%)	Liver (sp act.[a])	(%)
COOH	171.6	97.5	319.0	87.6
$CHNH_2$	3.7	2.1	28.9	7.7
CH_3	1.0	0.6	6.1	1.7
Av	58.8		118.0	
Total combustion	58.6		121.4	

[a] Corrected for dilution and expressed as microcuries per gram atom carbon.

TABLE III. Relative Specific Activity of Amino Acids Isolated from Protein Hydrolyzates of Chicks after Injection of Acetate-1-^{14}C.

Amino acid	Carcass	Liver
Alanine	1.0	1.0
Glutamate	12.0	10.0
Aspartate	5.0	2.0
Glycine	0.7	0.9
Serine	0.8	—

Similarly, the data in Table II can be utilized to evaluate the possibility that acetate is converted to butyrate, which then undergoes ω-oxidation to form succinate and finally pyruvate. If pyruvate were synthesized in this manner, acetate-1-^{14}C injection would result in uniform labeling of all three carbons of alanine. Again, the data are not consistent with significant amounts of acetate being utilized for such a synthesis.

The labeling pattern of alanine cannot be used to distinguish between utilization of acetate via the glyoxylate cycle and its metabolism in the citric acid cycle. Relative specific activity of amino acids (Table III) indicate, however, that the glyoxylate cycle is also of minor importance if it is present. In this cycle, oxaloacetate is an intermediate while alpha-ketoglutarate is not. Therefore, significant activity of the glyoxalate cycle would be expected to result in greater radioactivity in aspartate (the transamination product of oxaloacetate) than glutamate (the transamina-

tion product of α-ketoglutarate). Further, since glyoxylate is readily converted to glycine by transamination (17), glycine would also be expected to have a relatively high specific activity. Finally, the oxidation of injected glyoxylate was much more limited than acetate. These data are in agreement with the observations of Madsen (18), who was unable to demonstrate activity of key enzymes of this pathway in tissue of chick embryos just prior to hatching.

All of the data presented here are consistent with the metabolism of the injected acetate primarily via the citric acid cycle as it is in other animals. No evidence was obtained which would indicate that the chick has some mechanism to conserve glucose, since injected glucose was readily oxidized (Table I), although slightly slower than acetate. The relatively large amount of isotope associated with protein after acetate-1-^{14}C was injected probably reflects the rapid growth rate of the day-old chick. In the absence of any evidence of special pathways of acetate utilization, it is concluded that the lack of ketosis in the chicken is the result of a more subtle control mechanism of ketone body biosynthesis, either through reduced enzyme activity *in vivo* or control of substrate availability.

1. Deuel, H. F., Jr. and Morehouse, M. D., Advan. Carbohydrates Chem. **2**, 119 (1946).

2. Needham, J., "Biochemsitry and Morphogenesis," p. 62. Cambridge Univ. Press, London and New York (1942).

3. Renner, R. and Elcombe, A. M., J. Nutr. **93**, 31 (1967).

4. Brambila, S. and Hill, F. W., J. Nutr. **88**, 84 (1966).

5. Allred, J. B. and Upjohn, D. R., Federation Proc. **26**, 411 (1967).

6. Weinman, E. O., Strisower, E. H., and Chaikoff, I. L., Physiol. Rev. **37**, 252 (1957).

7. Mourkides, G. A., Hobbs, D. C., and Koppe, R. E., J. Biol. Chem. **234**, 509 (1959).

8. Milligan, L. P. and Baldwin, R. L., J. Biol. Chem. **242**, 1095 (1967).

9. Blixenkrone-Möller, N., Z. Physiol. Chem. **252**, 137 (1938).

10. Kornberg, H. L. and Madsen, N. B., Biochem. Biophys. Acta **24**, 641 (1957).

11. Kornberg, H. L. and Beevers, H., Biochem. Biophys. Acta **26**, 531 (1957).

12. Black, A. L. and Kleiber, M., Biochem. Biophys. Acta **23**, 59 (1957).

13. Moore, S. and Stein, W. H., J. Biol. Chem. **211**, 893 (1954).

14. Rosen, H., Arch. Biochem. Biophys. **67**, 10 (1957).

15. Van Slyke, D. D., Folch, J., and Plazin, J., J. Biol. Chem. **136**, 509 (1940).

16. Axelrod, B., "Metabolic Pathways," (D. M. Greenberg, ed.), Vol. 1, p. 205. Academic Press, New York (1960).

17. Weinhouse, S. and Friedman, B., J. Biol. Chem. **221**, 665 (1956).

18. Madsen, N. B., Biochim. Biophys. Acta **27**, 199 (1958).

Received Dec. 11, 1967. P.S.E.B.M., 1968, Vol. 129.

Vitamin B_6 Requirement in the Hypothalamic-Hyperphagic Rat* (33392)

PAMELA A. MARETT[1] AND JOHN R. BEATON[2]

Department of Physiology, University of Western Ontario, London, Ontario

Vitamin B_6 requirement has been related to the dietary intake of both protein and fat. Twenty-three years ago, Cerecedo and Foy (1) showed that increasing the dietary protein level caused a more rapid onset of typical acrodynia and a reduced survival time of rats provided with a diet deficient in vitamin B_6. In humans, Baker *et al.* (2) observed that xanthurenic acid excretion, a symptom

* Supported by a grant from the Medical Research Council of Canada.

[1] Based on a thesis submitted to the Faculty of Graduate Studies, The University of Western Ontario, in partial fulfillment of the requirements for the degree of Master of Science.

[2] Present address and address for reprints: Division of Nutrition, University of Hawaii, Honolulu, Hawaii 96822.