REFERENCES

Barber, M., Brooksbank, B. W. L. & Haslewood, G. A. D. (1948). *Nature, Lond.*, **30**, 701.
Brown, D. H., Dodgson, K. S., Sherwood, T. H. & Spencer, B. (1952). *Biochem. J.* **51**, xlvii.
Buehler, H. J., Katzmann, P. A., Doisy, P. P. & Doisy, E. A. (1949). *Proc. Soc. exp. Biol., N.Y.*, **72**, 297.
Derrien, M. (1911). *Bull. Soc. Chim. biol., Paris*, **9**, 110.
Dodgson, K. S., Lewis, J. I. M. & Spencer, B. (1952). *Biochem. J.* **51**, xlii.
Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **53**, 444.
Dodgson, K. S., Spencer, B. & Thomas, J. (1953). *Biochem. J.* **53**, 452.
Karunairatnam, M. C. & Levvy, G. A. (1951). *Biochem. J.* **49**, 210.
Kerr, L. M. H., Campbell, J. G. & Levvy, G. A. (1949). *Biochem. J.* **44**, 487.
Marsh, C. A., Alexander, F. & Levvy, G. A. (1952). *Nature, Lond.*, **170**, 163.
Mills, G. T. (1948). *Biochem. J.* **43**, 125.
Mills, G. T. & Paul, J. (1949). *Biochem. J.* **44**, xxxiv.
Moore, D. M. (1936). *J. Mar. biol. Ass. U.K.* **20**, 72.
Moore, D. M. (1937). *J. Mar. biol. Ass. U.K.* **21**, 721.
Morimoto, K. (1937). *J. Biochem., Tokyo*, **26**, 259.
Nagaoka, T. (1951). *Tohoku J. exp. Med.* **53**, 29.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Soda, T. (1936). *J. Fac. Sci. Tokyo Univ.* **3**, 150.
Soda, T. & Egami, F. (1933). *J. chem. Soc. Japan*, **54**, 1069.
Soda, T. & Egami, F. (1941). *J. chem. Soc. Japan*, **62**, 256.
Soda, T. & Koyama, S. (1935). *J. chem. Soc. Japan*, **56**, 1388.
Spencer, B. & Williams, R. T. (1951). *Biochem. J.* **48**, 537.
Tanaka, S. (1938). *J. Biochem., Tokyo*, **28**, 119.
Whitehead, J. E. M., Morrison, A. R. & Young, L. (1952). *Biochem. J.* **51**, 585.

The Effect of Cobalamin on the Quantitative Utilization of Serine, Glycine and Formate for the Synthesis of Choline and Methyl Groups of Methionine

By H. R. V. Arnstein and A. Neuberger
National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 3 *February* 1953)

It was observed by Du Vigneaud, Chandler, Moyer & Keppel (1939) that rats generally lost weight when kept on a synthetic diet containing homocystine but deficient in methionine and choline. The favourable effect on growth of the addition of choline or betaine indicated the occurrence of transmethylation which was conclusively proved later by isotope experiments carried out in Du Vigneaud's laboratory. In these early experiments it was noticed that occasionally some animals on the deficient diets grew, but this was ascribed to the action of the intestinal flora. These observations were greatly extended by Bennett and co-workers (Bennett, Medes & Toennies, 1944; Bennett & Toennies, 1946) who also obtained evidence suggesting that factors present in liver extract are involved in the synthesis of labile methyl groups. That such a synthesis indeed occurred was shown with the aid of deuterium by Du Vigneaud, Simmonds, Chandler & Cohn (1945). However, the relatively small amount of deuterium in the choline methyl groups indicated that only about 8% of them was derived from the body water, the rest presumably originating from the methionine also present in the diet. This incorporation was again ascribed to micro-organisms.

More precise information about this synthesis of methyl groups in the rat was obtained by the use of carbon isotopes. Thus, one of us (Arnstein, 1950, 1951) showed that formate, methanol, the β-carbon atom of serine and the α-carbon atom of glycine can be precursors of choline methyl groups. Similar results were reported about the same time by various groups of American workers (Jonsson & Mosher, 1950; Sakami & Welch, 1950; Weissbach, Elwyn & Sprinson, 1950; Du Vigneaud, Verly & Wilson, 1950). Sakami (1950) also demonstrated that the feeding of acetone labelled with ^{14}C in the methyl group gives rise to radioactivity in the methyl group of choline. Du Vigneaud, Verly, Wilson, Rachele, Ressler & Kinney (1951) used methanol labelled both with ^{14}C and deuterium and showed that the hydrogen was diluted to a greater extent than the carbon, indicating that the methyl group of methanol is not directly converted to that of choline. These workers obtained results similar to those reported for formate on feeding [^{14}C]formaldehyde, [^{14}C]formylphenylalanine and [^{14}C]methyl stearate. *In vitro* formation of choline methyl groups from various precursors has also been reported (Sakami & Welch, 1950; Siegel & Lafaye, 1950; Berg, 1951). These experiments and the important observations

on microbe-free rats by Du Vigneaud, Ressler & Rachele (1950; see also Du Vigneaud, Ressler, Rachele, Reyniers & Luckey, 1951) clearly established that the synthesis of methyl groups can occur in the tissues of the rat and need not be ascribed to the activity of the intestinal flora.

As mentioned above, the early experiments of Bennett and her colleagues indicated that certain growth factors, then unidentified, appeared to affect the dietary requirements of the rat for methionine and choline. Evidence has recently been obtained, independently by various workers, that at least one of these factors is cobalamin (Gillis & Norris, 1949; Schaefer, Salmon & Strength, 1949*a*, *b*; Bennett, 1950; Stekol & Weiss, 1950; Jukes & Stokstad, 1951). These studies, which were carried out with rats and chicks, showed that the dietary choline requirement for good growth was reduced and the incidence of kidney damage and mortality in young rats greatly diminished by supplying cobalamin in the diet; however, cobalamin had little beneficial effect if added to diets completely devoid of choline or other compounds containing labile methyl groups. The effect of various levels of choline in the diet on body weight and fatty infiltration of the liver was examined in great detail by Strength, Schaefer & Wilson (1951), and Schaefer & Knowles (1951) who also studied the effects of administration of methylaminoethanol, dimethylaminoethanol, betaine, glycine, serine and methanol on growth.

The aim of the present investigation was to ascertain quantitatively the extent to which synthesis of labile methyl groups, particularly those of choline, takes place in the rat from some of the known precursors mentioned above. In many experiments the radioactivity of the methionine methyl carbon atom was also measured.

The method generally used in tracer experiments consists of administering a labelled compound over a relatively short period. The quantitative interpretation of the results of such experiments requires a detailed knowledge of the nature of the intermediates between the precursor and the isolated product and of the rates of various reactions in which these compounds participate. Such information is not generally available. We have therefore fed over prolonged periods known amounts of labelled compounds as constituents of, in most experiments, synthetic diets. It was hoped that under such conditions, the effects of differences of reaction rates would be largely eliminated. It was also considered desirable to reduce or even eliminate from the diet compounds such as choline and methionine which contain biologically labile methyl groups. The effect of cobalamin in reducing the requirement for choline with purely synthetic diets was therefore investigated; at the same time it was hoped to obtain further information as to the possible relationship between this choline-sparing action of cobalamin and methyl-group synthesis. Some of the results of this investigation have been the subjects of two preliminary communications (Arnstein & Neuberger, 1951, 1952).

EXPERIMENTAL

Animals and diets. Except in Expts. 23 and 24, albino rats of Institute strain were used. The animals were placed on the experimental diets soon after weaning; their ages at the beginning of the experiment were 32 days (Expts. 1–3 and 16–22), 33 days (Expts. 14 and 15), 34 days (Expts. 6, 7 and 11–13; also one rat each in Expts. 16 and 17) and 36 days (Expts. 4, 5 and 8–10) respectively. In Expts. 23 and 24, four albino rats, specially bred from cobalamin-deficient stock, were used. These animals were supplied by Dr W. F. J. Cuthbertson, Glaxo Laboratories, Greenford, Middlesex. Their age at the beginning of the experiment was 46 days and they had been placed on the soya-flour diet (diet 3) described below at 21 days.

To test the efficiency of glycine, serine or formate as methyl-group precursors, one or more litters were distributed as evenly as possible with respect to sex and with respect to the dietary supplement such as cobalamin, choline or methionine. Unless otherwise stated, food consumption was not restricted.

Details of the basal amino-acid diets are given in Table 1. The soya-flour diet (diet 3), kindly supplied by Dr W. F. J. Cuthbertson, had the following composition: soya flour, 72%; lactose, 22%; salt mixture (Hubbell, Mendel & Wakeman, 1937), 4%; arachis oil, 2%; and the following vitamins/kg. diet: vitamin A, 4000 i.u.; vitamin D, 2000 i.u.; vitamin K, 2 mg.; α-tocopherol, 284 mg.; inositol, 220 mg.; nicotinic acid, 100 mg.; calcium D-pantothenate, 100 mg.; *p*-aminobenzoic acid, 75 mg.; thiamine, 30 mg.; riboflavin, 30 mg.; pyridoxine, 8 mg.; pteroylglutamic acid, 1 mg.; biotin, 0·2 mg.

In Expts. 1–3 the amino-acid diet AAD 1 containing 2% [α-^{14}C]glycine (57 μc./mole) was fed throughout the duration of the experiment (39 days). Both choline chloride (5 mg./rat/day) and cobalamin (2·5 μg./rat/day) were given orally. In later experiments (Expts. 4–13) the basal diet AAD 2, containing unlabelled glycine (0·5%), L-serine (0·7%) or sodium formate (0·1%), respectively, was given for an initial period of 12 days; [α-^{14}C]glycine (0·5%; 740 μc/mole), L-[β-^{14}C]serine (0·7%; 220 μc/mole) or sodium [^{14}C]formate (0·1%; 2·8 mc/mole) were then substituted for the respective unlabelled compounds and fed for the remainder of the experiments (20 days, except in the case of one of the two rats used in Expt. 9 which was fed the [β-^{14}C]serine for 16 days only). In Expts. 18–21, diet AAD 2 containing unlabelled glycine (0·5% or 2%) was fed for the first 8 days; 0·5% or 2% [α-^{14}C]glycine (160 μc/mole) were then substituted and fed for 21 days. Expt. 22 was carried out exactly as Expt. 11, except that the specific radioactivity of the sodium [^{14}C]formate fed was 1·1 mc/mole. In Expts. 14 and 15, the diet contained 1% DL-methionine as well as choline chloride (5 mg./rat/day). The animals in these and in Expts. 16 and 17 were pair-fed the respective diets (see Table 3), which contained 0·1% sodium [^{14}C]formate (1·1 mc/mole) throughout the experimental period (28 days).

In the above experiments (Expts. 4–22) cobalamin (2·5 μg./rat/day) was again given orally, but the choline chloride (5 mg./rat/day) was added to the diets daily. In Expts. 23 and 24, the animals, which were pair-fed, received no choline chloride but cobalamin was given both orally (5 μg./rat/day) and in the diet (5 μg./rat/day).

Table 1. *Composition of basal amino-acid diets used in feeding experiments*

(Amounts are given in g./kg. diet. The following vitamins were added to the two diets (mg./kg. diet): α-tocopherol, 40; vitamin K, 1; thiamine, 10; riboflavin, 10; pyridoxine, 10; nicotinic acid, 10; *p*-aminobenzoic acid, 10; calcium pantothenate, 50; inositol, 100; pteroylglutamic acid, 2; biotin, 0·1. 7200 i.u. of vitamin A and 1200 i.u. of vitamin D both/kg. diet were also added.)

Component of diet	Amino-acid diet 1 (AAD 1)	Amino-acid diet 2 (AAD 2)
DL-Valine	12	20
DL-Leucine	18	24
DL-Isoleucine	18	16
L-Arginine monohydrochloride	8	8
L-Histidine monohydrochloride hydrate	7	10
L-Lysine monohydrochloride dihydrate	28	18
DL-Threonine	16	14
DL-Homocystine	12	10
L-Cystine	5	7
DL-Phenylalanine	15	12
L-Tyrosine	10	6
DL-Tryptophan	4	8
[α-^{14}C]Glycine	20	5*
L-Proline	0	4
L-Aspartic acid	0	6
L-Glutamic acid	0	24
$NaHCO_3$	16	13
Maize starch	351	340
Salt mixture (Glaxo Laboratories) no. DL6	40	40
Cane sugar	340	340
Cod-liver oil	16	16
Arachis oil	64	64

* Only some of the animals of this group received [α-^{14}C]glycine, others had instead L-[β-^{14}C]serine or [^{14}C]-formate at levels indicated in the text.

The cobalamin used in all experiments was a highly potent concentrate ('Cytamen', made by Glaxo Laboratories Ltd.) kindly supplied by Dr W. F. J. Cuthbertson.

Food consumption and body weight. The food consumption of each animal was measured daily and the body weight on alternate days.

Radioactivity measurements. All measurements were carried out with a helium-filled bell-shaped Geiger-Müller counter, using 'infinite thickness' samples mounted on 1 sq.cm. Polythene disks (Popják, 1950). The combined errors, due to variation in sample preparation, small differences in disk size and statistical errors in the counting, were about ±5%. All counts were compared with a subsidiary standard and converted to μc. In experiments with synthetic [^{14}C]choline chloroplatinate (Arnstein, 1952) it was found that the back-scattering of the chloroplatinate, compared with $BaCO_3$, was not significant with our instrument and no correction for this effect was made in our calculations.

Isolation and degradation of choline. The animals were killed under anaesthesia and choline was extracted with ethanol separately from the minced pooled viscera (liver, kidney, spleen, reproductive organs, washed gastro-intestinal tract, heart and lungs) and from the minced carcasses respectively. In all experiments the choline (free and bound) was isolated as the reineckate which was converted to the chloroplatinate (Du Vigneaud, Cohn, Chandler, Schenck & Simmonds, 1941). In early experiments it was found that the crude choline chloroplatinate samples sometimes contained some ammonium chloroplatinate, which could be removed by filtration after dissolving the choline chloroplatinate in a little water. The choline chloroplatinate was crystallized from the filtrate by adding ethanol. Repeated purification of the choline chloroplatinate by this method afforded samples of constant radioactivity (Expts. 4–13, 18–21, 23 and 24). In some cases (Expts. 18–21) insufficient material was available for repeated recrystallization and the ammonia content was therefore calculated from the Pt analysis or by estimation using the Kjeldahl method. Later (Expts. 14–17 and 20–22), purification of choline by converting the reineckate into the mercurichloride complex ($C_5H_{14}ONCl.HgCl_2$, m.p. 170°), from which choline chloride was regenerated by H_2S before precipitation as the chloroplatinate, was found to be more satisfactory and to give samples which contained no ammonia as judged by the Kjeldahl method and Pt analysis.

During the course of this work it was found (Arnstein, 1952), that the oxidation of choline chloroplatinate with alkaline permanganate (Du Vigneaud *et al.* 1941) sometimes produces a relatively large amount of ammonia, which is precipitated as the chloroplatinate, together with the trimethylamine. The trimethylamine chloroplatinate obtained by this method was therefore combusted and assayed as $BaCO_3$ (Arnstein, 1952). Some of the values of the radioactivities of choline methyl groups, based on the direct assay of the 'trimethylamine' chloroplatinate, which were reported in one of our preliminary communications (Arnstein & Neuberger, 1951) may therefore be somewhat low. However, our present results show, on the whole, good agreement with our earlier experiments.

Radioactivity estimation of methionine methyl carbon atom and serine β-carbon atom. The ethanol-extracted viscera or carcasses were extracted three times with 6% (w/v) trichloroacetic acid, washed with water, hot ethanol and ether and dried at 80°. For the degradation of methionine, samples of the residue (3–10 g.) were hydrolysed with HI (sp.gr. 1·7; 3·6 ml./g. dry tissue), while N_2 was passed through the solution, essentially as described by Simmonds, Cohn, Chandler & Du Vigneaud (1943). The methyl iodide was absorbed in a 5% (w/v) solution of trimethylamine in methanol, which was cooled in solid CO_2. Crude tetramethylammonium iodide was isolated by evaporating the above solution to dryness. After recrystallization from water by adding ethanol, samples showing no change of radioactivity on further recrystallization were assayed directly for ^{14}C.

In most of the experiments reported in this paper, the radioactivity of the β-carbon atom of serine was measured on the formaldehyde dimedon derivative obtained by periodate oxidation as follows. The ethanol-extracted residues of the viscera were hydrolysed with 6 N-HCl for 24 hr. at 100°. The excess HCl was removed and the amino acids were precipitated as Hg complexes (Neuberg & Kerb, 1912; Campbell & Work, 1952). After regeneration with

H_2S, the mixture was oxidized with periodate (Rees, 1946) and the formaldehyde derived from the β-carbon atom of serine was precipitated as the dimedon derivative, which was recrystallized from ethanol to constant radioactivity and unchanged melting point (189°). The validity of this method was checked by comparing the radioactivity of the formaldehyde dimedon obtained as above with that of the dimedon derived from the periodate oxidation (Sakami, 1950) of chromatographically pure serine isolated from the same source (Arnstein & Neuberger, 1953).

RESULTS

The effect on growth of addition of cobalamin and/or suboptimal amounts of choline to basal diets lacking substances with preformed methyl groups

Experiments without restriction of food intake. In the main series of experiments the food intake of the rats was not restricted. Twelve rats altogether (group I) were fed diets which contained cobalamin and homocystine, but were devoid of methionine and choline. The diets contained, in addition to the essential amino acids (except methionine), cystine and one of the labelled substances to be tested, i.e. glycine or serine or formate. Three of the rats which received formate died in the first few days and showed haemorrhagic changes of the kidneys. All other animals survived for the duration of the experiments and appeared to be well. None of the rats which received cobalamin lost weight, but their average body weight increase was only 0·57 g./day (Table 2).

Another group of rats (group II) comprising fourteen animals received the same basal diet as group I, but cobalamin was replaced by choline chloride (5 mg./rat/day). There were no deaths in this group, but *post mortem* examination revealed old lesions in the livers and kidneys of several animals. The average weight gain was only 0·27 g./day and with some of the animals, weight at the end of the experiment was lower than the initial body weight. Statistical analysis (Table 2) suggests that the first diet which contained cobalamin, but no choline, is slightly better than the second diet which contained an insufficient amount of choline but no cobalamin. One animal (Expt. 6), which was not included in calculating the mean values given in Table 2, grew fairly well, the mean daily weight increase being 2·3 g. This was probably due to intestinal synthesis of cobalamin.

The addition of cobalamin to a diet containing the same amount of choline as that given to rats of group II (5 mg./day) resulted in an average weight increase of 1·87 g./day (Table 2). The growth rate found for this group (group III) is still about 30 % lower than that observed with rats of the same strain fed a diet containing casein (12 %) supplemented with cystine (0·3 %) and cobalamin. However, it is clear from the representative growth curves (Fig. 1) and from the statistical analysis (Table 2), that the differences in growth rate and food consumption between group III and groups I and II respectively are highly significant.

The growth rates on the three diets (groups I–III) appeared to be independent of the sex of the animal and not to be related significantly to initial weight. Growth was somewhat better on the second amino-acid diet (AAD 2, Table 1) which was used in most experiments than on amino-acid diet 1 (AAD 1, Table 1). There was no evidence that addition of glycine at the two levels of 2 and 0·5 % or of serine affected growth rate or food consumption, but formate at the level of 0·1 % had a slight but definite

Table 2. *Effect of administration of cobalamin (2·5 μg./rat/day) or of choline chloride (5 mg./rat/day) or of both together on body weight and food consumption of rats given a basal diet lacking substances containing labile methyl groups*

(For particulars of basal diet see Table 1; experimental periods were between 29 and 39 days; the initial ages of the animals are given in the experimental section.)

Group no.	No. and sex of animals		Presence (+) or absence (−) of		Average initial wt. (g.)		Average daily wt. change (g.)		Average daily food consumption (g.)	
	M.	F.	Cobalamin	Choline	Mean	S.D.	Mean	S.D.	Mean	S.D.
I	5	4	+	−	63	±9·3	+0·57	±0·33	4·88	±1·68
II	9	5	−	+	66	±7·9	+0·27	±0·33	4·59	±1·44
III	11	5	+	+	64·5	±6·2	+1·87	±0·56	7·22	±1·46

	Average daily change in body wt.	Average daily food consumption
Group I *v.* group II	$P<0·05$	$P>0·6$
Group I *v.* group III	$P<0·001$	$P<0·01$
Group II *v.* group III	$P<0·001$	$P<0·001$

P, the probability that difference between dietary groups is due to chance alone, was calculated from Student's tables using as value for the number of degrees of freedom the total number of animals of each pair of groups compared less 2.

growth-retarding effect with all three types of diet.

Experiments involving paired feeding. The average daily food intake of the animals which received both cobalamin and choline (group III) was about 50% greater than that found for either group I or II (Table 2). The increased growth found for rats receiving both supplements as compared with that observed with animals receiving choline alone or cobalamin alone might therefore be largely due to increased food intake. Two series of paired-feeding experiments (Table 3; rats 1–10 and 11–14 respectively) were therefore done: in the first the amino-acid diet (AAD 2) containing also choline and formate was used (Expts. 14–17), whilst in the second series (Expts. 23 and 24) the soya-flour diet was employed. In the first series there was either loss of weight or poor growth and the results were not affected significantly by the presence or absence of cobalamin. In the second series growth was relatively good, but again addition of cobalamin had no effect on weight changes.

It will be noticed that addition of methionine at the level of 1% to a diet already containing homocystine (0·8%) did not improve growth rate (Table 3, rats 1–4). Similar results, suggesting that combined administration of homocystine and methionine may result in depression of growth, have been obtained in other experiments not described here. The reasons for this effect are being investigated.

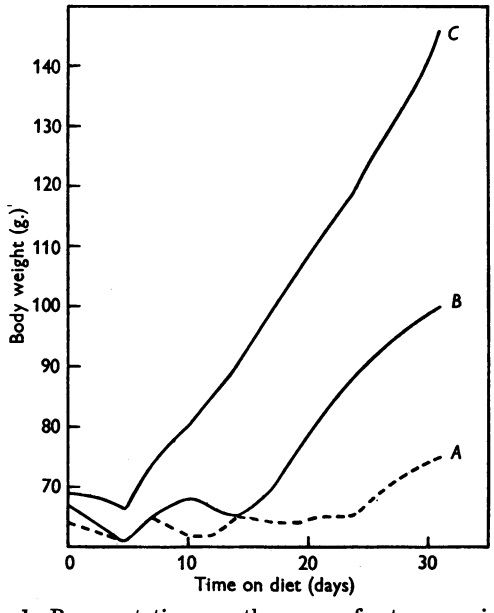

Fig. 1. Representative growth curves of rats on various diets, showing the effect of cobalamin with or without suboptimal amounts of choline. All rats received the basal amino-acid diet AAD 2 (Table 1). Curve *A* is the growth curve of a rat receiving AAD 2 + 5 mg. choline chloride/day without cobalamin; curve *B* gives the data of a litter mate receiving AAD 2 + cobalamin (2·5 μg./day) without choline; curve *C* is the growth curve of another litter mate given AAD 2 with both supplements.

Table 3. *Paired-feeding experiments on the effect of cobalamin on the growth rates of rats receiving various basal diets*

(Rats nos. 1–10 had an amino-acid basal diet (AAD 2, Table 1) containing homocystine; these rats also received sodium [^{14}C]formate (0·1% of the diet) and choline chloride (5 mg./rat/day). Rats nos. 1–4 were also given DL-methionine (1% of the diet) which replaced an equivalent amount of carbohydrate. Rats nos. 11–14 were given the soya-flour diet described in the Experimental section and [α-^{14}C]glycine (1% of the diet). All odd-numbered rats received cobalamin, whilst even-numbered animals received no cobalamin. The bracketed rats were pair-fed in such a way that the food intake of the odd-numbered animal was approx. equal to that of the corresponding even-numbered mate.)

Expt. no.	Rat No.	Rat Sex	Duration of experiment (days)	Body wt. (g.) Initial	Body wt. (g.) Final	Average daily food consumption (g.)
14	1	M.	17	96	91	5·7
15	2	M.	17	104	83	6·0
14	3	F.	28	92	81	6·2
15	4	F.	28	92	90	6·2
16	5	M.	28	100	88	6·0
17	6	M.	28	97	83	5·9
16	7	M.	28	79	85	6·6
17	8	M.	28	80	97	6·7
16	9	F.	28	77	86	6·9
17	10	F.	28	72	88	6·7
23	11	M.	10	129	152	12·3
24	12	M.	10	115	138	12·3
23	13	M.	10	96	115	11·0
24	14	M.	10	102	120	11·0

Quantitative aspects of the conversion of the α-carbon atom of glycine, the β-carbon atom of serine and of formate to methionine methyl groups

The present experiments (Table 4) show clearly that the extent to which the α-carbon atom of glycine is converted into the methyl groups of methionine is dependent on the level of glycine in the diet. The molar radioactivity of the methionine methyl groups obtained from the internal organs of rats fed a diet containing 2 % [α-^{14}C]glycine and cobalamin but no choline or methionine (Expt. 1), was about 13 % of that of the fed glycine. The latter had, however, been diluted by glycine formed through endogenous synthesis and it can be calculated from the observed radioactivity of the glycine of the internal organs that the dilution factor in this experiment was 2·9 (Arnstein & Neuberger, 1953). It would thus appear that at a dietary level of 2 % of glycine about 35–40 % of the methionine methyl groups of the internal organs are derived from the α-carbon atom of glycine. When the glycine level in the diet was only 0·5 %, the molar radioactivity of the methionine methyl groups of the internal organs was less than 2 % of that of the fed glycine (Expt. 4). In this experiment the dilution of the fed glycine by glycine formed through endogenous synthesis was about 8·0 (Arnstein & Neuberger, 1953) and thus, with this lower level of glycine, only about 14 % of methyl groups are derived from the α-carbon atom of glycine.

When L-[β-^{14}C]serine was fed at a level of 0·7 %, which is equivalent on a molar basis to 0·5 % glycine, the molar radioactivity in the methionine methyl groups (Expt. 8) was approximately 6 times that observed in the comparable glycine experiment (Expt. 4) and was about 70 % of that of the β-carbon atom of serine isolated from the internal organs of the same animals.

Sodium [^{14}C]formate was fed at a level of 0·1 %, equivalent to 0·16 % of serine. The results are therefore not strictly comparable, but it appears (Expt. 11) that its conversion into methionine methyl groups is at least as efficient as that of the β-carbon atom of serine.

In the experiments so far considered, no compounds containing labile methyl groups were present in the diet. When choline chloride was fed at amounts of 5 mg./rat/day, in addition to cobalamin, the radioactivity of the methionine methyl groups was reduced by 25–30 % (Expts. 5, 9 and 12) as compared with similar experiments in which cobalamin but no choline was supplied (Expts. 4, 8 and 11). On

Table 4. *The incorporation of radioactivity from* [α-^{14}C]*glycine,* L-[β-^{14}C]*serine and sodium* [^{14}C]*formate into the β-carbon atom of serine and the methyl carbon atom of methionine*

(For details of diets see Table 1 and the Experimental section. The average daily food consumption and growth of the animals are given in Table 2. The β-carbon atom of serine and the methionine methyl carbon atom were isolated from the hydrolysates of the mixed viscera proteins, as described in the Experimental section. In order to facilitate comparison, the specific radioactivities of the β-carbon atom of serine and the methionine methyl carbon atom are expressed as μc/g. atom and have been calculated for a specific radioactivity of labelled precursor in the diet = 1 mc/mole.)

Expt. no.	Basal diet	Labelled precursor	Supplement	Specific radioactivity (μc/g. atom): Serine β-carbon atom	Specific radioactivity (μc/g. atom): Methionine methyl carbon atom
1	AAD 1	2% [α-^{14}C]Glycine	Cobalamin	234	128
2			Choline + cobalamin	226	—
3			Choline	239	—
4	AAD 2	0·5% [α-^{14}C]Glycine	Cobalamin	33·6	17·8
5			Choline + cobalamin	30·1	11·0
6*			Choline	32·2	13·7
7			Choline	32·0	—
8	AAD 2	0·7% L-[β-^{14}C]Serine	Cobalamin	144	107
9			Choline + cobalamin	153	81·5
10			Choline	129	47·4
11	AAD 2	0·1% Sodium [^{14}C]formate	Cobalamin	9·4	40·6
12			Choline + cobalamin	7·0	33·8
13			Choline	6·4	20·0
14†			1% Methionine, choline + cobalamin	—	10·3
15†			1% Methionine, choline	—	8·3
16†			Choline + cobalamin	—	32·0
17†			Choline	—	28·6

— Signifies values not determined.
* The animal in this experiment grew abnormally well, possibly due to intestinal synthesis of cobalamin.
† The animals in these experiments were pair-fed.

diets containing choline chloride (5 mg./rat/day) but no cobalamin, the radioactivity of the methionine methyl groups was further reduced (Expts. 10 and 13). In one experiment (Expt. 6) there was no significant difference, but this animal grew exceptionally well (see p. 262) and it is presumed that extensive intestinal synthesis of cobalamin took place. This effect of cobalamin of increasing the radioactivity of methionine methyl groups was markedly reduced, but still significant, in experiments in which groups of rats were pair-fed (Expts. 14–17, Table 4; Expts. 23 and 24, Table 7) even when an adequate amount of methionine was given in the diet (Expts. 14 and 15, Table 4; Expts. 23 and 24, Table 7).

Table 4 also gives data for the radioactivity of the β-carbon atom of serine of the visceral proteins. In all experiments in which [α-^{14}C]glycine or [β-^{14}C]-serine were given, the serine β-carbon has a radioactivity which is 1·3 to 3·0 times that of methionine methyl carbon atom. By contrast, labelled formate gives rise to a radioactivity in the β-carbon atom of serine which is one-third to one-fifth that of the methionine methyl carbon atom.

In the glycine and serine experiments (nos. 1–10) the specific radioactivity of the β-carbon atom of serine was not affected significantly by administration of choline or cobalamin, but with formate as precursor addition of choline may cause a reduction in radioactivity (Expts. 11–13). It is also of interest that at the two levels of glycine the ratios of radioactivities of the β-carbon atom of serine to that of the methionine methyl groups are similar, but slightly lower than those found in comparable experiments in which [β-^{14}C]serine was the labelled precursor.

Comparison of the incorporation of radioactivity from [α-^{14}C]*glycine,* [β-^{14}C]*serine and sodium* [^{14}C]-*formate into choline methyl groups*

Tables 5 and 6 show the radioactivity of choline and the relative incorporation of ^{14}C into choline methyl groups from [α-^{14}C]glycine, [β-^{14}C]serine and [^{14}C]formate under various conditions. The [α-^{14}C]-glycine was fed at two different levels and, since results have been calculated on the basis of a uniform molar radioactivity of precursor (1 mc/mole), the specific radioactivity of the choline isolated from animals which had been fed glycine at a dietary level of 2% (Expts. 18 and 19) was, as expected, several times higher than in corresponding experiments in which 0·5% glycine was fed. The same applies to the specific radioactivity of the ethanolamine moiety of choline, which can be calculated by difference. The radioactivity of the trimethylamine moiety was affected to a greater extent than can be accounted for solely by the greater intake of radioactive precursor on the 2% glycine diet. This result is similar to the findings on the conversion of [α-^{14}C]glycine into methionine methyl groups described above.

At the 2% glycine level the administration of cobalamin appears to increase specifically the incorporation of radioactivity into the choline methyl groups (Expts. 18 and 19), which confirms our earlier findings (Arnstein & Neuberger, 1951). In a similar experiment, in which animals were pair-fed a diet containing 1% [α-^{14}C]glycine, in addition to an unknown amount of unlabelled glycine derived from the soya-flour protein (Table 7) for a shorter period (10 days), the addition of cobalamin increased the relative radioactivity in the choline methyl groups from 12 to 21%. However, this effect of cobalamin is not marked on the 0·5% glycine diet (Table 5; Expts. 5, 20 and 21).

The β-carbon atom of serine, fed at a level equivalent to 0·5% glycine, appears to be more effective as a precursor of the ethanolamine moiety of choline than the α-carbon atom of glycine. The incorporation of ^{14}C into the methyl groups is even more greatly increased, as indicated by the observation that in these experiments (nos. 9 and 10) more than 50% of the radioactivity of the choline is present in the trimethylamine moiety.

Formate, on the other hand, appears to be incorporated mainly into the methyl groups of choline, which contain between 60 and 85% of the total radioactivity.

Tables 5 and 6 also show that the radioactivity of the choline is reduced by about 50% when 5 mg. of choline chloride/day are supplied. A comparison of experiments in which animals received choline and cobalamin with those in which choline only was supplied shows a marked increase of choline synthesis from the labelled precursors when this vitamin is given. In paired feeding experiments using [^{14}C]formate (nos. 14–17) this effect of cobalamin, though less marked in the case of diets containing an adequate amount of methionine (Expts. 14 and 15), was still present.

In all experiments, with the exception of the high-glycine diets discussed above, the relative incorporation of ^{14}C from the labelled precursor into choline methyl groups compared with the ethanolamine moiety was hardly affected by the administration of cobalamin, indicating that the latter stimulated conversion of the labelled carbon atoms into the two moieties to an approximately equal extent.

It is of interest that although the labelled precursors were fed over periods of as long as 5 weeks the radioactivity of the carcass choline was always considerably less than that of the visceral choline (Tables 5 and 6). Differences of the same order were observed for the methionine methyl groups derived from carcass and visceral proteins (Table 7), but in

Table 5. *Conversion of the α-carbon atom of glycine and the β-carbon atom of* L-*serine into choline*

(The composition of the basal diet (AAD 2) which was used in these experiments is given in Table 1. Details of the dietary supplements, the food consumption and growth of the animals are given in Table 2 and in the Experimental section. The choline was isolated either separately from the pooled viscera and from the carcass or from the total body. The isolation and degradation methods are described in the Experimental section. The specific radioactivities of the isolated compounds have been calculated for a specific radioactivity of precursor = 1 mc/mole in order to facilitate comparison of different experiments.)

Expt. no.	Labelled precursor	Supplement to basal diet	Specific radioactivity (μc/mole) of choline* isolated from: Viscera	Carcass	Total body	Specific radioactivity (μc/mole) of trimethylamine obtained by degradation of choline from: Viscera	Carcass	Total body	Percentage of radioactivity of choline in methyl groups: Viscera	Carcass	Total body
18	2% [α-^{14}C]Glycine	Choline + cobalamin	512[a]	326[b]	—	208	135	—	41	41	—
19		Choline	182[a]	88·0[b]	—	37·4	26·2	—	21	30	—
4	0·5% [α-^{14}C]Glycine	Cobalamin	—	—	103[b]	—	—	24·0	—	—	23
5		Choline + cobalamin	—	—	47·6[b]	—	—	8·5	—	—	18
20		Choline + cobalamin	119[d]	86·0[a]	—	31·8	20·6	—	27	24	—
6†		Choline	—	—	44·8[b]	—	—	10·4	—	—	23
7		Choline	—	—	23·1[b]	—	—	—	—	—	—
21		Choline	59·3[d]	25·6[c]	—	9·4	6·2	—	16	24	—
8	0·7% L-[β-^{14}C]Serine	Cobalamin	—	—	217[b]	—	—	—	—	—	—
9		Choline + cobalamin	—	—	110[b]	—	—	61·8	—	—	56
10		Choline	—	—	69·5[b]	—	—	36·0	—	—	52

— Signifies value not determined.

* The radioactivity of the choline was calculated from the assay of the chloroplatinate (see Experimental section) as follows:
(*a*) After correction for the ammonium chloroplatinate content, as determined by Pt analysis.
(*b*) After recrystallization of the choline chloroplatinate to constant radioactivity.
(*c*) After correction for the ammonium chloroplatinate content, as determined by Pt analysis and Kjeldahl estimation.
(*d*) After purification of the choline through the $HgCl_2$ complex.

† The animal in this experiment grew abnormally well, possibly due to intestinal synthesis of cobalamin.

Table 6. *Conversion of formate to choline*

(In all the experiments, diet AAD 2 described in Table 1 containing sodium [^{14}C]formate (0·1%) was fed (see Experimental section). The choline was isolated either separately from the pooled viscera and carcass or from the total body. The isolation and degradation methods used are given in the Experimental section. The specific radioactivities of the choline and trimethylamine have been calculated for a specific radioactivity of precursor = 1 mc/mole in order to facilitate comparison of different experiments.)

Expt. no.	Supplements	Specific radioactivity (μc/mole) of choline* isolated from: Viscera	Carcass	Total body	Specific radioactivity (μc/mole) of trimethylamine obtained by degradation of choline from: Viscera	Carcass	Total body	Percentage of radioactivity of choline in methyl groups: Viscera	Carcass	Total body
11	Cobalamin	—	—	47·5[a]	—	—	—	—	—	—
22	Cobalamin	89·0[b]	64·8[b]	—	67·2	46·2	—	75	71	—
12	Choline + cobalamin	—	—	23·1[a]	—	—	18·8	—	—	81
13	Choline	—	—	9·3[a]	—	—	7·85	—	—	84
14†	1% Methionine + choline + cobalamin	19·3[b]	15·1[b]	—	—	10·5	—	—	58·5	—
		18·0[b]‡	11·2[b]‡	—	—	6·55‡	—	—	69·5‡	—
15†	1% Methionine + choline	16·2[b]	11·6[b]	—	—	7·65	—	—	66	—
		11·6[b]‡	—	—	—	—	—	—	—	—
16†	Choline + cobalamin	52·5[b]	37·0[b]	—	39·9	22·8	—	76	62	—
17†	Choline	31·4[b]	18·8[b]	—	25·7	16·0	—	82	85	—

— Signifies value not determined.

* The radioactivity of the choline was calculated from the assay of the chloroplatinate (see Experimental section) as follows: (*a*) After recrystallization of the choline chloroplatinate to constant radioactivity. (*b*) After purification of the choline through the $HgCl_2$ complex.

† The animals in these experiments were pair-fed (see Table 3).

‡ Sample isolated from animal which was killed one week earlier than the other animals in this experiment.

Table 7. *Conversion of the α-carbon atom of glycine into choline and into the methyl group of methionine under paired-feeding conditions*

(In these experiments four rats, specially bred from cobalamin-deficient stock, were pair-fed (see Table 3) for 10 days a soya-flour diet (diet 3, Experimental section), which contained 1% [α-^{14}C]glycine. The methyl carbon atom of the methionine, derived from the proteins of either the pooled viscera or the carcass, and the choline from the total body were isolated as described in the Experimental section and recrystallized to constant radioactivity. The specific radioactivities of the compounds isolated have been calculated for a specific radioactivity of precursor = 1 mc/mole in order to facilitate comparison with other experiments.)

Expt. no.	Supplement given	Specific radioactivity (μc/mole or μc/g. atom): Methionine methyl carbon atom from: Viscera	Carcass	Choline (from total body)	Trimethylamine moiety of choline	Percentage of radioactivity of choline in methyl groups
23	Cobalamin (5 μg./day added to diet + 5 μg./day orally)	7·4	3·4	34·4	7·1	21
24	None	5·9	3·2	35·4	4·4	12

this case the experimental period was relatively short (10 days) and the results are therefore not strictly comparable.

DISCUSSION

The effect of cobalamin on the growth of rats fed diets deficient in substances containing labile methyl groups

The growth rates obtained in experiments in which food intake was not restricted (Table 2) are in general agreement with those of Schaefer & Knowles (1951) who used diets containing peanut meal and oxidized casein, and those of Stekol & Weiss (1950) who used amino-acid diets similar to those employed in the present work. A diet containing cobalamin, homocystine and cystine, but no methionine or choline, was found consistently to support very slow growth. On the other hand, a diet of similar composition, but in which cobalamin was replaced by a suboptimal amount of choline chloride (5 mg./rat/day) was less regular in its effect; some animals lost weight, whilst others grew at a very slow rate. Combined administration of the same suboptimal amount of choline and of cobalamin produced moderately good growth. It would appear that the supply of 2·5 μg. of cobalamin in our experiments is equivalent to the administration of somewhat more than 5 mg. of choline chloride. We have also found, confirming the observations of Schaefer & Knowles (1951), that the addition to the diet of serine, glycine

or formate, substances which are known to be precursors of labile methyl groups, has no stimulating effect on the growth with any of the diets used.

The growth-promoting effect of cobalamin was absent in experiments in which paired feeding was used (Table 3). Similar observations showing that the marked effect of cobalamin on the growth of rats can be largely attributed to increased food intake have recently been reported by Black & Bratzler (1952) who used somewhat different diets.

The relative importance of glycine, serine and formate as precursors of methyl groups

As already stated above, the labelled substances were fed over relatively long periods in the hope that a steady state would be reached at the end of the experiment which would enable us to assess quantitatively the contributions made by the various precursors to the synthesis of methyl groups. The concept of the steady state implies that the turnover and 'mixing' times of all components of the system under consideration are infinitely small compared with the period of the experiment. Under such conditions the isotope content of a reaction product compared with that of one of its precursors should yield a quantitative measure of the proportion of the product derived from the given precursor.

The present results indicate that this steady state has not been reached, even in experiments in which the labelled compound was administered for 28 days. Thus the isotope content of the choline derived from the viscera always exceeded that of the choline obtained from the carcass, the differences varying between 25 and 100% (Tables 5 and 6). Similar differences were also observed between the glycine isolated from the viscera and carcass proteins respectively (Arnstein & Neuberger, 1953). It thus appears that equilibration between the internal organs and the rest of the body has not been achieved. However, in studies on the metabolism of proteins and other constituents of the internal organs, such as liver and kidney, turnover times have been found which were appreciably shorter than the experimental periods used in most of the present work. It may be accepted therefore that a comparison of isotope data based on results obtained with compounds isolated from the viscera should yield at least semiquantitative conclusions about the metabolic relationships under investigation.

In all experiments the isotope content of the methionine methyl groups was appreciably higher than that of the choline methyl groups (Table 8). The most reasonable explanation of this finding is that the various precursors give rise, in the first place, to methyl groups in methionine and that these are then incorporated into choline by transmethylation. Such an interpretation agrees with the findings of Berg (1951) who used *in vitro* systems. The alternative explanation that the methyl groups are incorporated independently into both choline and methionine and that the former has a much

Table 8. *Comparison of the specific radioactivity of the methyl groups of methionine and choline*

(Details of the isolation and degradation of choline and methionine and of the radioactivity determinations are given in the Experimental section. The specific radioactivity of the methyl groups is expressed as μc/g. atom, calculated for a specific radioactivity of precursor = 1 mc/mole.)

				Specific radioactivity (μc/g. atom)				
				Choline methyl carbon atom			Methionine methyl carbon atom	
Expt. no.	Basal diet	Labelled precursor	Supplement	Viscera	Carcass	Total body	Viscera	Carcass
1	AAD 1	2% [α-^{14}C]Glycine	Cobalamin	—	—	—	128	—
18	AAD 2		Choline + cobalamin	69	45	—	—	—
19	AAD 2		Choline	12·5	8·7	—	—	—
23*	Diet 3	1% [α-^{14}C]Glycine	Cobalamin	—	—	2·4	7·4	3·4
24*	Diet 3		None	—	—	1·5	5·9	3·2
4	AAD 2	0·5% [α-^{14}C]Glycine	Cobalamin	—	—	8·0	17·8	—
5	AAD 2		Choline + cobalamin	—	—	2·8	11·0	—
6†	AAD 2		Choline	—	—	3·5	13·7	—
9	AAD 2	0·7% L-[β-^{14}C]Serine	Choline + cobalamin	—	—	20·6	81·5	—
10	AAD 2		Choline	—	—	12·0	47·4	—
12	AAD 2	0·1% Sodium [^{14}C]formate	Choline + cobalamin	—	—	6·3	33·8	—
13	AAD 2		Choline	—	—	2·6	20·0	—
14*	AAD 2		1% Methionine + choline + cobalamin	—	2·2	—	10·3	—
15*	AAD 2		1% Methionine + choline	—	3·5	—	8·3	—
16*	AAD 2		Choline + cobalamin	13·3	7·6	—	32·0	—
17*	AAD 2		Choline	8·6	5·3	—	28·6	—

* The animals in these experiments were pair-fed.
† The animal in this experiment grew abnormally well (see footnote, Table 4).

slower turnover rate, at least under conditions of a dietary methyl-group deficiency, is not excluded. Table 4 also shows clearly that formate differs strikingly from [β-^{14}C]serine and [α-^{14}C]glycine, in giving rise to a relatively low activity in the β-carbon atom of serine and a relatively high activity in the methionine methyl carbon atom. It would follow that the conversion of formate into methyl groups cannot proceed by a pathway involving serine as an intermediate. The earlier suggestion made by one of us (Arnstein, 1951) and based on short-term experiments, that serine is concerned in this transformation, is therefore incorrect. The radioactivity of the methionine methyl groups after feeding labelled formate was high (Table 4), taking into account the relatively small amount of formate supplied and the fact that the results are calculated on the basis of a constant radioactivity of substance administered. The significance of this observation is difficult to assess, as it is still uncertain whether formate itself is an important physiological intermediate. Moreover, the extent to which the dietary formate was diluted by endogenous 'formate' is not accurately known. Weinhouse & Friedmann (1952) have estimated that endogenous formation of 'formate' by the rat may be of the order of 0·5–1·5 m-moles/day. A calculation based on these figures would suggest that the importance of formate as precursor of methyl groups is of the same order as that of serine.

In the case of serine, however, the degree to which the dietary amino acid was diluted by endogenous synthesis can be assessed by comparison of the radioactivities of the fed serine and of the serine isolated from the viscera (Arnstein & Neuberger, 1953). It can be deduced that, in the absence of dietary methyl groups and in the presence of cobalamin, about 70–75% of the methionine methyl groups of the proteins of the viscera is derived from the β-carbon atom of serine. This calculation is based on the assumption that the amino acid used for methyl group synthesis is derived from the same 'pool' as that incorporated into proteins of the viscera and that a steady state has been reached in the viscera.

The efficiency of the α-carbon atom of glycine as methyl-group precursor depends on its level in the diet (Tables 4 and 5); it increases with the level as does conversion of glycine to the β-carbon atom of serine (Arnstein & Neuberger, 1953). Moreover, the ratio of specific activities of the methionine methyl carbon atom to that of the β-carbon atom of serine was similar to that found on feeding [β-^{14}C]-serine. It would thus seem that the conversion of the α-carbon atom of glycine into methionine methyl groups proceeds through the β-carbon atom of serine.

The results of the present investigation, together with the observations of other workers, are compatible with the following general scheme. The immediate precursor (X) of methyl groups is a compound or group of compounds related to, or, less likely, identical with, formaldehyde or formate. X may be derived from a variety of sources such as acetone, methanol, histidine (Reid & Landefeld, 1951; Soucy & Bouthillier, 1951; Toporek, Miller & Bale, 1952), tryptophan (Knox & Mehler, 1950), serine or glycine; but some of these precursors may not be quantitatively important under physiological conditions. The present work suggests that the β-carbon atom of serine is quantitatively the most significant of these precursors; this is presumably due to the fact that serine is, unlike histidine or tryptophan, a non essential amino acid and is synthesized in relatively large quantities by the rat. Since some of these precursors, especially serine, are readily available to the animal, the rate-determining step in methyl-group synthesis must be the conversion of the precursors into X or that of X into methyl groups.

The present results would be quantitatively compatible with the view that formate, or a close derivative, is the intermediate (X) in the conversion of the β-carbon atom of serine to labile methyl groups; however, the findings of Elwyn, Weissbach & Sprinson (1951) suggest that formate is not an intermediate in this reaction and we have therefore postulated that different one-carbon intermediates may be concerned in methyl-group synthesis from formate and serine.

The effect of cobalamin on methyl-group synthesis

The choline- or methionine-sparing effect of cobalamin found with rats kept on diets deficient in substances containing labile methyl groups is suggestive evidence, but does not constitute a rigorous proof that cobalamin is concerned with methyl-group synthesis. The present experiments lend further weight to this hypothesis. Rats not supplied with cobalamin synthesized labile methyl group to a significant extent (Tables 4–7), but this may be due to the persistence of cobalamin stores laid down before the dietary restriction was imposed or to microbial synthesis of the vitamin in the intestine. Addition of cobalamin to the deficient diet greatly increased the isotope contents of the methyl groups of both methionine and choline (Tables 4–8) independently of the nature of the labelled precursor, provided food intake was not restricted. In the paired-feeding experiments (Tables 4, 6 and 7) this effect of cobalamin was reduced, but still present, indicating that the effect of cobalamin of increasing the incorporation of labelled carbon into methyl groups cannot be explained entirely as being due to increased food consumption leading to enhanced rate of growth.

The observation that this effect of cobalamin was found with all three precursors suggests that the reaction catalysed by cobalamin is either similar for or common to the various precursors and represents the rate-determining step discussed above. The finding that the ratio between the specific radioactivity of the choline and that of the methionine methyl carbon atom is not markedly affected by cobalamin (Table 8) excludes the possibility that this vitamin is concerned with transmethylation.

The distribution of radioactivity within the choline molecule

The relative amount of the total radioactivity of choline present in the methyl groups appears to be relatively constant and characteristic of the labelled precursor fed. Thus with formate 60–85 % of the total radioactivity was found in the trimethylamine moiety and this ratio was not significantly affected by the presence of cobalamin in the diet (Table 6). With [β-^{14}C]serine the activities in the two fragments of choline were about equal and again there was no marked effect of cobalamin (Table 5). These ratios are not easy to explain in the absence of any detailed knowledge of the turnover rate of choline, the speed of transmethylation from methionine and possible re-utilization of methyl groups. The lack of effect of cobalamin on the ratios, at least with serine and formate as precursors, may seem surprising. It might have been expected that, with the absence of cobalamin and consequent diminution of methyl-group synthesis, the undiminished transformation of serine to ethanolamine should lead to a relative decrease of the radioactivity in the trimethylamine moiety of choline. However, it is possible that, in the absence of cobalamin, very little choline is synthesized, by transmethylation or otherwise, and the utilization of the labelled ethanolamine is therefore reduced. But the possibility that cobalamin is also concerned in the conversion of serine to ethanolamine cannot be completely excluded.

When glycine was fed at a level of 0·5 %, the proportion of the radioactivity present in the trimethylamine moiety was about 20 % and again there was no effect of cobalamin (Table 5). But with a level of 1 % (Table 7) or 2 % (Table 5) the relative radioactivity in the methyl groups was definitely increased by cobalamin. This finding led us to suggest (Arnstein & Neuberger, 1952) that this vitamin stimulates the transformation of glycine into serine and the same idea was put forward by Stekol, Weiss & Weiss (1952). However, the direct measurement of the radioactivity of the β-carbon atom of serine (Table 4; see also Arnstein & Neuberger, 1953) gives no support to this hypothesis. The synthesis and metabolism of choline is particularly complicated owing to the intricate inter-relationship between serine, ethanolamine, methyl-group synthesis and transmethylation. With methionine methyl groups the position is simpler and the conclusion reached in the present work that cobalamin affects methyl-group synthesis is mainly based on the methionine results, particularly those where possible effects of different growth rates were eliminated by paired feeding.

SUMMARY

1. The conversion of the β-carbon atom of serine, the α-carbon atom of glycine and the carbon atom of formate into the methyl groups of choline and methionine has been studied quantitatively by feeding completely defined diets containing these precursors, suitably labelled with ^{14}C, to young rats over prolonged periods.

2. In all experiments the radioactivity of the methionine methyl groups greatly exceeded that of the choline methyl groups, suggesting that most of the newly synthesized methyl groups are first incorporated into methionine and are then transferred to choline by transmethylation.

3. With diets devoid of preformed methyl groups, but containing [β-^{14}C]serine and cobalamin, the methyl group of the visceral methionine had 70 % of the radioactivity of the β-carbon of serine isolated from the same source. This indicates that the hydroxymethyl group of serine is quantitatively the most important precursor of synthesized methyl groups.

4. In similar experiments with [α-^{14}C]glycine, the α-carbon atom of glycine was found to be relatively inefficient as a precursor of methyl groups, particularly when the level of glycine in the diet was not unduly high.

5. Formate was found to be an efficient precursor of methyl groups, but its conversion to the hydroxymethyl group of serine was relatively poor. The results are compatible with the assumption that formate is extensively converted to a substance which is an intermediate in the synthesis of methyl groups from serine.

6. Cobalamin increased the conversion of all three precursors into the methyl groups of methionine and into both the methyl groups and the ethanolamine moiety of choline. The stimulating effect of cobalamin on methyl-group synthesis was reduced, but still present, in paired-feeding experiments. With diets containing 2 % [α-^{14}C]glycine, cobalamin increased the relative incorporation of isotope into the methyl groups of choline, compared with that into the ethanolamine moiety.

We wish to thank Mrs F. Higginson and Mr A. Spiers for technical assistance and Dr W. F. J. Cuthbertson for supplying the animals and diet used in some of the experiments.

REFERENCES

Arnstein, H. R. V. (1950). *Biochem. J.* **47**, xviii.
Arnstein, H. R. V. (1951). *Biochem. J.* **48**, 27.
Arnstein, H. R. V. (1952). *J. chem. Soc.* p. 4527.
Arnstein, H. R. V. & Neuberger, A. (1951). *Biochem. J.* **48**, ii.
Arnstein, H. R. V. & Neuberger, A. (1952). *Biochem. J.* **50**, xxxviii.
Arnstein, H. R. V. & Neuberger, A. (1953). *Biochem. J.* **55**, 271.
Bennett, M. A. (1950). *J. biol. Chem.* **187**, 751.
Bennett, M. A., Medes, G. & Toennies, G. (1944). *Growth*, **8**, 59.
Bennett, M. A. & Toennies, G. (1946). *J. biol. Chem.* **163**, 235.
Berg, P. (1951). *J. biol. Chem.* **190**, 31.
Black, A. & Bratzler, J. W. (1952). *J. Nutr.* **47**, 159.
Campbell, P. N. & Work, T. S. (1952). *Biochem. J.* **50**, 449.
Du Vigneaud, V., Chandler, J. P., Moyer, A. W. & Keppel, D. M. (1939). *J. biol. Chem.* **131**, 57.
Du Vigneaud, V., Cohn, M., Chandler, J. P., Schenck, J. R. & Simmonds, S. (1941). *J. biol. Chem.* **140**, 625.
Du Vigneaud, V., Ressler, C. & Rachele, J. R. (1950). *Science*, **112**, 267.
Du Vigneaud, V., Ressler, C. & Rachele, J. R., Reyniers, J. A. & Luckey, T. D. (1951). *J. Nutr.* **45**, 361.
Du Vigneaud, V., Simmonds, S., Chandler, J. P. & Cohn, M. (1945). *J. biol. Chem.* **159**, 755.
Du Vigneaud, V., Verly, W. G. & Wilson, J. E. (1950). *J. Amer. chem. Soc.* **72**, 2819.
Du Vigneaud, V., Verly, W. G. L., Wilson, J. E., Rachele, J. R., Ressler, C. & Kinney, J. M. (1951). *J. Amer. chem. Soc.* **73**, 2782.
Elwyn, D., Weissbach, A. & Sprinson, D. B. (1951). *J. Amer. chem. Soc.* **73**, 5509.
Gillis, M. B. & Norris, L. C. (1949). *J. biol. Chem.* **179**, 487.
Hubbell, R. B., Mendel, L. B. & Wakeman, A. J. (1937). *J. Nutr.* **14**, 273.
Jonsson, S. & Mosher, W. A. (1950). *J. Amer. chem. Soc.* **72**, 3316.
Jukes, T. H. & Stokstad, E. L. R. (1951). *J. Nutr.* **43**, 459.
Knox, W. E. & Mehler, A. H. (1950). *J. biol. Chem.* **187**, 419.
Neuberg, C. & Kerb, J. (1912). *Biochem. Z.* **40**, 498.
Popják, G. (1950). *Biochem. J.* **46**, 560.
Rees, M. W. (1946). *Biochem. J.* **40**, 632.
Reid, J. C. & Landefeld, M. O. (1951). *Arch. Biochem. Biophys.* **34**, 219.
Sakami, W. (1950). *J. biol. Chem.* **187**, 369.
Sakami, W. & Welch, A. D. (1950). *J. biol. Chem.* **187**, 379.
Schaefer, A. E. & Knowles, J. L. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 655.
Schaefer, A. E., Salmon, W. D. & Strength, D. R. (1949*a*). *Proc. Soc. exp. Biol., N.Y.*, **71**, 193.
Schaefer, A. E., Salmon, W. D. & Strength, D. R. (1949*b*). *Proc. Soc. exp. Biol., N.Y.*, **71**, 202.
Siegel, I. & Lafaye, J. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 620.
Simmonds, S., Cohn, M., Chandler, J. P. & Du Vigneaud, V. (1943). *J. biol. Chem.* **149**, 519.
Soucy, R. & Bouthillier, L. P. (1951). *Rev. canad. Biol.* **10**, 290.
Stekol, J. A. & Weiss, K. (1950). *J. biol. Chem.* **186**, 343.
Stekol, J. A., Weiss, S. & Weiss, K. W. (1952). *Arch. Biochem. Biophys.* **36**, 5.
Strength, D. R., Schaefer, A. E. & Wilson, W. D. (1951). *J. Nutr.* **45**, 329.
Toporek, M., Miller, L. L. & Bale, W. F. (1952). *J. biol. Chem.* **198**, 839.
Weinhouse, S. & Friedmann, B. (1952). *J. biol. Chem.* **197**, 733.
Weissbach, A., Elwyn, D. & Sprinson, D. B. (1950). *J. Amer. chem. Soc.* **72**, 3316.

The Synthesis of Glycine and Serine by the Rat

By H. R. V. Arnstein and A. Neuberger
National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 3 *February* 1953)

It has been known for almost 50 years that glycine can be synthesized by mammals. Thus Wiechowski (1906) and Magnus-Levy (1907) showed that animals fed benzoate over long periods excreted more hippuric acid than could be accounted for either by the glycine present in their body proteins or that ingested. Later, McCoy & Rose (1937) and Rose, Burr & Sallach (1952) using diets containing pure amino acids demonstrated clearly that neither glycine nor serine has to be supplied to the young rat in order to obtain a good rate of growth. It has also been shown that other mammalian species, including man, can synthesize amounts of glycine sufficient to maintain body weight and nitrogen equilibrium and to support a normal growth rate. The hen also can synthesize glycine, but not at a rate permitting optimum growth, as shown by the fact that growth can be greatly improved by addition of glycine to a diet deficient in this amino acid (Almquist, Stokstad, Mecchi & Manning, 1940; Almquist & Grau, 1944). The turkey, too, requires glycine for optimum growth (Jukes, Stokstad & Belt, 1947), although the amounts needed appear to be lower than in the hen (Kratzer & Williams, 1948). It thus appears likely that glycine is a 'semi-essential' amino acid for all birds.