APPENDIX

No.	Samples from	Description of slices	Cooking quality, taste etc.	Moisture content of chips Per cent
1	Coimbatore Paddy Station	Brownish white thin slices rind intact	Insipid darkened on cooking	5·74
2	Kasargod	do.	Insipid	8·21
3	Palur	White thin slices	Inspid, darkened slightly	10·28
4	Ambasamudram	Brownish white rind intact	Inspid	9·10
5	Taliparamba	do.	do.	7·36
6	Gudiyattam	Grey white	No taste	6 04
7	Central Farm, Coimbatore	Thin white slices	Taste not bad	5·84
8	Pilicode	Brownish white	Insipid, darkened	7·95
9	Aduthurai	White big slices	Sweet, darkened	8·65
10	Tindivanam	White thin chips	Good—No colouration	5·77
11	Nileshwar	Light brown thin slices	Inspid—darkened	7·64
12	Pattukottai	White small thick slices	Insipid, turned brown	7·81
13	Koilpatti	Brownish thin slices	Insipid, darkened completely	9·05
14	Pattambi	Dull white big slices	Not palatable	7·14
15	Kallar	White thick slices	Insipid, darkened	8·10

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Transmethylation and Methyl Synthesis

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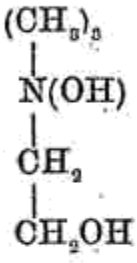
Introduction: Converging lines of research by nutritonists, chemists and clinicians lead to something entirely new in dietary requirements. Nutritional requirement had usually been in terms of whole complex compounds like the fats, carbohydrates or vitamins or in terms of simple elements like calcium, phosphorus etc. But for the first time and for probably the only unique instance of dietary requirement a group like the methyl group emerges out as an entity in Nutrition. Its discovery came about in the study of the diverse metabolic reactions of apparently totally unrelated compounds like casein, cysteine, methionine, choline, folic acid and recently Vitamin B_{12}.

Research on the amino acids on the one side, with the search for lipotropic substances on the other and lately the vitamin studies soon

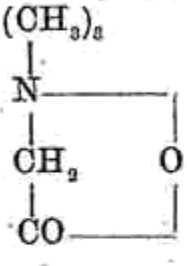
focussed particular attention to this group as of such great importance in dietary requirements that as a result of numerous further studies since done, it has come to stay as an essential nutrient which has to be supplied to the animal for growth and metabolic function.

Source of Methyl Groups: This biologically important group of compound is found in a number of compounds which have been called methyl donors:

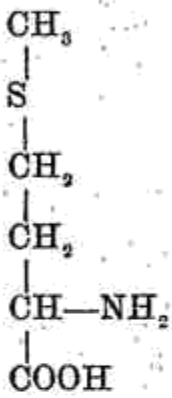
Choline



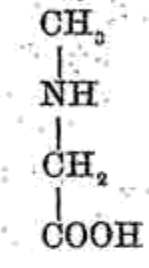
Betaine



Methionine

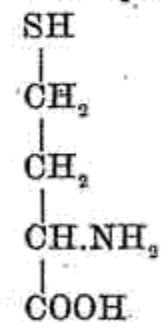


Sarcosine

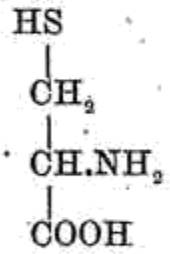


These compounds have in common what is now known as "biologically labile methyl groups." By this is meant, that all methyl groups as a class are not all biologically useful unless they are found in specific compounds which allows the methyl group to be labile or transferable from them to what are now known as "methyl acceptors." Under the class of methyl acceptors or receptors the following could be mentioned:

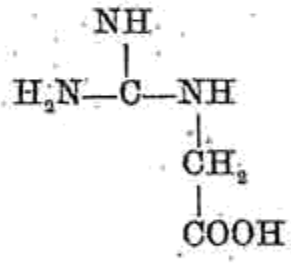
Homocystine



Cysteine



Guanido acetic acid



These could take on methyl groups to form other biologically important compounds and this process of transferring methyl groups is known as transmethylation. Thus having defined the main terms involved in this study and the compounds that play a role in the nutrition of the methyl group a chronological review of the works that lead to the discovery of the group would make the picture complete.

Transmethylation: The phenomenon of transfer of methyl groups in biological systems came to be identified as a result of work done in almost entirely different fields as mentioned at the outset. These could be classified and discussed under: 1. Amino acid research; 2. Search for lipotropic and anti-lipotropic substances; 3. Vitamin research.

Amino Acid Research: Jackson and Block (17) working on diets low in casein for rats, found improved growth with the addition of methionine. Meanwhile to check some contrary findings of the time on fatty livers in relation to low casein and high casein diets, with and without the amino acid cystine, Tucker and Eckstein (28) set up an experiment to find the effect of added cystine in a low protein high fat diet. They found that cystine caused fatty livers when added to a high fat ration and that if the other sulfur containing amino acid viz., methionine was supplied this condition was prevented. Subsequently Rose and his associates (24) using purified amino acids showed that the addition of cystine alone to a diet deficient in both cystine and methionine was practically without any effect while on the other hand the incorporation of methionine in such a diet permitted rapid growth. Here was a poser which needed clarification, for, if the body needed sulfur containing amino acids to replenish the high content of sulfur in keratin proteins such as wool, hair, nails and hooves, in hormones such as insulin, etc., why should there be a difference in the growth response of methione and cystine, which were both sulfur containing amino acids?

A clue in the metabolism of the two sulfur containing amino acids was brought about by the work of Tarver and Schmidt, who fed methioine with labelled sulfur and isolated cystine with the labelled sulfur (29), thus showing the conversion of methionine to cystine. So, if the methionine could supplant the function of cystine, by synthesising it in *vivo*, it soon became evident that sulfur was not the key note for the dietary efficiency of methionine. It should therefore have something which cystine does not contain.

It was known at this time that the phospho-creatine is hydrolysed and re-synthesized during muscle action. Du Vigneaud et al (2, 10) showed by means of isotopic elements in D. L. Methionine that this amino acid contributes its methyl group for the conversion of guanido acetic acid to creatine. Thus, it became evident by 1941, that the methyl group was the important factor in the varied reactions obtained with the sulfur containing amino acids.

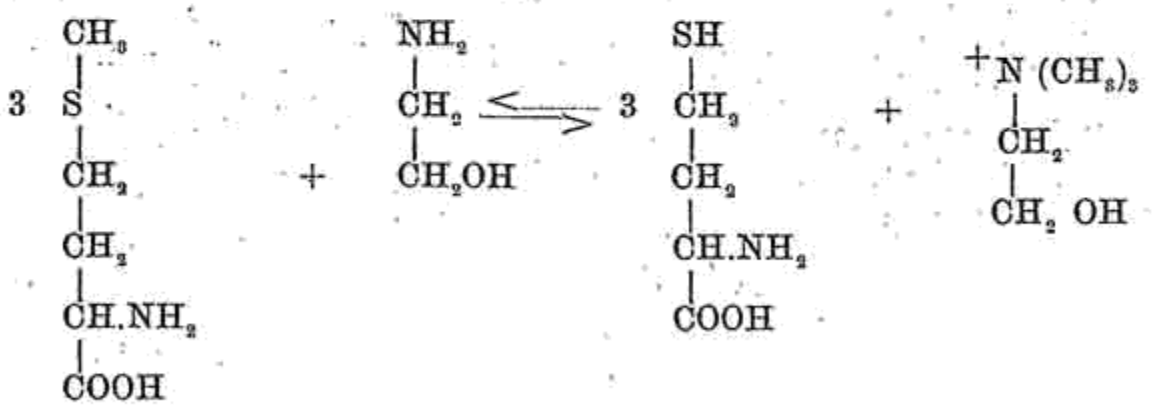
Search for Lipotropic and Anti-Lipotropic Substances: Side by side with the knowledge accumulating on amino acids, facts began to accumulate on substances which cause certain clinical symptoms in the animal system. These symptoms were invariably found to be (i) severe degeneration of liver cells concommittant with hemmorhage and intestinal inflamation (ii) degeneration occurring in the convoluted tubules in the kidneys (iii) deposition of fat in the liver.

Best, Hershey, and Huntsman (1, 2) had observed as early as 1932 that a deficiency of choline, betaine or methionine in the diet of young rats or dogs causes the deposition of fat in the liver. Du Vigneaud

(8) proposed homocystine as a possible intermediate in the conversion of methionine to cystine. This was found to cause fatty livers in the absence of methionine or choline, i. e., it was anti-lipotropic. Glynn, Hinswoth, and Neuberger (14) kept rats on a diet deficient in methionine and cystine and observed the development of massive hepatic necrosis which was prevented by methionine. Best and his co-workers (2) had reported that lecithin in the egg yolk was capable of preventing fatty livers and that the active lipotropic agent in lecithin is choline. Thus it became established that methionine and choline were lipotropic while homocystine and cystine were anti-lipotropic. Sulphur was not the incriminating factor in fatty livers for the sulphur containing compounds were ranged on either side. The answer to this enigma came from Du Vigneaud in 1942 and may be best put in his own words:

"This striking difference in lipotropic activity between homocystine and methionine was not inconsistent with the idea that the methyl group of methionine played an important role in the synthesis of choline by the body. Thus what was otherwise a perplexing similarity of action on the part of two apparently totally unrelated compounds such as methionine and choline, was, according to this hypothesis of transmethylation, readily understandable. The possession of a labile methyl group as the characteristic which choline and methionine had in common was not suspected as the explanation of this lipotropic activity until homocystine-methionine studies indicated a metabolic inter-relationship between choline and methionine" (11).

Thus transmethylation—the transfer of a methyl group from one compound to another—seemed to answer the apparent enigma. The scheme of transmethylation may then be pictured as follows:



Transmethylation has now been proved beyond doubt by a number of subsequent works, but the subject of methyl groups did not however become a closed chapter. It soon became interlaced with the subsequent studies of the B-Vitamins.

B-Vitamins and Methyl Groups: **Niacin:** Handler and Dann (16) found that 1% nicotinic acid or its amide caused toxic manifestations

with concomittant loss of weight, which was counteracted by methionine but not by cystine or homocystine. Apparently nicotinic acid created a labile methyl group deficiency which was corrected by methyl donors.

Folic Acid and Vitamin B_{12}: Toennies, Bennett and Medes (30, 31) repeatedly came across conflicting results with rats in that they were able to obtain growth with methyl free homocystine containing diets. Analysis of the body showed an actual increase of methionine during the experimental period while the diet was free of labile methyl groups.

When a sulfasuxidine, along with eight B vitamins was given, the growth ceased (3). It seemed therefore that the sulfasuxidine had inhibited the formation of a methylation factor distinct from the essential growth vitamins. This lead them to conclude that "there may be vitamin factors of either dietary or intestinal origin, the presence of which may enable the animal to compensate for the absence of dietary methyl donors by biosynthetic means of its own or intestinal bacteria." When liver extract was added to such a methyl free homocystine diet in place of the B vitamins, growth was obtained.

Patton and co-workers (22) noted in chicks that the growth promoting effects of methionine as a supplement to a corn soybean diet was no longer observed when 2% sardine meal was added to basal diet. Cunha et al (6) working with pigs found that Vitamin B_{12} and APF were similar from the standpoint that methionine did not help in growth when fed in addition to either of them and that methionine improved growth of pigs only in the absence of an APF supplement. Shive (27) found that methionine and vitamin B_{12} could function interchangeably in enabling growth of *Escherchia coli* to take place on a medium containing sulfanilamide. In rats, Schaeffer and coworkers (26) demonstrated the protection afforded by B_{12} against kidney damage produced by a diet low in choline and methionine. Drill (7) showed the lipotropic effect of B_{12} in rats. Gills and Norris (15) find that the inclusion of a source of APF in their basal diet obviated the need for supplementary methylating compounds for chicks. All these studies seemed to indicate a definite relationship between methylating compounds and B_{12}.

Next came the observations on folic acid by Bennett (4) which partly cleared up the exact relationship. She reported that folic acid in the absence of sulfasuxidine promoted growth on a methyl-donor-free-diet, but there were some cases of growth in the presence of sulfasuxidine also. This indication of the rat's ability to utilise homocystine even though the intestinal folic acid synthesis had been checked by the sulfa drug was interpreted by her to mean that folic acid was not the only factor involved and that some other factor probably vitamin B_{12}, by being stored in varying degrees in the pre-experimental period might probably

be involved. In a subsequent experiment (5) she reported that B_{12} plus folic acid gave an effect similar to that obtained earlier with crude liver extracts with rat's on the methyl-free-homocystine diet.

Patrick (23) found in chicks that methionine and B_{12} gave the same growth response and when both were added the growth response was no greater than either. Jukes, Stokstad and Broquist (18) report B_{12} as involved in both the methionine and choline requirement of chicks.

So, from all these it was evident that vitamin B_{12} was somehow concerned in the synthesis of methyl groups in the animal system. Whether these were formed in the enzyme system of the tissues or effected through the intestinal bacteria was the next aspect to be investigated. Oginsky (21) using rat liver homogenate found that liver from B_{12} deficient rats exhibit a lower ability to form methionine from homocystine and either choline or betaine, as compared with those from animals dosed with Vitamin B_{12}. In vitro activation of the methionine forming system by addition of solutions of crystalline Vitamin B_{12} (Merck), proved unsuccessful. Hence though B_{12} is concerned in vivo synthesis of methyl groups, its mechanism is still not clear. That the biological synthesis of methyl groups in the presence of B_{12} was only by tissues was shown by the use of germ free animals (12).

Vitamin B_{12} and folic acid therefore seems to bear some relationship to labile methyl metabolism and its synthesis in the animal system.

The source of the carbon from which the synthesised methyl group is obtained has been studied in recent times and though the reports are conflicting, it may be said to be probably derived from glycine or serine.

Sakami and associates (25) report the incorporation of the methyl group of labelled acetone and of formate into the methionine and choline methyl groups. While Jonsson and Mesher (19) fed serine labelled with C^{14} in beta position and isolated choline with radio activity in the methyl group and believes there is reason to state that pteroylglutamic acid and Vitamin B_{12} may be involved in these transformations.

Weissbach et al (32) reports that L-serine is not only a source of ethanol amine portion of the choline but also of its methyl carbon atoms.

Elwyn and Sprinson (13) showed the extensive synthesis of methyl group of thymine in the adult rat. The activity of the methyl carbon atom of thymine following the administration of beta labelled serine accounted for 90% of the total activity of the molecule and was about 2·5 times that of the methyl groups of choline. The authors suggest that the reported role of folic acid and Vitamin B_{12} in the synthesis of methyl groups and the common origin of the methyl groups of choline

and thymine and the 2 and 8 positions of uric acid offers an explanation for the known replaceability of folic acid or Vitamin B_{12} by thymine in certain deficient micro-organisms.

These results undoubtly show that synthesis of the methyl group is possible in the animal system and need not be an essential supplement, provided fairly high levels of Vitamin B_{12} and folic acid are furnished.

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