On the utilization of hydrogen isotopes for the study of the metabolism of the methyl group

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Proceedings of the Third International Congress of Biochemistry, Brussels 1955
edited by C. LIÉBECQ
Academic Press Inc., Publishers — New York 1956

Contents and list of contributors : see back cover

On the utilization of hydrogen isotopes for the study of the metabolism of the methyl group

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(*Received* 20 *September* 1955)

I wish to congratulate Dr. Cantoni on his excellent lecture on labile methyl metabolism. Dr. Cantoni has clearly distinguished between transmethylation and labile methyl synthesis. I remember that, when labile methyl synthesis was first demonstrated to occur from one carbon units such as formate or formaldehyde (1), it was suggested that transmethylation always occurred through such intermediates. Dr. du Vigneaud then insisted that transmethylation means a transfer of the carbon of the methyl group with its three hydrogens from one molecule, the methyl donor, to another one, the methyl acceptor. The concept of transmethylation precludes the existence, as intermediates, of substances like formate, formaldehyde or their equivalents which no longer possess three hydrogens attached to the carbon.

The problem was thus to prove that transmethylation reactions occur in living cells. The nutritional experiments which showed that, in the diet given to rats, choline could be replaced by methionine, or methionine by choline and homocystine, and the tracer experiments which followed either the carbon or the hydrogen of the methyl group from one molecule to another, were highly suggestive and consistent with the concept of transmethylation. It is very good to have a fair hypothesis that is not contradicted by any fact, but it is still better to have a proof of its correctness. The nutritional and tracer experiments that I have so far mentionned, were not sufficient to eliminate the alternative hypothesis of formate or formaldehyde being intermediates in, for instance, the synthesis of choline from methionine and aminoethanol.

Du Vigneaud and his coworkers have given the much needed proofs of the reality of one biological transmethylation (from methionine to choline). You all know the two classical experiments of du Vigneaud :

(*a*) In one experiment, rats were fed, over a long period, a diet containing, as the only source of labile methyl groups, methionine labelled with deuterium in the methyl group; the animals were then sacrificed and the choline isolated from the carcass. The deuterium concentration of the methyl of the carcass choline rose to about 90 % of the deuterium concentration of the methyl of the methionine fed (2). In this experiment, less than one third of the hydrogen atoms of the methyl groups were lost when the carbon migrated from methionine to choline; in other words, many carbon atoms migrated with their three hydrogens : this is, by definition, a transmethylation reaction.

(*b*) In another experiment, rats received methionine labelled with ^{14}C and deuterium in the methyl group; the ratio D/^{14}C in the methyl group of the choline isolated from the carcass was identical with that of the methyl of the methionine fed; the conclusion is again that the carbon atoms of the methyl groups migrated from one compound to the other with their three hydrogens (3, 4).

When Dr. Cantoni says that methionine must be activated to S-adenosylmethionine to give its methyl group to an acceptor, it seems logical to admit that this sequence of bioreactions intergrates within the concept of transmethylation. But I wonder whether a direct proof of it might not be useful; I suggest to Dr. Cantoni to doubly label the methyl of the S-adenosylmethionine and to test, in his *in vitro* system, whether the isotope ratio in the methyl of the methylated acceptor is the same as in the labelled donor.

We know that the labile methyl group can be oxidized to CO_2 through the intermediary oxidation steps of formaldehyde and formate (5); we also know that these intermediates can be used for methyl synthesis (1). Between the labile methyl groups of two compounds two pathways seem possible : transmethylation, and oxidation followed by reduction. If one believes that, in a particular case, the reaction is one of transmethylation, it is still not possible to omit to prove that it is so.

I have recommended the use of the double labelling technique to prove transmethylation; I have now to add a few words of caution. To doubly label a methyl group, one has to use an isotope of hydrogen, either deuterium or tritium, and these isotopes do not behave like protium, hydrogen mass 1, which is nearly the only isotope present in ordinary hydrogen : the bond between C and H is much stronger when the hydrogen is deuterium than when it is protium, and still stronger when it is tritium.

If, as is the case in transmethylation reactions, there is no breaking of the C-H bonds, one may hope to have no isotope effect and, in this particular case, deuterium or tritium is a true tracer.

The situation is very different if some of the C-H bonds are split : the C-protium bonds are preferentially split and the C-D or C-T bonds are preferentially retained; finally the number of bonds broken is in excess of what the deuterium or tritium content of the reaction product indicates.

The possible magnitude of this isotope effect is really startling. In 1951, in Dr. du Vigneaud's laboratory, we injected subcutaneously to rats a mixture of $^{14}CH_3OH$, CD_3OH and CH_2TOH and we measured the ^{14}C, D and T in the methyl groups of the choline isolated from the carcass; we knew that methanol was a precursor of the labile methyl group (6) and that conversion of methanol to choline methyl occurred through oxidation of the methanol methyl followed by reduction (4). If,

during these bioreactions, there had been no isotope effect, the ratio T/D ought to have been the same in the isolated choline methyl group as in the methanol methyl group; but we found it to be three times higher in the choline methyl than in the methanol methyl : the ratio D/^{14}C in the choline methyls was 22 % and the ratio T/^{14}C 72 % of what they were in the methanol methyl (7); this comparison gives an idea of the possible magnitude of the isotope effect in the very kind of biochemical reactions that we are studying in this colloquium.

This work on hydrogen isotope selection in connection with methyl metabolism which I initiated in 1951, was continued in Dr. du Vigneaud's laboratory by Dr. Rachele and later by Dr. Aebi from Switzerland. Dr. Rachele and E. Kuchinskas, with the collaboration of Dr. Eidinoff from the Sloan Kettering Institute, injected subcutaneously to rats a mixture of $^{14}CH_3OH$, CH_2DOH and CH_2TOH; the difference between this experiment and the preceeding one is that CD_3OH has been replaced by CH_2DOH; in the second experiment, the ratio D/^{14}C in the choline methyls was 66 % and the ratio T/^{14}C 84 % of what they were in the methanol methyl (8).

We shall try to interpret these experiments. When a hydrogen labelled methyl group undergoes oxidation, the isotope content of the reaction product depends on which hydrogen isotope has been used (deuterium or tritium) and on how the molecule has been tagged (whether one or the three hydrogens of the methyl group have been labelled). The results obtained with the CD_3 label are probably the most representative of what normally happens to the CH_3 group, because there is no competition, within the same methyl group, between the hydrogen atoms; but the oxidation rate of $-CD_3$ is smaller than the oxidation rate of the normal $-CH_3$. I recall Thorn's experiment on the oxidation rate of deuteriosuccinic acid by the succinic-dehydrogenase : the reaction rate was smaller for dideuteriosuccinic acid than for normal succinic acid, and still smaller for tetradeuteriosuccinic acid (9).

The reduction of the oxidation reaction rate due to the deuterium label, is important in experiments where the double labelling technique is used; the doubly labelled compound is usually prepared by mixing a carbon labelled species and a hydrogen labelled species, for instance a $^{14}CH_3$- species and a CD_3- species; when the methyl is oxidized, the reaction rates are different for the two molecular species and the ratio D/^{14}C does not tell what happens to a normal CH_3- group. It would seem much better to have one molecular species labelled simultaneously in the carbon and the hydrogens, for instance a $^{13}CD_3$- compound; although the rate of oxidation of this species is certainly different from that of normal $^{13}CH_3$-, it might be hoped that the reaction mechanism would not be altered and that the change of D/^{13}C ratio would be parallel to the change of H/^{13}C ratio during the oxidation steps.

The least desirable label to use to trace the fate of a methyl group is CH_2T, because T is so different from protium; not only oxidation reaction rates are different for the CH_2T- and CH_3- species, but there is, within the grouping itself, a competition between protium and tritium. But that label, bad as it is, is the only one that can be used to solve certain problems; we had to employ it to study the biological origin of the methyl group of adrenaline.

In conclusion, I shall say that it is almost impossible to know by the isotope technique what happens, quantitatively and qualitatively, to the normal-CH_3 group on oxidation. Qualitatively, the best approach is to use the $^{13}CD_3$-label : it can probably tell the level of oxidation reached; but it does not tell how many methyl groups have undergone oxidation. Moreover, there is always the danger that oxidation of the methyl group followed by reduction, might be interpreted as transmethylation by people unaware of the isotope selection, because the heavy hydrogen isotopes are so firmly attached to the carbon.

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