# Influence of N-Methylformamide on $C^{14}$-Formate Incorporation III. Effect of Time of Administration of Isotope. (25964)

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Our previous work(1,2,3) has shown that N-methyl-formamide (NMF) has marked effects on formate utilization in rat livers and tumors. These effects, under particular conditions of the experiments, consist of a large stimulation of incorporation of formate into the nucleic acids, glycogen and protein of the liver, and inhibition of incorporation of formate into nucleic acids of tumor tissue. The conditions under which these effects were observed were: the isotope was given 24 hours after a single large dose of NMF, and the animals sacrificed 24 hours after administration of isotope. When this work was first presented (2) the question was raised as to whether the same type of result would have been obtained at short time intervals after injection of isotope during period of most rapid incorporation. Our data show that the NMF effect is as striking 2 and 6 hours after administration of isotope as at 24 hours. In addition, it was observed, and confirmed, that incorporation of radioactive formate into rat liver nucleic acids follows a multi-peak curve, with a minimum around 4 to 6 hours after injection of isotope.

*Methods.* Male Wistar strain rats (Carworth Farms) weighing 150 ± 10 g were used. Nine rats were injected, at approximately the same time, with a single dose of 2 g/kg of body weight of NMF* administered as 50% solution in physiological saline). Each rat was injected, in groups of 3, with 4 $\mu$c of $C^{14}$-formate,† one group 24 hours after NMF, another at 42 hours and the other 46 hours after NMF injection, appropriate controls of 3 rats/group being injected with isotope at corresponding times. They were all sacrificed 48 hours after injection of NMF and livers of each group pooled in dry ice. Livers were first homogenized in alcohol, alcohol-ether, and ether in Waring blendor and these extracts discarded. The solids were then extracted in the cold 3 times with 5% trichloroacetic acid (TCA) and glycogen isolated from pooled TCA extracts by alcohol precipitation. Glycogen samples were recrystallized to constant radioactivity, and assayed for purity by the anthrone reaction(4). They all varied between 98-101%, using glucose as standard. Nucleic acids were next extracted and individual purines isolated as described previously (1,3). The residues from this extraction were stirred well with 5% TCA at 85°, filtered and washed well with TCA, water, alcohol and ether. These final residues were ground to a fine powder, suspended in 25% ethanol and plated on planchets for determining protein radioactivity. Activities are presented as relative specific activities (RSA) where

$$\text{RSA} = \frac{\text{counts/min./}\mu\text{M isolated compound}}{\text{counts/min./}\mu\text{M injected compound}} \times 100$$

in the case of purines. In the case of combined nucleic acids, glycogen and protein, RSA was calculated on a mg basis.

*Results.* Fig. 1 shows results of NMF treatment on incorporation of formate into combined nucleic acids (Na, counted as sodium salt), glycogen (glyc.), and protein (prot.) of rat liver at 2 hours, 6 hours and 24 hours after administration of isotope. Since all experimental groups had received NMF 48 hours before sacrifice, variations in levels at different time intervals may reasonably be attributed to differences in incorporation of formate at different time intervals after administration. In both nucleic acids and proteins, both control and experimental, lowest levels of incorporation are found at 6 hour interval after administration of isotope.

The level of incorporation of formate into glycogen 2 hours and 6 hours after adminis-

* From Rohm and Haas Co., Philadelphia, Pa.

† Obtained from Technical Assn., Burbank, Calif. Diluted with sodium formate to give specific activity of 1 $\mu$c/$\mu$M.

tration was too low to be of significance in the controls, although NMF apparently increased incorporation or storage of formate in glycogen at these time intervals. However, one cannot rule out the possibility that NMF has no effect on actual rate of utilization of formate for glycogen synthesis, since higher activity may be due to lowered amount of glycogen present. In both the 2 hour and 6 hour experiment we isolated from NMF-treated livers about 40-50% of amount of glycogen isolated from controls, and in the 24 hour experiment about 20% of amount isolated from controls was obtained. Although this was not done quantitatively, the procedures were the same and the livers approximately equal in weight.

Fig. 2 gives comparisons of isolated adenine of the DNA and RNA. The striking NMF effect previously reported(1,2) is evident at all 3 time intervals, and it is obvious that the results 2 and 6 hours after injection of isotope are no more informative in regard to effect of NMF, than the results 24 hours after injection of isotope. The results with isolated guanine were qualitatively identical.

In controls specific activity 6 hours after isotope is significantly lower than either the 2 hour interval or the 24 hour interval, just as

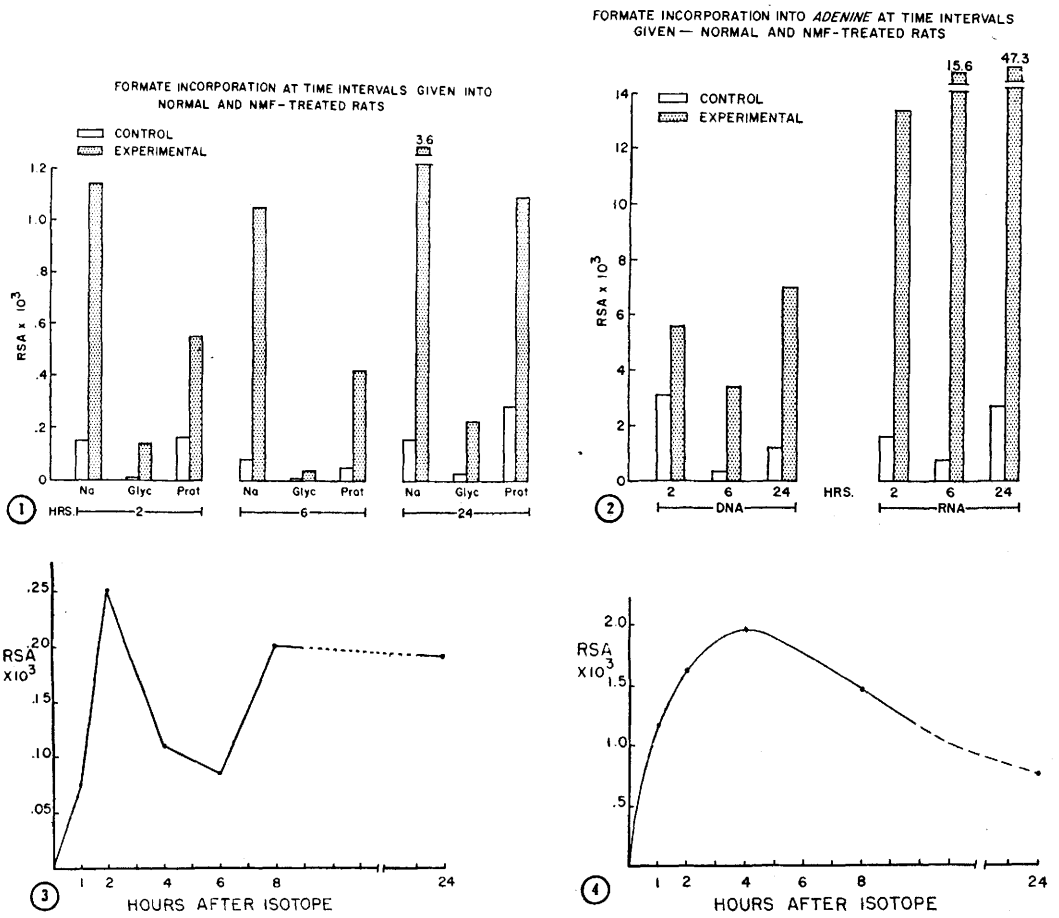


FIG. 1. Formate incorporation at time intervals given into normal and NMF-treated rat livers.

FIG. 2. Formate incorporation at time intervals given into rat liver adenine of normal and NMF-treated animals.

FIG. 3. Formate incorporation at time intervals noted into nucleic acids of normal rat liver.

FIG. 4. Formate incorporation at time intervals noted into nucleic acids of normal rat intestine.

is the case in unfractionated nucleic acids (Fig. 1).

These results prompted us to do the following experiment to see if this was a real effect: 12 rats were each given 4 $\mu$c $C^{14}$-formate; 2 rats were sacrificed at 1 hour, 2, 4, 6, 8, and 24 hours after administration of isotope. Nucleic acids were isolated from combined livers and intestines as described, redissolved in hot 5% NaCl and reprecipitated with ethanol, and counted. The results in liver nucleic acids are graphed in Fig. 3, and in intestinal nucleic acids in Fig. 4.

In the liver, initial incorporation of formate into nucleic acids reached its highest level at 2 hours after injection, with a second peak at 8 hours. The lower RSA at 6 hours than at 2 hours confirms the observations presented in Figs. 1 and 2, although the actual value for 2 hour incorporation was slightly higher in experiment in Fig. 3 (0.25 compared to 0.17 in Fig. 1). The RSA values for 6 hour samples agreed almost exactly (0.1 and 0.09).

The pattern of multiple peaks in the incorporation curve is reminiscent of results presented by Hammarsten *et al.*(5), although the conditions of incorporation which they used are not at all comparable. These investigators injected $N^{15}$-glycine 5 and 4 hours before sacrifice at various time intervals after hepatectomy. Since the time interval from isotope injection to death was constant, they were measuring relative uptake of $N^{15}$ into purines at progressive stages of liver regeneration. They observed 2 peaks of incorporation: 14 hours and 26-52 hours into RNA, and 26-32 hours and 56 hours into DNA. The double peaks may be due, as Hammarsten pointed out, to the complex heterogeneity of the nucleic acids.

Incorporation of formate into intestinal nucleic acids shows a peak of incorporation 4 hours after administration. There are not enough points after that to know whether there is a second peak after the 8 hour value (the 6 hour sample was inadvertently lost). However, it is of interest (and unexplainable at present) that in the same animals liver nucleic acids show this multi-peak effect but intestinal nucleic acids do not.

It seems, therefore, that choosing a time interval after administration of isotope is largely up to the purpose of the investigator. Two main points to consider in comparing the effect of a drug to control values in experiment of this type are: is action of drug discernible at particular time of examination; and is level of incorporation of isotope high enough to give significant counts at this time? In the present case, it had previously been determined that NMF action is present to a high degree at 48 hours(1). The interpretation of this effect remains the same(1,2) whether the isotope is available to the system for 2, 6, or 24 hours.

*Summary.* 1) The effect of NMF on rat liver metabolism has been shown to be qualitatively the same at 2 and 6 hour intervals after administration of the isotope as at 24 hours after administration. 2) The first peak of incorporation of formate into rat liver nucleic acids occurs 2 hours after administration, with a second peak 8 hours or after. Peak of incorporation into intestinal nucleic acids is around 4 hours after administration.

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