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NOTE

Quantitative Evaluation of Metabolites in the Study of Labelled Barbiturics and Hydantoines

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A description of an experimental plan about the use of labelled drugs in drug metabolism studies has already been given [1]. In that paper, we described the procedure and the techniques for the metabolic study of labelled drugs, and gave also advices about the interpretation of the results of these techniques.

In the present paper, we are giving some information about the use of liquid scintillation counting [2] for the quantitative evaluation of the urinary metabolites in the study of labelled barbiturics and hydantoines.

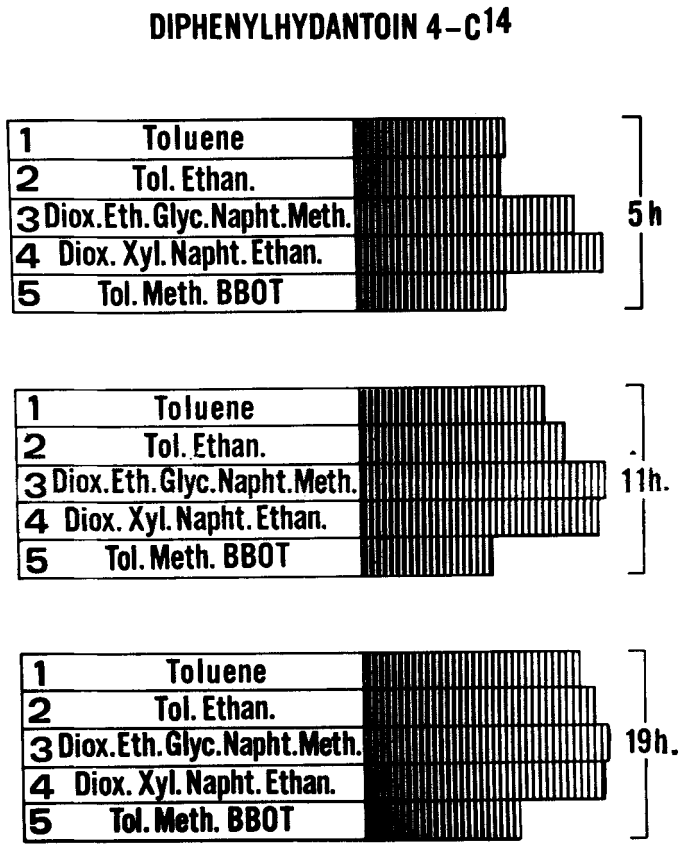
TOTAL URINE RADIOACTIVITY.

It is chiefly through the urine that the organism eliminates the administrated drugs and the products of the metabolism.

In the study of the metabolism of labelled drugs, the method of measuring the radioactivity in the urines is therefore very important. In our laboratory the radioactivity of the urines is measured in two manners, through G. M. counter with thin window, and through scintillation counting. The measures through liquid scintillation counting are more reproducible, especially for the easy preparation of the samples, and they make it possible to calculate the absolute activity. However, it is often necessary, to make parallel measures by G. M.

In fact, the values obtained through liquid scintillation counting are sometimes at variance with those obtained through G. M., especially in the measures of the urines of the first hours after the injection. This can be explained by the fact that the ratios of the metabolites in the urine change, particularly during the first hours.

* From the communication presented at the Symposium on Liquid Scintillation Conuting at the National Physical Laboratory, Teddington, 18-19 October 1966.



1. PPO (4 g) + POPOP (0.05 g) + Toluene - - - 1,000 ml.
2. PPO (4 g) + POPOP (0.05 g) + Toluene - - - 1,000 ml + 100 ml Ethanol.
3. Naphtalene (60 g) + PPO (4 g) + POPOP (0.2 g) + Methanol (100 ml) + Ethylene Glycol (20 ml) + Dioxane - - - 1,000 ml.
4. Naphtalene (80 g) + PPO (4 g) + POPOP (0.05 g) + ((Xylene (5 vol.), dioxane (5 vol.), Ethanol (3 vol.)) - - - 1,000 ml.
5. BBOT (Ciba) (6 g) + Toluene (500 ml) + Methanol (500 ml).

FIG. 1. Relatives values of the activity, measured in different scintillator systems, of the rat urine of three different hours, after administration of DPH-4-^{14}C.

We have measured through liquid scintillation counting the urine of different hours in different scintillator systems, after administration of Diphenylhydantoin-4-^{14}C.

From the Figure 1, we can see that, being the urine a watery system, the best results are always given by the dioxane-naphthalene scintillator

systems, but the differences of counting in the different scintillator solutions change from hour to hour.

This fact also confirms our precedent statement and stresses the importance of measuring the urines parallely through G. M. and liquid scintillation counting.

RADIOACTIVITY OF URINARY METABOLITES.

One of the main purposes of the analysis of the urines, after administration of labelled drugs, is the quantitative and qualitative determination of the urinary metabolites. Particularly, the determination of the amounts of the metabolites as function of time allows us to state the kinetics of the transformation. The quantitative measurement of the metabolites through the planimetrical method and through the disc integration of the peaks present in the recordings of the chromatograms, does not give satisfactory results in our case, on account of the large differences among the peak heights.

The method of the elution of each active sector of the chromatogram for the quantitative analysis, has therefore been chosen. This method allows the quantitative analysis and the production of the metabolites, but it is long and laborious.

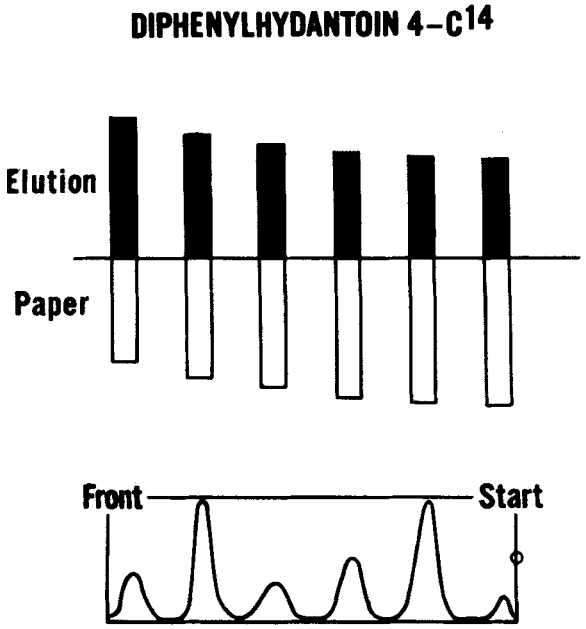
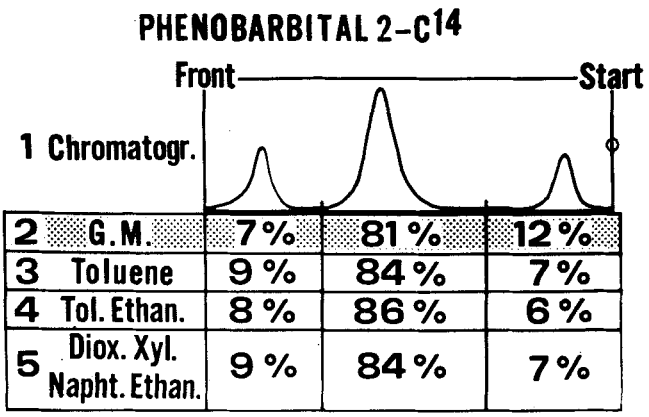
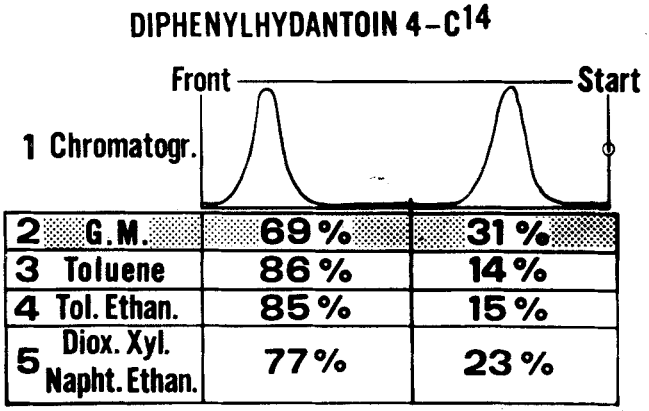


FIG. 2. Ratio calculated by different techniques:
1) Elution of active sectors;
2) Sectors on the paper,
for 6 metabolites present in the urine of rat, after administration of DPH-4-^{14}C.

It would be much easier and save a lot of handling to put the active sectors directly in the scintillation vials.

For this purpose, we compared the values obtained for each metabolite, whether each active sector is eluted and then measured by scintillation counting, or each active sector is cut and then directly put in the scintillator system.





1) Registration of radioactivity after chromatography.
2) Geiger-Müller counter
3) PPO (4 g) + POPOP (0.005 g) + toluene, - - - 1,000 ml.
4) PPO (4 g) + POPOP (0.005 g) + toluene, - - 1,000 ml + 100 ml ethanol;
5) Naphtalene (80 g)' PPO (4 g) + POPOP (0.005 g) + ((xylene (5 vol.), dioxane (5 vol.), ethanol (3 vol.)), - - - - 1,000 ml.

FIG. 3. Comparison of the different amounts of the metabolites of 2 drugs, by G. M. counting and in different scintillator systems.

To make a correct comparison between the values of the radioactivity obtained through these two different techniques, we investigated if there was any absorption of the photons by the chromatographic paper, but this absorption proved to be negligible.

For the Diphenylhydantoin-4-^{14}C, the results are described in the Figure 2.

We have calculated the ratio between the radioactivity values of the metabolites after elution and the radioactivity values of the metabolites on the chromatographic paper. This ratio should be constant, but on the contrary it appears to increase from "start" to "front" on the chromatogram.

This may be probably explained on account of the different chemical structures and the different physical properties of the labelled metabolites, particularly the different solubility in the scintillator system.

We have also tested if each metabolite, after its separation by paper chromatography and successive elution, has different counting efficiency in the different scintillator solutions.

As it is shown in the Figure 3, the results are the following : for the phenobarbital-2-^{14}C we do not observe differences greater than 2% among the relative amounts of the metabolites, when they are measured in different systems, while for the diphenylhydantoin-4-^{14}C differences are observed up to 10%.

In conclusion, in drug metabolism studies, liquid scintillation counting is a very important technique for an easy and quick preparation of the samples, either urine or urinary metabolites must be measured; this technique makes it possible to calculate the absolute activity and to measure the radioactivity of products containing both ^{14}C and ^{3}H.

The disadvantage of this technique in drug metabolism studies consists above all in the impossibility of recovering the samples, metabolites in particular, after they have been measured.

Nevertheless, the liquid scintillation counting is expected to have a great extent in pharmacology, it can also be associated to the gas chromatography technique.

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2. BIRKS, J. B. — *The theory and practice of scintillation counting*. Pergamon Press (1964).