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SHORT COMMUNICATION

ARE LIQUID SCINTILLATION PARAMETERS RELATED TO STEROID STRUCTURES? A PRELIMINARY REPORT

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During many years of *in vitro* experimentation with double-labelled steroid precursors it became clear to us that "overlapping" of ^{14}C counts into the channel whose gains and windows had been set for tritium counting occurred to a different degree in each of the radiometabolites formed. Moreover, while polarity seemed to be one of the factors affecting overlapping—less polar steroids exhibiting the phenomenon to a smaller degree than the more polar ones—the differences could not always be explained by this property and hence, by solubility in organic solvents. In an attempt to gain information on other factors affecting relative counting efficiencies in "upper" and "lower" channels‡, the following assays were performed:

Duplicate samples containing approx. 10,000 d.p.m. of six steroids labelled with ^{14}C at C-4 were purified by usual paper chromatographic and thin-layer techniques and dissolved in two types of scintillation liquors: Mixture 1 contained 3 g of PPO (2,5 Diphenyloxazole) and 100 mg of dimethyl POPOP (1,4-bis-2(4-Methyl-5-Phenyloxazolyl)-Benzene) per l. toluene. Mixture 2 contained 5 g of PPO and 300 mg of dimethyl POPOP per l. toluene. The total volume of each sample was equal to 10 ml. The fluorescent solutes, as well as the radioactive steroids were obtained from New England Nuclear, Boston, Mass. The toluene was of "Electronic Grade" (Carlo Erba, Milano). The specific activity of the steroids was equal to 40 nm/mmol.

A three-channel automatic liquid scintillation counter UNILUX II (Nuclear Chicago) was utilized in all assays. All three channels were calibrated for exact coincidence and the same linearity. This was carried out as follows: first the gain of one channel was set to obtain maximal counts for a ^{14}C standard supplied with the equipment. Then the remaining channels were calibrated until identical readings were obtained. During the procedure windows were kept wide open (100 mV to 10 V). The use of this wide dynamic range amplifier with a 100:1 range, avoids the need for a separate channel calibrated for ^{3}H. Because of this wide dynamic range, a 45% efficiency is still obtained with the tritium standard in channels A and B. It must be considered that the highest efficiency for ^{3}H in this instrument is 58%. Channel A has a better efficiency for the lower pulse height than channel C. Thus the more quenched the sample is, the more "spill-over" counts will be registered in channel A. Standards of Amersham/Searle with different degrees of quenching were measured and showed to give identical readings in the three channels.

Once the proper gains were obtained, channel B was chosen as the one covering the whole spectrum, channel A as the one covering the "lower" region and channel C, the "upper" region. The efficiency of the ^{14}C standard supplied with the instrument in channel B was 90% and that of most steroids assayed was near this value, with a minimum of 82%.

Conditions for the choice of "A" and "C" were the following: (1) B = A + C, (2) the lower level of channel C and the upper level of channel A were chosen so as to obtain maximal variations in channel ratios between one steroid and another (C/A was equal to 10/1).

The counter was allowed to count the samples three times for five minutes, so that for each sample three countings, separated in time, became available. One ^{14}C and one tritium standard were inserted in order to detect any efficiency variation during the course of the measurements. Such variations did not occur.

Once all measurements were averaged, ratios A/B, A/C and C/B were calculated. Of all these parameters, the one in which a correlation with chemical structure could be found was A/C.

Table 1 lists these ratios for the six steroids. Numbers between parentheses indicate increasing polarities of these steroids in the toluene–propylene glycol system [1]. A second number between parentheses, in the case of 11-deoxycorticosterone and dehydroepiandrosterone indicates that a change in the relative polarity position took place when both steroids were chromatographed in petroleum ether–toluene–methanol–water, 5:5:7:3 by vol [2]. The values for all these metabolites were either taken from [3] or from [4].

Three tentative conclusions can be drawn from the above data: (a) When the less efficient scintillation liquor (mixture 1) was employed, a more intense overlapping into the "lower" channel occurred. (b) When this same scintillation mixture was employed, this overlapping increased, as a general rule, with increasing polarity. (c) 11-deoxycorticosterone, in

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‡ The term "quenching" is omitted in most instances and replaced by more precise descriptions of the specific phenomenon occurring in each case.

Table 1. A/C Ratios

Mixture 1 (3 g PPO and 100 mg dimethyl POPOP)		Mixture 2 (5 g PPO and 300 mg dimethyl POPOP)	
Dehydroepiandrosterone (2) (1)	0·0957	Testosterone (3)	0·0905
	0·0957		0·0905
Testosterone (3)	0·0958	11-deoxycorticosterone (1) (2)	0·0915
	0·0960		0·0930
11-deoxycorticosterone (1) (2)	0·0970	17-hydroxyprogesterone (4)	0·0930
	0·0980		0·0930
11-deoxycortisol (5)	0·0978	Dehydroepinadrosterone (2) (1)	0·0945
	0·0994		0·0945
Cortisol	0·1005	11-deoxycortisol (5)	0·0946
	0·1010		0·0948

See explanations in the text. Values for 17-hydroxyprogesterone in Mixture 1: 0·0960 and 0·0990. Values for cortisol in Mixture 2: 0·0935 and 0·0969.

this first series, exhibited a higher degree of overlapping than could be expected from its relative polarity position. (d) When the more efficient scintillation liquor (mixture 2) was employed, two exceptions to the polarity rule became noticeable: 11-deoxycorticosterone and dehydroepiandrosterone. The latter was more striking than the former because of the fact that dehydroepiandrosterone, one of the two less polar steroids listed, exhibited almost the highest degree of overlapping while its more polar isomer, testosterone, possessed the lowest A/C ratio of all steroids analyzed.

This latter observation prompted further assays, this time with the pair progesterone-pregnenolone, utilizing mixture 2 as scintillation liquor. Although the dispersions between duplicates were repeatedly high, an average of 0·0898 the lowest overlapping so far obtained was found for progesterone (0·0910 and 0·0876) while the mean value for pregnenolone was 0·0932 (0·0922 and 0·0942), i.e. between 17-hydroxyprogesterone and dehydroepiandrosterone.

Both progesterone and pregnenolone are less polar than the steroids listed on Table 1, with only little differences between their mobilities in most chromatographic systems.

The evidence obtained from the pair testosterone-dehydroepiandrosterone and, to a minor degree, from the pair progesterone-pregnenolone, speaks in favour of a role of 4-en-3 keto group (conjugated double bond) in avoiding the "quenching" of β particles emitted by ^{14}C atoms, at least when these are introduced into the C4 position of steroid molecules. The alternative or concomitant role of "favoured" positions of the hydroxyl, such as positions C21 in 11-deoxycorticosterone and C17 in dehydroepiandrosterone, cannot be outruled.

Future investigations will be aimed at this goal. Also, A/C ratios will be measured for each of the steroids used in a series of vials containing the same amount of β-activity but increasing amounts of radioinert steroid, so as to approach the amounts expected in typical *in vitro* studies.

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