

## V. Miscellaneous (Metabolism)

### Biosynthesis of $^3\text{H}$ -Bilirubin with the Use of $^3\text{H}$ - $\delta$ -ALA and Studies on the Conditions for Liquid Scintillation Counting

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$^3\text{H}$ -SALA, 265  $\mu\text{Ci}/\text{mM}$  was injected i.v. to rats and days with a biliary fistula, in a dose of 40  $\mu\text{Ci}$  and 500  $\mu\text{Ci}$ , respectively.

Bile was collected at one hour intervals for a total of 24 hours and each sample was counted for  $^3\text{H}$  by a Beckman LS233 type liquid scintillation counter.

Each sample was diluted 10 times with 0.1N NaOH and placed at 10 cm distance and exposed to a Toshiba mercury lamp to effect photodecomposition of bilirubin with the purpose to prevent color quenching in counting  $^3\text{H}$  in bile.

About 18% of the injected  $^3\text{H}$ -labeled- $\delta$  ALA was recovered in bile of rats in a 24 hour collection and also about 18% in bile of dogs in 8 hours.

Radioassay of  $^3\text{H}$  in bile was performed in a Beckman LS233 liquid scintillation spectrometer with use of a toluene/dioxane/cello-solve/naphthalene/PPO.

Counting efficiency of  $^3\text{H}$  in 0.1N NaOH was 29%.

Chromatographic analysis of azopigments which were prepared by adding the diazo

reagent and methanol to bile, showed two bands which seemed to be azopigment A and B, and most of the radioactivity coincided with the azopigment.

Furthermore,  $^3\text{H}$ -labeled crystalline bilirubin was obtained from bile by the method of Ostrow, et al.

This bilirubin showed the same absorption spectrum as authentic crystalline bilirubin in chloroform and the azopigment obtained by the diazotization which coincided with the radioactivity, had the same Rf values as the standard, i.e. Rf 0.62–0.63, on a paperchromatogram.

For counting  $^3\text{H}$ -labeled crystalline bilirubin, bilirubin was dissolved in benzene and exposed to light, then 5 Vol. of bilirubin-benzene solution added to 10 Vol. of a toluene/PPO.

Counting efficiency was 43.7%.

The obtained  $^3\text{H}$ -labeled crystalline bilirubin had a specific activity of 1.6  $\mu\text{Ci}/\text{mg}$  which was about the same as that by Ostrow, et al.