Interaction of Technetium-99m-HM-PAO with Glutathione

TO THE EDITOR: A number of publications have recently appeared in *The Journal of Nuclear Medicine* which dealt with the decomposition of the stereoisomers of technetium-99m-hexamethyl-propyleneamine oxime (^{99m}Tc-HM-PAO), with the lipophilic-hydrophilic transformation, with kinetic analyses of the decomposition, and with the role of glutathione (GSH) in these processes (*1–3*).

During the development of an HM-PAO kit destined for use in our own laboratory, we have already encountered a considerable proportion of the problems mentioned in the publications referred to above. The bulk of our experimental results are in accord with those described in the above publications. In the present communication, I should like to mention briefly those of our results which supplement the published data or which put them in a somewhat different light.

It was reported by Ballinger et al. (*3*) that the stereoisomers of HM-PAO interact to different extents with GSH, and they suggest that this might be the reason for the differences in cerebral retention. We have repeated the experiments they describe and have found that they were perfectly reproducible (effects of GSH concentration and incubation time on the extractability of d,l and meso-HM-PAO from an aqueous GSH phase), but we do not agree completely with the conclusions drawn from the experiment. Ballinger et al. appear to have worked with a non-buffered aqueous medium (at least their article makes no mention of a buffer). Under the experimental conditions they applied, interval 0-10 mg/ml the pH varied between 6.5 and 2.75. We have performed an experiment with Tc-d, l-HM-PAO in which the Ballinger method was used and the pH of the aqueous medium was varied between 6.5 and 2.9 with 0.05 *M* HCl. After a mixing time of 4 min, the following results were obtained:

At pH 6.5, the aqueous phase contained 3.4% ± 0.82% of the radiopharmacon. At pH 2.9, the aqueous phase contained 12.72% ± 1.61% of the radiopharmacon.

In these experiments, n = 5. This result demonstrates that in their experiment Ballinger et al. measured the resultant of the effects of GSH and pH. However, there is no doubt that the pH has only a negligible effect on the lipophilic-hydrophilic transformation. For at a GSH concentration of 10 mg/ml, where the pH was 2.75, the aqueous phase contained 84.6% ± 2.8% of the test substance.

Acknowledging the fact that the pH is approximately neutral under physiologic conditions, we carried out experiments under the previous conditions, using an aqueous medium containing various GSH concentrations, but neutralized with $NaHCO_3$. We obtained the following results:

At a GSH concentration of 0.1 mg/ml and pH 4.4, the aqueous phase contained 78.4% ± 1.9% of the radiopharmacon and contained 4.29% ± r0.627% of the radiopharmacon at pH 7.59 (buffered).

These results demonstrated that in neutral or mildly basic medium GSH had no effect at all on the lipophilic-hydrophilic transformation.

When GSH was replaced by L-cysteine HCl under otherwise identical conditions, we obtained the following results:

At an L-cysteine HCl concentration of 0.1 mg/ml and pH 3, the aqueous phase contained 68.2% ± 2.3% of the radiopharmacon and at pH 7.4 (buffered), the aqueous phase contained 5.87% ± 1.1% of the radiopharmacon.

Since GSH and cysteine exert similar effects, there is a high probability that GSH does not have a specific effect on the lipophilic-hydrophilic transformation.

In our view, these experimental results strongly suggest that, under in vitro conditions, GSH acts on the lipophilic-hydrophilic transformation merely as a reductant, and that this effect can be suspended by neutralization of the medium. This view is supported by our experimental results which (similarly to those of Hung et al.) indicated that increase of the quantity of Sn^{2+} in the HM-PAO kit accelerates transformation of the lipophilic Tc-d, l-HM-PAO complex to the hydrophilic complex (*4,5*).

We consider that the foregoing in vitro experiments demonstrate only that the lipophilic-hydrophilic transformation of Tc-d, l-HM-PAO is greatly accelerated by reductants. Accordingly, any redox system present physiologically in the organism and operating at physiologic pH can be "suspected" for the lipophilic-hydrophilic transformation of Tc-d, l-HM-PAO.

REFERENCES

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REPLY: We thank Dr. Lang for his interest in our work (*1*) and welcome this opportunity to respond to his letter (*2*).

Dr. Lang is quite correct in assuming that our glutathione (GSH) solutions were not buffered and therefore varying pH