terferences as judged by the run-to-run variance expected with new rotors.

In general, end-point tests suffer more from inter-test contaminations than enzymatic tests. For example, triglycerides (10) cannot be run after an ammonia test (11) because of the difficulty of removing glycerol, which is used in high concentrations in one of the reagents in the ammonia test. Thus we do not recommend that washed rotors that originally have been used in one end-point test be reused with a different end-point test.

The washer–dryer system as described in this paper can be an effective means of reducing laboratory costs with no loss of analytical precision or accuracy if used within the limits described. A normal 5-min wash requires about 32 L of water. Thus a substantial savings is realized (the current price of new rotors is $1.95 each). The use of washed rotors combines the advantages of a disposable rotor, by maintaining cleanliness, and the advantages of a permanent rotor, by keeping replacement costs down.

References

Alan H. B. Wu1
James Ohneck2
Eric Klaus
Robert B. McComb2
Hartford Hospital
Hartford, CT 06115

1 Present address: Dept. of Pathol. and Lab. Med., Univ. of Texas, Houston, TX 77025.
2 Present address: Beckman Instruments, Inc., Brea, CA 92621.
3 Address correspondence to this author.

Radioiodinated Hormones Purified by Hydrophobic Interaction Chromatography

To the Editor:

Recently, Baxter and Brown (1) reported the advantageous purification, by hydrophobic interaction chromatography (HIC), of an iodinated tracer for use in a somatomedin C radioimmunoassay. We have compared the performance of HIC with that of the classical gel-exclusion chromatography for purification of radioiodinated polypeptide hormones, and give here our results further evidencing the usefulness of HIC.

Pituitary and placental hormones from Radiolysis Systems (Carson, CA 90746) were iodinated enzymically with 125I, according to Thornell and Johansson (2). We divided the iodinated mixtures into two portions. The first was purified by exclusion chromatography (3) on Ultrogel AcA 54 (LKB, Bromma, Sweden). The second was promptly desalted, then purified by HIC on Phenyl-Sepharose-CL 4B (Pharmacia, Uppsal, Sweden). We passed this second portion through a 1 × 15 cm column packed with Sephadex G-50 and equilibrated with phosphate buffer (pH 7.5, 50 mmol/L) containing 5 g of bovine serum albumin per liter. We eluted with this buffer, collecting 1-mL fractions. We then applied fractions corresponding to the protein peak to a 1 × 20 cm column packed with Phenyl-Sepharose-CL 4B, equilibrated in Tris–NaCl (25 mmol/L Tris, 775 mmol/L NaCl, pH 8.6). We eluted 50 mL at this same ionic strength, then a further 80 mL at another ionic strength (0.25 mmol Tris, 7.75 mmol NaCl, pH 8.6). We collected 4-mL fractions. To restore acceptable ionic strengths and protein contents in these fractions, we promptly diluted them to 200 000 cpm/mL with, according to their ionic strengths, either dis-

Table 1. Decrease in B/T Observed for Some 125I-Labeled Polypeptide Hormones during Storage in Lyophilized Form after Purification by Conventional Gel Filtration (AcA 54), and Hydrophobic Interaction Chromatography (HIC) *

<table>
<thead>
<tr>
<th>Weeks of storage</th>
<th>125I-labeled chorlogonadotropin</th>
<th>125I-labeled follicitropin</th>
<th>125I-labeled placental lactogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AcA 54</td>
<td>HIC</td>
<td>AcA 54</td>
</tr>
<tr>
<td>0</td>
<td>100.0</td>
<td>100.0</td>
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</tr>
<tr>
<td>1</td>
<td>89.0</td>
<td>90.5</td>
<td>91.1</td>
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<tr>
<td>2</td>
<td>72.7</td>
<td>87.7</td>
<td>79.8</td>
</tr>
<tr>
<td>3</td>
<td>62.1</td>
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</tr>
<tr>
<td>4</td>
<td>48.9</td>
<td>58.1</td>
<td>58.1</td>
</tr>
</tbody>
</table>

* The decrease in B/T is expressed relative to the initial B/T, considered as 100% binding.

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Fig. 1. Elution profile of 125I-labeled human placental lactogen (hPL) from a Phenyl-Sepharose-CL 4B column. Shaded areas represent the extent of antibody-binding of the recovered materials; ionic strength of eluent indicated by arrows.
tilled water or phosphate buffer (pH 7.5, 100 mmol/L), both containing 10 g of bovine serum albumin per liter.

As an example, Figure 1 shows the elution profile of $^{125}$I-labeled human placental lactogen from a Phenyl-Sepharose column. The extents of antibody-binding of the recovered materials, as measured by radioimmunoassay (4), give evidence that the purification procedure separates poorly immunoreactive materials from immunoreactive hormone. Indeed, the former materials account for 40% of the total radioactivity applied to the column. Purification of the same starting material by chromatography on Ultrogel AcA 54 allows the separation of immunologically unreactive hormone polymers and enzyme from immunoreactive hormone. The former accounts for only 25% of the total radioactivity applied to the column. Evidently, from an immunological point of view, HIC more effectively purifies the tracer-labeled hormone than does gel filtration. Furthermore, as exemplified in Table 1 for several iodinated hormones, those purified by HIC are likely to be more stable during storage than those recovered from gel-exclusion chromatography.

Thus, HIC seems to us to be an attractive means of obtaining high-quality tracers, an interesting alternative to more sophisticated purification techniques recently promoted, such as polyacrylamide gel electrophoresis (5).

References

Patrick Englebienne
Gerald Doyen

Center for Res. and Diagnosis in Endocrinol.
12, rue Gualbert
B-7540 Kain, Belgium