THE USE OF RANEY’S NICKEL IN A DIFFERENTIAL 
ASSAY FOR OXYBIOTIN AND BIOTIN*

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A method for the determination of oxybiotin in the presence of biotin in biological materials has recently been described (1). This differential assay was based upon the stability of oxybiotin towards dilute potassium permanganate and the inactivation of biotin by this reagent. This paper describes another differential assay which is based on the contrasting behavior of biotin and oxybiotin towards Raney’s nickel catalyst.

Du Vigneaud et al. (2), applying the Mosingo reaction (3), have shown that biotin undergoes hydrogenolysis when treated with Raney’s nickel, and is thereby converted into desthiobiotin. It could be expected that the tetrahydrofuran moiety of oxybiotin would be resistant to this treatment. This was actually found to be the case.

In materials containing both biotin and oxybiotin, treatment with Raney’s nickel converts the biotin into desthiobiotin without affecting the oxybiotin.₁ Desthiobiotin is inactive for Lactobacillus arabinosus (5). Therefore, the activity remaining after treatment with Raney’s nickel is due to the oxybiotin present. These considerations served as the basis for a quantitative method for the determination of oxybiotin in the presence of biotin. A similar procedure has already been applied by Stokes and Gunness (6) to demonstrate the conversion of desthiobiotin to biotin.

Methods and Materials

Preparation of Solutions for Assay—Solutions of d-biotin and dl-oxybiotin were prepared in 0.1 N sodium hydroxide at concentrations of 2 and 4 millimicrograms per ml., respectively. A solution containing 1 millimicrogram of biotin and 2 millimicrograms of oxybiotin per ml. was also employed.

The natural materials were first hydrolyzed with acid, according to standard procedures. They were then neutralized and additional sodium hydroxide added to a concentration of approximately 0.1 N. The final

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₁ dl-Oxybiotin has 50 per cent the activity of d-biotin for Lactobacillus arabinosus (4).
concentration of biotin in the alkaline extracts should not exceed 2 millimicrograms per ml.

*Preparation of Catalyst*—A 200 mg. sample of Raney's nickel alloy was suspended in 5.7 ml. of 4 per cent sodium hydroxide in a 50 ml. volumetric flask and heated for 45 minutes on a steam bath. The liquid was decanted, 5.7 ml. of 4 per cent sodium hydroxide were added, and the suspension again heated for 15 minutes. The catalyst was washed by decantation with three 5 ml. portions of water and was then ready for use.

*Inactivation Procedure*—A solution prepared as described above (10 ml.) was added to the catalyst and the suspension was mechanically shaken in

<table>
<thead>
<tr>
<th>Material</th>
<th>After Raney's nickel treatment (4.0 millimicrograms before treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Biotin</td>
<td>0.0</td>
</tr>
<tr>
<td>dl-Oxybiotin</td>
<td>4.0</td>
</tr>
<tr>
<td>d-Biotin + dl-oxybiotin†</td>
<td>2.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.0</td>
</tr>
<tr>
<td>&quot; &quot; + d-biotin‡</td>
<td>0.0</td>
</tr>
<tr>
<td>&quot; &quot; + dl-oxybiotin§</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* All activities are expressed as oxybiotin equivalents. These equivalencies are obtained by referring growth activities to an oxybiotin standard curve.

† A solution containing 1 millimicrogram of d-biotin and 2 millimicrograms of dl-oxybiotin per ml.

‡ The yeast extract was diluted with an equal volume of a solution containing 2 millimicrograms of d-biotin per ml.

§ The yeast extract was diluted with an equal volume of a solution containing 4 millimicrograms of dl-oxybiotin per ml.

a boiling water bath for 10 minutes, made up to volume with distilled water, and filtered. 10 mg. of Raney's nickel alloy should be employed for each millimicrogram of d-biotin present. It is essential to conduct the inactivation procedure in an alkaline solution, since at neutrality oxybiotin is strongly adsorbed by Raney's nickel catalyst. 25 ml. of the filtrate were placed in a 50 ml. volumetric flask, adjusted to pH 6.8 with 0.5 N hydrochloric acid with brom-thymol blue as an internal indicator, and made up to volume with distilled water. The growth-promoting activity of this solution was then determined with *Lactobacillus arabinosus* (7). The original activity of the solution was determined by applying the identical procedure in the absence of the catalyst. The results obtained are given in Table I.
The results reported in this paper may be summarized as follows.

1. Biotin in pure solution or in the presence of yeast extract is quantitatively inactivated by Raney's nickel.

2. Under similar conditions, oxybiotin is unaffected by this treatment.

3. The activity remaining after treatment of a mixture of biotin and oxybiotin is due to the oxybiotin present.

4. All of the activity present in a yeast extract is inactivated by the treatment with Raney's nickel.

A quantitative procedure for the determination of oxybiotin in the presence of biotin in natural materials has been developed.

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BIBLIOGRAPHY

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