DETERMINATION OF HISTAMINE AS AN ISOTOPIC DERIVATIVE

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The isotope derivative technique was devised by Keston and his co-workers and applied to the quantitative analysis of amino acids (1). The method involves the quantitative reaction of the amino group1 with p-iodophenylsulfonyl chloride (pipsyl chloride) which can be labeled either with I\(^{131}\) or S\(^{35}\). Following the quantitative formation of this radioactive derivative, the amount of amino acid can be determined by use of carrier (2) or by chromatography (3). The carrier technique for amino acid determination was abandoned by these workers owing to the difficulties encountered in removing radioactive impurities (3).

We have applied this method to the quantitative analysis of histamine in biological samples and find the carrier technique to be very satisfactory. Purification of pipsylhistamine carrier is much easier than purification of pipsylamino acids, since the former can be recrystallized from several different organic solvents; pipsylamino acids are not readily soluble and must be precipitated by addition of acid to an aqueous ammoniacal solution.

Current methods for the determination of histamine are based on pharmacological activity or on colorimetry. These methods do not offer proof that the substance being measured is histamine. It is believed that the present method offers a more reliable identification of histamine than do existing methods.

EXPERIMENTAL

Pipsyl chloride, both non-isotopic and I\(^{131}\)-labeled,\(^2\) was prepared in the usual manner (2). Material of low activity is satisfactory for the analysis reported in this paper.

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1 p-Iodophenylsulfonyl chloride also reacts with hydroxyl groups.
2 In the preparation of the radioactive reagent, purification by sublimation is essential to eliminate an impurity which is extremely difficult to remove by crystallization.
DETERMINATION OF HISTAMINE

Reaction of Pipsyl Chloride with Histamine on Large Scale

Histamine dihydrochloride, 18.4 gm. (100 mmoles), was dissolved in 200 ml. of water, and a solution of 76 gm. of pipsyl chloride (125 mmoles) in 800 ml. of dioxane was added. The flask was flushed out with nitrogen, and 50 gm. of sodium bicarbonate, suspended in 200 ml. of water, were added. The flask was shaken. Very soon an oil separated and crystallized. More water was added to precipitate the remaining dipipsyl derivative of histamine. After recrystallization by dissolving in hot acetone, filtering, and adding a 0.5 volume of warm water, 56.4 gm. of derivative, a yield of 88 per cent, were obtained. The pure compound melts at 176.5°. Calculated (for dipipsylhistamine), C 31.7, H 2.35, N 6.53 per cent; found C 32.1, H 2.39, N 6.58 per cent.

Reaction of Pipsyl Chloride with Microgram Amounts of Histamine

Under experimental conditions to be described later, samples of C¹⁴-histamine (4) in the presence of tissue extracts, when treated with non-isotopic pipsyl chloride, were constantly converted in 93 to 96 per cent yield to an ether-extractable compound. This radioactivity could not be removed from carrier dipipsylhistamine in repeated recrystallizations; it produced only one radioactive peak on paper chromatograms. Hence the products of pipsylation of histamine on both micro and macro scales are identical.

Method of Tissue Analysis—Tissues were ground with sand in a mortar with trichloroacetic acid plus 1 drop of 0.1 N hydrochloric acid per gm. of wet tissue. After 30 minutes the extract was filtered into a glass-stoppered graduate, and the tissue residues were extracted three additional times with small amounts of 5 per cent trichloroacetic acid. The latter was removed by ether extraction (done in the graduate), residual ether was blown off with nitrogen, and the volume measured. Generally the volume was adjusted so that 0.20 ml. of extract would be equivalent to 0.10 gm. of wet tissue. There is no loss of histamine by this treatment (5).

For samples, 0.20 ml. of tissue extract was used. Standards for each tissue were prepared by adding a known amount of histamine to 0.20 ml. of tissue extract.

In glass-stoppered test-tubes of about 18 ml. capacity were combined 0.20 ml. of tissue extract, 0.10 ml. of water (for samples) or 0.10 ml. of histamine solution (for standards), and 0.30 ml. of purified dioxane containing 5 mg. of T¹⁸-pipsyl chloride. The tubes were flushed with nitrogen, 0.10 ml. of sodium bicarbonate solution (90 mg. per ml.) was added, and the tubes stopped and shaken gently. After standing at room temperature for 30 minutes with occasional mild shaking, the tubes were placed in a 50° water bath for 15 minutes to accelerate the hydrolysis of excess pipsyl chlo-
ride. 3 ml. of water were added, and each sample and standard extracted five times with 7 ml. portions of ether. The ether layers were transferred with a dropper (with care to avoid contamination by the highly radioactive aqueous portion) to a 125 ml. Erlenmeyer flask containing 500 mg. of carrier pipsylhistamine dissolved in acetone. The solution was evaporated to a small volume on a hot-plate, diluted to about 30 ml. with acetone, warmed, treated with about 0.7 gm. of Norit, filtered, washed, and adjusted to about 40 ml. with acetone. Then 15 ml. of warm water were added, mixed, and crystallization permitted to occur without further agitation. The pipsylhistamine was filtered and washed with a small amount of cold methanol.

For the second recrystallization the sample was dissolved by heating with 10 to 15 ml. of acetone plus 15 to 20 ml. of ethanol. 0.7 gm. of Darco was added, the mixture filtered, and the filtrate evaporated to a volume of about 15 ml. The crystals were permitted to form slowly. They were washed with a small volume of cold ethanol.

For the third recrystallization the sample was dissolved in 20 ml. of acetone, and then 15 ml. of toluene and 0.7 gm. of Nuchar were added. After filtration the solution was evaporated to a volume of about 10 ml. Benzene and Skellysolve, 1:1, were used for washing the crystals.

The fourth crystallization was the same as the first, but required proportionally less solvent and adsorbent.

The fifth and sixth recrystallizations, when carried out, were similar to the second and third. In every type of biological material tried, the radioactivity was invariably constant after the fourth crystallization and often constant after the third. Therefore, in routine assays a count was made only after the fourth crystallization, and the histamine content of the sample was calculated from that value.

For counting, samples weighing between 48 and 52 mg. were plated and counted; then the material on the plate was redistributed and recounted until good checks were obtained. After subtracting background, the observed count was corrected for the difference between the actual weight and 50 mg. Then the corrected counts per minute are directly proportional to the histamine content of the samples and standards. In this procedure self-absorption errors are negligible.

In Table I a tissue analysis performed in quadruplicate is presented, in which a large number of standards were employed. These data are in-

3 The recrystallization procedures are designed to produce losses of carrier sufficient to insure efficient removal of impurities.

4 In routine work standards are not essential for each group of samples. The histamine equivalent of a batch of radioactive pipsyl chloride is determined on a certain date; then samples can be assayed on another date and correct values obtained by correction for radioactive decay.
tended to show the reproducibility of the values and the constancy of the count after the fourth recrystallization.

Values for various organs obtained by the isotope derivative method, expressed as micrograms of histamine per gm. of fresh tissue, are as follows: rat liver 1.0, 1.0, 1.1; rat abdominal skin 21, 23, 25, 33; rat small intestine 6, 7, 11; rat stomach (average of six combined) 20; rat abdominal muscle 6, 7, 9; rat large intestine (average of six combined) 5; rat lung 2.5, 2.5, 2.9; rat kidney 0.5, 0.6, 0.9; guinea pig lung 11, 12, 13, 16.

Values for some of these organs obtained by other assay techniques are of comparable magnitude. Feldberg and Talesnik (6) report the following values: rat liver 0.7, rat abdominal skin 24, rat duodenum 5, rat stomach 14 μg per gm. A survey of the literature reported by Guggenheim (7) lists the following values: rat liver 0.5 to 1.3, rat lung 3.5 to 10.2, and guinea pig lung 12 to 23 μg per gm.

**TABLE I**

Histamine Assay of Combined Large Intestines of Six Rats

Total weight, 6.4 gm.; total volume of extract, 12.8 ml.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Description</th>
<th>4th crystallization</th>
<th>5th crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.20 ml. extract</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>0.20 &quot; &quot;</td>
<td>200</td>
<td>Lost</td>
</tr>
<tr>
<td>3</td>
<td>0.20 &quot; &quot;</td>
<td>190</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>0.20 &quot; &quot;</td>
<td>170</td>
<td>180</td>
</tr>
<tr>
<td>5</td>
<td>0.20 &quot; + 0.50 ml. histamine</td>
<td>330</td>
<td>370</td>
</tr>
<tr>
<td>6</td>
<td>0.20 &quot; + 0.50 &quot;</td>
<td>410</td>
<td>380</td>
</tr>
<tr>
<td>7</td>
<td>0.20 &quot; + 1.00 &quot;</td>
<td>520</td>
<td>490</td>
</tr>
<tr>
<td>8</td>
<td>0.20 &quot; + 1.00 &quot;</td>
<td>530</td>
<td>540</td>
</tr>
<tr>
<td>9</td>
<td>0.20 &quot; + 2.00 &quot;</td>
<td>930</td>
<td>970</td>
</tr>
<tr>
<td>10</td>
<td>0.20 &quot; + 2.00 &quot;</td>
<td>940</td>
<td>970</td>
</tr>
</tbody>
</table>

Calculations (based on data of the fourth crystallization); average of quadruplicate samples, 190 c.p.m.; average values of standards after subtraction of 190 c.p.m. due to histamine in sample, 0.50 μg = 180 c.p.m.; 1.00 μg = 340 c.p.m.; 2.00 μg = 760 c.p.m.; 1.00 μg of histamine = 360 c.p.m., average. 190/360 = 0.53 μg of histamine in 0.20 ml. of extract = 5.3 μg of histamine per gm. of tissue. The deviation from the mean (from data of both the fourth and fifth crystallizations) is for samples 5 per cent, for 0.50 μg standards 6 per cent, for 1.00 μg standards 3 per cent, and for 2.00 μg standards 2 per cent.

* Corrected.

DISCUSSION

The isotope derivative technique for the determination of histamine has several advantages over existing methods. It is believed to be more nearly
specific, since it involves the isolation of a compound which is crystallized to a well defined criterion of purity; i.e., constant specific activity. It is difficult to conceive of minute amounts of impurity surviving the rigorous regimen of adsorption and crystallization without a loss which would be reflected by a drop in the specific activity of the sample. There are no known tissue constituents which interfere with this assay. No acidic compound including the free amino acids forms a pipsyl derivative which is extracted by ether from basic solution. Urea labeled with C\(^{14}\), when treated under these conditions, did not produce an ether-extractable compound. Pipsylamide and a small amount of unchanged pipsyl chloride are to be expected as contaminants, but they are readily removed during crystallization. Trial experiments showed that, when microgram amounts of the pipsyl derivatives of C\(^{14}\)-labeled tyramine, tryptamine, cadaverine, and epinephrine were added to carrier pipsylhistamine, they were completely eliminated by four crystallizations.

Another advantage is that almost all purification is carried out after the labeled pipsylhistamine is mixed with carrier. From this point on, loss of material has no effect on the final analytical value.

The method can be used over an extremely wide range of concentrations. Since pipsyl chloride can be prepared which has a very high specific activity, the method has potentialities for determining histamine in minute fragments of tissue.

Finally, the method may be useful for the determination of any amine which forms a crystalline pipsyl derivative. Preliminary work has shown that crystalline derivatives are obtained from tyramine, tryptamine, phenethylamine, cadaverine, epinephrine, norepinephrine, and hydroxytyramine.

**SUMMARY**

A method for the quantitative determination of histamine in biological samples is described. It is based on the conversion of histamine to a radioactive derivative with iodine-labeled \(p\)-iodophenylsulfonyl chloride (pipsyl chloride) and the removal of interfering substances by repeated recrystallization of added carrier.

**BIBLIOGRAPHY**


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\(^{5}\) Certain samples which reached a constant specific activity during three crystallizations have been subjected to three additional crystallizations, with no significant change in specific activity. Furthermore, tissues such as rat liver and kidney, which might be expected to be high in interfering substances, rapidly drop during a few crystallizations to very low specific activities.
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