THE ESTIMATION OF BUTAZOLIDIN IN BLOOD

BY

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Butazolidin, or 3,5-dioxo-1,2-diphenyl-4-n-butyl-pyrazolidine, has recently been given extensive trials in the treatment of rheumatoid arthritis. Estimations of drugs in blood are often of some interest during the early period of their use, as it may be found possible to relate both the therapeutic response and the toxicity (if any) to the blood concentration. Toxicity, for example, may be related to a cumulative effect of the drug as is the case in bromide therapy.

Butazolidin was first estimated in blood by Pulver (1950), who hydrolysed the substance, with formation of hydrazobenzene, which then rearranged to benzidine. This latter substance was estimated colorimetrically by a diazo reaction. Brodie and Burns (1952) extracted acidified plasma with heptane, and, after returning the butazolidin to aqueous alkali, determined the optical density at 265 m\(\mu\) with an ultra-violet spectrophotometer. The method was modified by Stanfield, Brodie, and Yeoman (1953), and it is simple and accurate. It requires, however, the use of an expensive instrument which is not generally available. The present method was based on that of Pulver (1950) and makes use of the following chain of reactions:

\[
\text{C}_8\text{H}_8\text{CHCO} + \text{Acid} \xrightarrow{100^{\circ}} \text{NH} + \text{NH} + \text{NH}_2
\]

\[
\text{Butazolidin} \rightarrow \text{Hydrazobenzene} \rightarrow \text{Benzidine}
\]

Preliminary experiments on normal serum, fortified by the addition of "butazolidin," showed that considerable losses of the drug occurred on protein precipitation by the commonly used reagents. Using trichloracetic acid, the recovery was 31%.

A mixture of zinc sulphate and sodium hydroxide, as used by King (1946), gave a recovery of 45%. These losses are probably to be explained by a strong binding of "butazolidin" to plasma proteins, which has been reported by Burns (1953). Extraction of blood or serum by organic solvents, however, gives good recoveries. Acetone was preferred in the present method, but its use requires precautions against loss of solvent by evaporation.

Pulver used somewhat drastic hydrolysis conditions for the conversion of "butazolidin" to benzidine, involving heating to dryness with p-toluene sulphonic acid. Hydrolysis by dilute sulphuric acid, however, has been substituted in the present technique, as it was found to give more reproducible results. Fig. 1 shows the formation of benzidine plotted against hydrolysis time under these conditions. The maximum yield of benzidine was obtained after eight hours' hydrolysis, and corresponded to 83% of the stoichiometric yield. After four hours' hydrolysis the yield was 58% of the theoretical.
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The benzidine produced was estimated by diazotization, followed by coupling with N-sulphatoethyl-m-toluidine, this reagent being preferred to the N-N-dimethyl-a-naphthylamine used by Pulver.

The absorption spectrum of the purple pigment formed in the present method is shown in Fig. 2. The wavelength of maximum light absorption was 525 mμ and was the same for both the colour derived from a solution of benzidine and from hydrolysed "butazolidin." A linear relation was found between optical extinction, measured at 525 mμ, and concentration of "butazolidin," up to a concentration equivalent to a serum level of 25 mg./100 ml. Normal serum gives a negligible colour by the method. Recoveries of "butazolidin" added to normal serum are shown in Table I.

![Absorption spectra of the purple pigments derived from](image)

**Table I**

<table>
<thead>
<tr>
<th>Added (μg.)</th>
<th>Found (μg.)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>43</td>
<td>86</td>
</tr>
<tr>
<td>100</td>
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<td>150</td>
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<td>200</td>
<td>190</td>
<td>95</td>
</tr>
<tr>
<td>250</td>
<td>236</td>
<td>95</td>
</tr>
</tbody>
</table>

**Method**

**Reagents.**—The following are required:

1. A.R. acetone.
2. Normal sulphuric acid.
3. 0.5% Sodium nitrite, which is stored at 0° C.
4. 1.8% N-Sulphatoethyl-m-toluidine* in water, stored at 0° C., keeps for several weeks, but should be discarded if a brown tint appears.
5. Standard "butazolidin" solution, 3.0 mg.% in A.R. acetone, which should be stored in a tightly stoppered bottle.

**Procedure**

Serum, 1 ml., is added to 4 ml. acetone in a glass-stoppered centrifuge tube of 8 ml. capacity. After shaking well, the mixture is stood for 30 minutes and is then centrifuged, the stopper being retained in the tube during this procedure. Duplicate portions of 1 ml. of supernatant solution are removed and transferred to conical pyrex centrifuge tubes of 1 ml. capacity graduated in tenths of a ml. A duplicate standard is set up simultaneously by taking two portions of 1 ml. of standard "butazolidin" solution in similar graduated centrifuge tubes.

The bulk of the acetone is now cautiously evaporated off in a hot-water bath, the tubes being agitated gently to prevent loss by spurtting or bumping. N sulphuric acid, 1 ml., is added to each tube, and the tubes are placed in a boiling-water bath for four hours. At half-hourly intervals the volumes in the tubes are restored to 1 ml. by the addition of distilled water. The final volume is 1 ml. After hydrolysis the tubes are chilled in ice-water, and 0.5 ml. of ice-cold sodium nitrite solution, followed by 2 ml. of ice-cold N-sulphatoethyl-m-toluidine, is added, the solution being mixed well after each addition. The purple colours are allowed to develop for 30 minutes at room temperature. The serum tubes have a slight turbidity at this stage due to the presence of unsaponified lipid material. This turbidity is removed by adding 0.2 ml. of 40 to 60° light petroleum, shaking briefly, and centrifuging. Then 3 ml. of the aqueous phase is removed. The standards are similarly treated with light petroleum. The coloured solutions are read in a spectrophotometer at 525 mμ, or in a colorimeter with appropriate filter. The standards correspond to a serum level of 15 mg./100 ml.

The reproducibility of the method is rather poor, due to slight differences in the conditions of hydrolysis. In a series of 50 estimations carried out in duplicate on sera of patients receiving the drug the paired results differed from their individual means by amounts varying from ±0.0 to ±0.8 mg./100 ml., the average difference being ±0.3 mg./100 ml. For a serum level of 10 mg./100 ml. this would correspond to an error of ±3%.

Estimations of "butazolidin" in blood, plasma, or serum have been reported by Pulver (1950), Gsell and Müller (1950), Donenjoz (1952), Burns, Schulert, Chenkin, Goldman, and Brodie (1952), Currie, Peebles Brown, and Will (1953), Burns, Rose, Chenkin, Goldman, Schulert, and Brodie (1953), Meanock (1953), Stanfield et al. (1953),

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* Supplied by Imperial Chemical (Pharmaceuticals), Ltd., Manchester.
Yü, Sirota, and Gutman (1953), and Pemberton (1954). As some of these analyses were carried out on whole blood and some on plasma or serum,

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Haematocrit</th>
<th>Plasma Level (mg.%)</th>
<th>Whole Blood Level (mg.%)</th>
<th>Concentration Calculated in Red Cells (mg.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>4.5</td>
<td>3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>6.5</td>
<td>4.5</td>
<td>0.0</td>
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<tr>
<td>3</td>
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<td>7.5</td>
<td>5.0</td>
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<tr>
<td>4</td>
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<td>0.5</td>
</tr>
<tr>
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<td>11.0</td>
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<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>15.5</td>
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<td></td>
</tr>
</tbody>
</table>

it was considered desirable to examine the distribution of the drug between the red cells and the plasma. Specimens of heparinized blood were collected from patients receiving "butazolidin," and simultaneous estimations were carried out on whole blood and plasma, the haematocrit being determined on a separate specimen. The concentration of "butazolidin" in the red cells was then calculated from the equation:

$$x = \frac{100b - 100p - ph}{h}$$

where b, p, and x are concentrations of "butazolidin" (in mg.%) in whole blood, plasma, and red cells respectively, and h is the haematocrit. The results are shown in Table II. The drug was found to be absent or present only in traces in the red cells.

**Discussion**

There appears to be general agreement that the blood concentration of "butazolidin" is related to its therapeutic effect. Currie et al. (1953) considered a plasma level of 8 to 11 mg.% to be required for the suppression of symptoms in rheumatoid arthritis, and Burns et al. (1952) say "about 10 mg.\%." Meanock (1953) considered the therapeutic range to be 5–10 mg.\% in whole blood, and stated that levels in excess of these did not give an additional response. Pemberton (1954), whose analyses were carried out on serum, considered levels of 12 to 16 mg.% to be desirable. A plasma level of about 10 mg.\% appears to be of some physiological significance in the treatment of gout, as Yü et al. found uricosuria to be induced at this level. The drug is not cumulative, and Burns, Rose, Chenkin, Goldman, Schulert, and Brodie (1953) found a ceiling plasma level to exist for a given person. The present investigations confirmed this; the highest serum level of a series of about 300 estimations was 22.5 mg.\%.

The serum or plasma level is not proportional to the dosage and increases by a proportionately smaller amount as the dosage is increased. For a given daily dosage, however, the serum level is generally quite constant over a considerable period of time. After administration has been stopped the "butazolidin" is slowly eliminated from the body, and may still be detected in the serum three weeks later. These points are illustrated by Fig. 3, which shows daily estimations on two patients with rheumatoid arthritis.

![Graph showing daily serum "butazolidin" estimations on two patients with rheumatoid arthritis. Patients (1) received 800 mg. per day until the eighth day, when a toxic reaction occurred, and the drug was discontinued. Patient (2) received 400 mg. per day until the twenty-first day and 600 mg. per day after that.](http://jcp.bmj.com/Downloaded from)

Complete and rapid absorption of "butazolidin" from the alimentary tract has been reported by Currie et al. (1953) and by Burns et al. (1952). We were not able to reproduce these findings, however, and after administration of a single dose of 400 mg. of the substance in the form of enteric-coated tablets to two patients with rheumatoid arthritis, maximum serum levels of less than 1 mg.% were found. Stanfield et al. (1953) and others have described the finding of such tablets intact in the stools. In addition to the possibility of incomplete absorption, the rate of metabolism of "butazolidin" varies greatly from patient to patient, and the serum level is not generally predictable from a consideration of the dosage schedule. Serum "butazolidin" estimations are therefore of some value in those patients who fail to give an adequate response to the drug. If the serum level is found to be within the required therapeutic range administration of "butazolidin" can be discontinued as being ineffective. If, however, the
serum level is undesirably low, an increased dosage can be considered.

No data relating the blood level of "butazolidin" with its various toxic manifestations have yet been published, though Meanock (1953) considered such a relationship to exist. Of eight patients in the present series who reached serum levels of over 20 mg.%, only one developed what was considered to be a toxic reaction. This was of a mild character (sore throat and excess salivation). Of six patients who developed gastro-intestinal disturbances, and whose serum "butazolidin" level was known at the time of the reaction, two had levels below the range 12–16 mg.%, two in that range, and two slightly above (both 17.5 mg.%). This limited number of cases, therefore, does not suggest that the estimation is of value in the avoidance of toxic reactions.

I am much indebted to Dr. H. S. Barber and Dr. Lois Stent, in cooperation with whom the work was carried out. A specimen of purified "butazolidin" was supplied by Geigy, Ltd.

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