An Evaluation of Blood Nitrate Levels

By JOHN W. BERRY, M.D., THOMAS C. ROACH, M.D.

The lack of a suitable objective yardstick to measure the effect of anti-anginal drugs has led to confusion in their clinical evaluation. A method of quantitating the nitrate level in the serum has furnished a means of correlating the subjective relief with this group of drugs. Representative samplings of serum from noncardiac and cardiac patients were analyzed to establish control nitrate levels. The cardiac patients were selected for their good responses to nitroglycerin and pentaerythritol tetranitrate. A good subjective response to the drugs correlated with the increased blood nitrate levels.

The difficulties inherent in evaluating anti-anginal drugs have been stressed by a number of authors. In general the lack of an objective yardstick for determining a subjective response remains the problem. It has been noted that favorable reports of a variety of drugs when subjected to the double blind technic of administration fail to bear out the initial enthusiasm. On the other hand, carefully controlled clinical studies have indicated that at least one drug—pentaerythritol tetranitrate (PETN)—is helpful in the chronic management of anginal patients. In an attempt to resolve some of the discrepancies alluded to, a study was undertaken to measure blood nitrate levels in a group of patients. The development of a quantitative method for the microanalysis of blood nitrates is the basis for this report. It was felt that differences in individual absorptions of the nitrate radical might explain the variable clinical findings in the evaluation of these drugs. Glyceryl trinitrate (nitroglycerin) and pentaerythritol tetranitrate (PETN) were chosen for the study. The latter drug was evaluated in 2 dosage forms—tablets and in a timed disintegration capsule (PETN-TD) shown to have a more prolonged and even release of the medicament.

METHOD

Selected patients from the medical wards and the outpatient clinic of the St. Louis City Hospital were utilized for these studies. The patients were divided into 2 groupings: a “control” group and a “cardiac” group. The control group of patients had been hospitalized for reasons other than coronary artery disease. All were recovering from their illnesses and were in good clinical condition at the time of the studies. None of the control patients had received any nitrate drugs during this illness. The cardiac group consisted of selected patients from the Cardiac Out Patient Clinic on whom the diagnosis of angina pectoris had been made by the usual clinical history of chest pain brought on by exertion. All of the selected cardiac patients had experienced relief within 10 minutes of the acute attacks of angina pectoris with sublingual nitroglycerin. All of the selected cardiac patients had been maintained on PETN tablets in varying doses of 40 to 80 mg. per day for 1 to 3 months with good control of the anginal attacks. A patient was considered to have good control when there had been a 75 per cent reduction in the number of daily angina attacks from the pre-nitroglycerin control period. A deliberate effort was made to exclude from the cardiac group angina pectoris that might be associated with factors other than coronary artery disease. This was accomplished by excluding by the appropriate clinical methods all patients with valvular heart disease, myocardial infarction, and cardiomegaly greater than 25 per cent of the expected norm, and who did not have the expected subjective response to nitroglycerin. It is emphasized that the “cardiac” group of patients was selected by the typical therapeutic response to nitroglycerin and to PETN administered chronically. The selected anginal patients were ambulatory throughout the study period and hospitalized only for 24 hours to obtain the blood sampling for the nitrate determinations.

From each of the 2 groups of patients under specified conditions, whole blood was collected for nitrate determinations. The collected blood was centrifuged and the sera stored in a commercial type deep freeze at —4 C. until used. The analysis for serum nitrates was done by a modification of the Yogoda method, on a Beckman spectrophotometer with readings at 408 m.4,16

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TABLE 1.—Normal Limits of Serum Nitrate

<table>
<thead>
<tr>
<th>Subject</th>
<th>Determination 1</th>
<th>Determination 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Grey)</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>B (White)</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>C (Young)</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>D (Schaeffer)</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>E (Hazeltine)</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Ave. 4.8</td>
<td>Ave. 4.8</td>
<td></td>
</tr>
</tbody>
</table>

Range 4.0 to 5.7 µg. per ml.

RESULTS

From the control patients the normal limits for serum nitrates were determined. This was accomplished by obtaining fasting blood specimens on 5 different subjects. Table 1 shows the results of these studies. The normal range of blood nitrates by the methods used was 4.0 to 5.7 µg. per ml. of serum. To check the reproducibility of our technics, determinations on the stored sera were run another time. Of the paired determinations on the sera, little variation is noted; the greatest difference being 0.5 µg. per ml. of serum. The effect of diet upon the blood nitrate levels was determined on the blood specimens from 5 other control patients. The patients all received the regular hospital diet and were ambulatory on the ward. Blood was obtained, on each patient, at 2-hour intervals during the day. The serum nitrate level subsequently was determined for each patient. Figure 1 shows the average nitrate determinations on the sera of the 5 control patients for the 12-hour observation period. It is evident that a slight diurnal pattern is present with the highest peak in the late afternoon.

The effect of nitrate drugs with an anti-anginal action upon the nitrate blood levels of control patients was determined. Figure 2 graphically shows the effect of nitroglycerin. In this experiment, 3 control patients were given 0.0012 Gm. (1/50 gr.) of nitroglycerin sublingually. Blood samples were drawn at 2, 4, 6, 10, 12, and 15 minutes following the medication. The average serum nitrate levels for the 3 patients are shown in figure 2. The peak level was reached at 6 minutes, with dissipation by 15 minutes. The figures obtained are consistent with the fairly rapid action of nitroglycerin for the acute treatment of angina pectoris. It is of interest that the blood nitrate levels lag behind the expected time for clinical relief of the anginal pain.

The results of PETN on the blood nitrate levels of the control group of patients were obtained. For this experiment, 6 patients were selected and divided into 3 groups of 2 each. Each pair of patients were given respectively an identically colored capsule containing a placebo of lactose, 30 mg. PETN plain capsules, and 30 mg. PETN timed-disintegrating capsules. The capsules were given to each pair of patients at 12-hour intervals for 3 days. The capsules were distributed by one of us without prior knowledge of their content. The contents of the capsules were subsequently made known after the laboratory data had been completed. At the conclusion of the third day of treatment, blood samples from each pair of patients were
collected at variously spaced timed intervals for 24 hours. The blood nitrate curves obtained are shown in figure 3. The average range for the blood nitrates on the 2 patients receiving the placebo was 3.8 µg. per ml. to 4.5 µg. per ml. These values were in agreement with previous determinations. On inspection of the absorption nitrate curve (fig. 3) it is noted that the placebo curve differs from the diurnal curve previously shown on the "control" subjects. The explanation for this apparent discrepancy is the difference in the time of collection of the blood specimens. The specimens were collected at 4-hour intervals, in this experiment, rather than at 2-hour intervals (fig. 1).

The effect of PETN upon the nitrate blood levels is apparent in figure 3. The 2 patients receiving 30 mg. PETN, plain capsules, reached a peak nitrate level at 8 hours with a 24-hour level of 5.0 µg. per ml. being obtained. A difference in the curve of the blood nitrate levels is seen with the 2 patients receiving PETN-TD capsules. A smoothing out the blood nitrate absorption curve is noted, consistent with the more uniform release of the medication. In the latter 2 patients, the average peak intake level of 8.5 µg. per ml. was obtained 12 hours after the last dose of PETN-TD.

**Observations on Cardiac Patients**

The selected cardiac patients had been on PETN for at least 1 month, with good control of the symptom of angina pectoris. The dosage schedule for each patient was 20 mg. 4 times daily, before meals and at bed time. At the time of their hospitalization the medications were continued. The patients remained ambulatory on the wards and received the regular hospital diet during the 24-hour observation period. The regularly prescribed medication was given at bed time, usually between 10 and 11 p.m. The following morning, 7 to 8 a.m., an overnight blood specimen was drawn for subsequent nitrate analysis. This fasting specimen represented a 6- to 10-hour abstinence from the nitrate medication. Table 2 records the morning fasting nitrate values from the sera obtained on 10 patients who had received PETN, 20 mg. after meals and at bed time for 2 months. It is noted that the range of the individually determined nitrate levels is considerably greater than the nitrate levels obtained in the control group. Furthermore, the individual nitrate levels on the serum are higher, indicative of a cumulative effect for the chronically administered nitrate drugs.

Figure 4 illustrates the effects of PETN given in a timed dosage schedule on the blood
TABLE 2.—Blood Nitrate Levels of Patients with "Chronic" Ingestion

<table>
<thead>
<tr>
<th>Subject</th>
<th>µg./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Sehorski)</td>
<td>8.1</td>
</tr>
<tr>
<td>B (Vogel)</td>
<td>8.6</td>
</tr>
<tr>
<td>C (Sanders)</td>
<td>7.8</td>
</tr>
<tr>
<td>D (Divien)</td>
<td>8.1</td>
</tr>
<tr>
<td>E (Earls)</td>
<td>4.6</td>
</tr>
<tr>
<td>F (Frost)</td>
<td>9.2</td>
</tr>
<tr>
<td>G (Satisophalis)</td>
<td>5.2</td>
</tr>
<tr>
<td>H (Brerman)</td>
<td>6.2</td>
</tr>
<tr>
<td>I (Holmgren)</td>
<td>7.2</td>
</tr>
<tr>
<td>J (Rogers)</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Range 4.6 to 9.2 µg. per ml.

nitrate levels of patients with coronary artery disease. Blood specimens were obtained every 2 hours during the day for subsequent nitrate analysis of the sera. The curve shown represents the average values for the sera nitrate determinations from 5 anginal patients given 10 and 20 mg. of PETN before their meals and at bed time. The picket-fence type of nitrate absorption curve is typical of that obtained with PETN tablets. The effect of the higher dosage schedule (20 mg.) is merely to exaggerate the peaks of the absorption curve. It is of interest that despite the drop or the fluctuation of the blood nitrate level, angina was not precipitated in these subjects during this experiment.

Figure 5 shows the results obtained from the sera of 10 patients with angina pectoris who were given a timed disintegrating capsule of 30 mg. of PETN administered at 12-hour intervals. The marked variations in the absorption curve seen with the PETN tablets is dampened and the resultant curve smoother. The observed nitrate levels fall within the range of 6 to 9 µg. of nitrate per ml. of serum. From our preliminary data we are of the opinion that a serum nitrate range of 6 to 9 µg. correlates with good control of the anginal syndrome.

A subjective trial of PETN-TD capsules was undertaken to evaluate a dosage schedule shown to provide a nitrate blood level of

between 6 to 9 µg. per ml. Sixteen patients with angina pectoris were selected from our out-patient department by the previously defined criteria. As accurately as possible, the anginal episodes of each patient were evaluated as to severity of pain, frequency of attacks, precipitating factors, exercise tolerance (subjective observations only; no Master tolerance tests were done), and good response to PETN tablets. Each patient kept a daily log of the character and number of attacks of angina that occurred, also
noting the precipitating factor. After 4 weeks of clinical observation the patients were switched from the PETN tablets to PETN-TD capsules, 30 mg.* every 12 hours, and continued to maintain a daily log. They were also asked to note any other effects of the capsules, especially drowsiness, anorexia, insomnia, lassitude, nausea, vomiting, or palpitations.

Subjective Observations

Fifteen of the 16 patients thought that the number and severity of their anginal episodes were generally decreased when they were placed on PETN therapy. Eight of the 16 who initially were started on 10 mg. of PETN, 4 times a day, noted a further improvement when the dosage was increased to 20 mg. of PETN 4 times a day. The side effects were limited to 3 patients who complained of gastric upset after taking the tablets.

On PETN-TD capsules, 14 of the 16 patients reported that their anginal episodes were further decreased in severity and frequency. It should be noted that these patients were receiving 2 30-mg. TD capsules (60 mg.) as compared to 4 20-mg. tablets or a total of 80 mg. of PETN daily. From these data it would appear that 60 mg. of PETN in TD capsule form is as effective as 80 mg. of PETN in tablet form, in controlling the anginal attacks.

Each PETN 30-mg. TD capsule included 50 mg. of secobarbital. Two patients complained that drowsiness was marked, but 8 patients preferred the capsules because they noted less "nervousness" and better ability to sleep. The remainder of the group (4 patients) did not note any effect of the barbiturate. The capsules were not associated with other side effects. Therefore, 12 of the 16 patients preferred the TD capsules, 3 of these noting further decrease in anginal episodes; 8 decreased "nervousness" attributable to the secobarbital, and all preferring the dosage schedule of 2 capsules per day to the 8 tablets daily of the plain PETN.

Discussion

It is generally accepted that the nitrites are the only specific therapeutic coronary dilators for the treatment of angina pectoris. Glyceryl trinitrate (nitroglycerin) is of proved value for the relief of the acute episodes of anginal pain. This relief is so dramatic that authors have incorporated the anti-anginal effect of nitroglycerin into their definitions of angina pectoris. The prophylactic use of nitroglycerin has also been accepted by clinicians as being helpful in the management of coronary artery disease. Because of the subjective nature of angina pectoris it is admittedly difficult to assess the value of any medicament in the syndrome. Objective evidence supporting the role of the nitrates has been presented by Starr utilizing ballistocardiographic changes after nitroglycerin in patients with angina pectoris. In these studies, the abnormal ballistocardiograms became more normal within 7 minutes after nitroglycerin was given, with dissipation of the effect by 15 minutes. The time effects of nitroglycerin correlate very closely to our own findings of the changes of the blood nitrates. It is logical to speculate that the ballistocardiographic changes can be attributable to the increase of nitrate blood level with its pharmacologic action on the coronary bed. Our studies would also indicate that a blood nitrate level of 6 to 9 µg. may be critical in the control of the anginal pain. Further observations along these lines are planned. We are particularly interested in correlating patients with poor control of the anginal attacks after nitroglycerin and PETN. It may well be that this small percentage of patients with angina pectoris may have some unknown failure to absorb the medication.

Summary

Nitrate blood levels can be influenced by nitroglycerin or pentaerythritol tetranitrate (PETN). There is an abrupt rise and fall

in nitrate blood levels following administration of each dose of PETN in tablet form. By combining PETN in timed-disintegration capsules, a more uniform release of the drug is obtained. PETN in timed-disintegrating capsule form provides a uniform, sustained release of the drug and a prophylactic effect for 10 to 12 hours’ duration, in the treatment of angina pectoris.

SUMMARIO IN INTERLINGUA
Le nivellos de nitrato del sanguine pote esser influentiate per nitroglycerina o pentaerythritol tetranitrato (PETN). Il occorre un abrupte augmento e descendita del nivellos de nitrato del sanguine post omne administration de un dose de PETN in le forma de tablettas. Per includer PETN in capsulas a disintegration relentate, un plus uniforme liberation del droga es effectuate. PETN in iste modo de administration resulta in le uniforme e continue liberation del droga e in un effecto prophylactic de un duration de 10 a 12 horas in le tractamento de angina de pectore.

REFERENCES
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