# Phenacetin-induced hemolytic anemia

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**Summary: The hematological features of phenacetin-induced hemolytic anemia are presented in order to make the physician aware of the abnormalities which suggest the use of an oxidant drug. The presence of "bitten out" red cells is the commonest initial clue to the existence of drug-induced hemolytic anemia. The diagnosis is confirmed by the demonstration of Heinz bodies and sulfhemoglobinemia. Early recognition of this form of drug-abuse may avert the development or progression of analgesic nephropathy.**

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Of the hemolytic anemias, the drug-induced form is by far the most common. In Canada phenacetin (acetophenetidin) continues to be one of the agents most frequently implicated, despite the fact that its deleterious hematological and renal effects are widely recognized.[1-4] The hematological effects of phenacetin usually are part of the "analgesic abuse syndrome" which consists of psychiatric disturbances, gastrointestinal ulceration (often necessitating surgery), nephropathy and anemia. This syndrome has been well-discussed by Gault *et al.*[1] We will be concerned primarily with the hemolytic anemia involved.

There are at least two mechanisms by which a drug can lead to lysis of the red cell: (1) induction of an immune-hemolytic process; (2) oxidative denaturation of hemoglobin with or without hemoglobin abnormality or erythrocyte enzyme defect, ultimately causing a Heinz body hemolytic anemia. Both these mechanisms lead to alterations of the red cell membrane, which cause premature destruction of the cell.

Although there are isolated reports of phenacetin acting via an immunological mechanism,[5-7] by far the most common effect is Heinz body production.

It is the purpose of this paper to discuss the hematological aspects of phenacetin-induced Heinz body anemia. Clinical findings of the "analgesic abuse syndrome" which was present in many of the patients, will be reported also.

## Mechanism of Heinz body formation and oxidative degeneration of hemoglobin

Oxidant substances may be divided into two groups: (1) one group causes the oxidation of red cell constituents via the production of hydrogen peroxide ($H_2O_2$); (2) the other group oxidizes hemoglobin and other red cell components directly without the production of an intermediary.[8]

There is good evidence that the effect of a drug that is oxidative by virtue of its $H_2O_2$-generating capacity is "buffered" by reduced glutathione (GSH). The level of GSH in the erythrocyte is determined by the hexosemonophosphate (HMP) pathway of oxidative glycolysis (Fig. 1). When $H_2O_2$ is produced in the red cell, the sulfhydryl groups of the GSH molecules preferentially undergo oxidation, thus protecting hemoglobin (as well as certain enzymes and perhaps the red cell mem-

brane) against irreversible degenerative changes. The production of adequate amounts of GSH to provide this defense, requires glutathione (GSSG) reductase and reduced triphosphopyridine nucleotide (NADPH) (Fig. 1). In the red cell the latter is available only from the first step of the HMP shunt which is dependent on normal glucose-6-phosphate dehydrogenase (G6PD) activity. Under conditions of oxidant stress the increased rate of oxidation of GSH to GSSG (oxidized glutathione) stimulates the HMP pathway.[8] Consequently, vastly increased amounts of NADPH potentially can be produced. By the NADPH-catalysed reduction of GSSG a corresponding increment in GSH is generated.

The hemolytic susceptibility of G6PD-deficient red cells is explained by the fact that the enzyme deficiency limits the rate at which the first step of the HMP pathway takes place. Hence, in response to an oxidant stress, levels of reduced glutathione are not maintained, permitting oxidative degeneration of hemoglobin. Other deficiencies (glutathione, glutathione reductase, glutathione peroxidase) can compromise the red cell in a similar way (Fig. 1).[9]

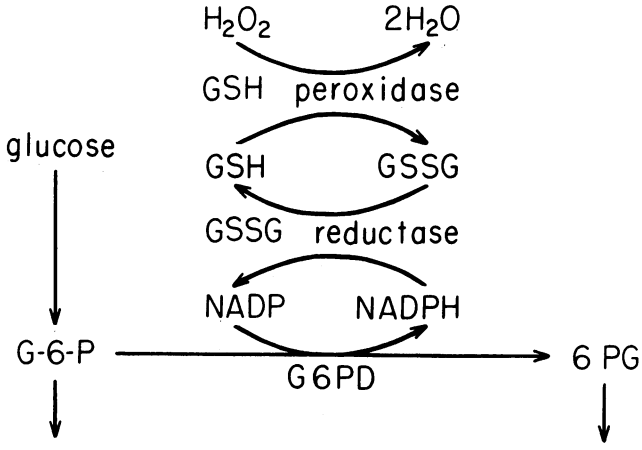


FIG. 1—Hexosemonophosphate (HMP) pathway of oxidative glycolysis
G-6-P = glucose-6-phosphate.
6-P-G = 6-phosphogluconate.
NADPH = reduced triphosphopyridine nucleotide.
NADP = oxidized triphosphopyridine nucleotide.
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**Table I**
**Clinical aspects of 25 cases of subacute and chronic analgesic intake**

| | |
|---|---|
| Sex: | 8 male, 17 female |
| Age (years): | 23-69 |
| Daily consumption of phenacetin: | 0.9-4.0 g. |
| Total calculated intake of phenacetin: | 1.0-9.0 kg. |
| Duration: | 2 weeks to more than 10 years. |
| Reason for analgesia: Headaches | 14/25 (56%) cases |
| Abdominal pain | 7/25 (28%) cases |
| Other pain (Ca breast, multiple myeloma) | 4/25 (16%) cases |
| Psychiatric illness: | 11/25 (44%) cases |
| Peptic ulceration with total or subtotal gastrectomy: | 14/25 (56%) cases |
| Renal disease: Renal failure | 8/25 (32%) cases |
| Urinary tract infection | 6/25 (24%) cases |

Substances that directly oxidize NADPH, hemoglobin and GSH, notably acetylphenylhydrazine and methylene blue, exert their effect despite normal function of the HMP shunt and a normal GSH level. These chemicals are highly potent oxidants, almost equally destructive to normal as well as to enzyme-deficient red cells.[8] It is not known by which mechanism the metabolites of phenacetin exert their oxidative effect.

Once the buffering action of GSH is lost, heme and globin undergo oxidative changes. Heme is affected by the conversion of ferrous to ferric iron resulting in the reversible production of methemoglobin. Concomitantly, but at a slower rate, the SH-groups of the globin molecule undergo oxidation, leading to irreversible denaturation of the molecule with formation of "sulfhemoglobin" (pyridine hemochromagen), and ultimately precipitation and polymerization of the denatured protein resulting in what is morphologically recognizable as Heinz bodies.[10, 11] The red cells containing these inclusions are rapidly cleared from the blood by entrapment and destruction in the spleen. The mechanism of entrapment may be related to alterations of red cell membrane SH-groups or, more likely, to the fact that the presence of one or more Heinz bodies renders the affected cell less deformable. Such a stiff red cell may become entrapped during its passage through the spleen, either in the Billroth cords or while passing through the fenestrations separating the cords from the sinusoids. The Heinz body, together with a piece of red cell membrane, may then simply be pinched off, leading to deformation of the cell and spherocytosis. During their prolonged sojourn in the spleen the damaged red cells are exposed to and engulfed by macrophages of the reticulo-endothelial system, and disposed of by intracellular digestion (erythrophagocytosis).[12]

## Methods

The hematological methods used are routine in our laboratories and have been reported previously. Red cells were stained for Heinz bodies with methyl violet, erythrocyte G6PD content was determined by the method of Zinkham and GSH stability by the method of Beutler.[13] G6PD and GSSH reductase spot tests were carried out using Beutler's method.[14] Met- and sulfhemoglobin levels were determined by the method of Evelyn and Malloy.[15] Hemoglobin electrophoresis was carried out on starch block at pH 8.6.[16]

## Material

The patients were drawn from 81 cases of drug-induced hemolytic anemia (DHA) encountered in the years 1965 to 1969 at the Royal Victoria Hospital. Most cases were discovered on routine hematological examinations, a few were found by reviewing the charts of patients with sulfhemoglobinemia. Twenty-six patients were studied. Of these, there was one case of acute intoxication which will be discussed separately. In one other patient there was a two-week history of phenacetin intake, and in the remaining 24 cases the history extended over many years. The renal aspects of four cases of chronic abuse have been reported in detail elsewhere.[1]

### Clinical aspects of chronic and subacute cases (Table I)

Eight of the patients were males, 17 were females; their

ages ranged from 23 to 69 years; all patients were Caucasian except one negro female. The daily consumption of phenacetin varied between 0.9 and 4.0 g. in the subacute and chronic cases, with a duration of two weeks in the subacute case and lasting from two to more than 10 years in the 24 chronic abusers. The total intake of phenacetin ranged from 1.0 to 9.0 kg. The majority of patients consumed, in addition to phenacetin, large amounts of caffeine and acetylsalicylic acid (ASA) in the form of a common proprietary tablet. One patient had ingested a preparation containing acetanilid, phenacetin and acetylsalicylic acid. In 56% of patients the analgesics were used for relief of headaches and in 28% for relief of abdominal pain. In three patients analgesia was necessary for the relief of pain associated with malignant disease. Forty-four per cent of the patients had frank psychiatric disease ranging from personality disorder to schizophrenia. A history of peptic ulcer with total or partial gastrectomy was present in 56% of cases. Malnutrition was present in many patients and malabsorption was proven in four. Eight patients had progressed to the stage of renal failure as evidenced by increased creatinine and BUN. In six patients without evidence of renal failure, acute or chronic urinary tract infection was diagnosed. None of the patients had undergone splenectomy.

**Hematological data (Table II).**

**Red cell morphology**

The red cell morphology, as assessed in the course of routine hematological studies, was often the clue that led to the diagnosis of DHA. The morphological pattern characterizing this anemia is the presence of "bitten out" and irregularly contracted, densely stained erythrocytes, accompanied by anisocytosis and polychromasia. These so-called "bitten out" red cells, which occurred with a frequency of 80%, appear to have had one or more "bites" made in their cell membrane as shown in Fig. 2.

**Table II**

**Hematological data in 25 cases of subacute and chronic analgesic intake**

| | | Present in | |
|---|---|---|---|
| | | Number of cases/total number examined | % |
| Anemia: Initial Hb. conc. (g. per 100 ml.) | mean 9.5 range 5.4-16.6 | 25/25 | 100 |
| Reticulocytosis: Initial retic. count (%): | mean 3.2 range 0.6-43.6 | 25/25 | 100 |
| "Bitten out" red cells | | 20/25 | 80 |
| Heinz bodies: Initial percentage: | mean 1.2 range 0-85 | 21/25 | 84 |
| Methemoglobinemia (> 2.5% of total Hb.) | | 5/25 | 20 |
| Sulfhemoglobinemia | | 25/25 | 100 |
| G6PD deficiency | | 0/18 | 0 |
| Positive Coombs' test | | 1/19 | 5 |
| Fe deficiency | | 12/22 | 54 |
| Folate deficiency | | 7/15 | 47 |
| Abnormal hemoglobin | | 0/9 | 0 |

The presence of these cells with or without spherocytosis and reticulocytosis should alert the hematologist to the possible existence of a hemolytic process caused by use of oxidant drugs.

Because of the association of iron and folate deficiencies, hypochromia, poikilocytosis, oval macrocytes and hypersegmented neutrophils sometimes were present in addition to the characteristic morphological picture just described. In the patients with renal failure burr cells were regularly observed.

**Anemia**

All 25 patients were anemic. On the basis of sulfhemoglobinemia, Heinz bodies and reticulocytosis, approximately 84% of patients had evidence of hemolysis as the cause of or as an important contribution to the anemia. Of the remaining four patients, three had a reticulocytosis, although Heinz bodies were not observed. Serum bilirubin was not elevated in any of the patients. In 12 (54%) out of 22 patients adequately studied, iron deficiency, diagnosed by absent bone marrow iron stores and/or less than 10% saturation of transferrin, contributed to the anemia. Seven patients showed evidence of folate deficiency (serum folate less than 4 ng./ml.). Six of these had had total or partial gastrectomy and four had proven malabsorption. In the patients with chronic

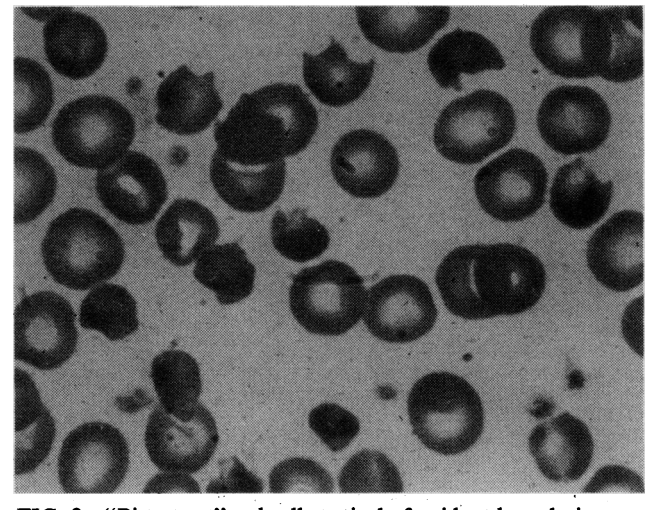

FIG. 2—"Bitten out" red cells typical of oxidant hemolysis.

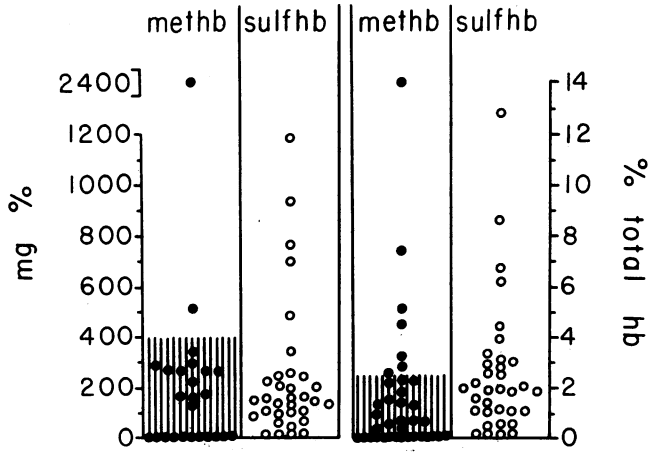


FIG. 3—Levels of methemoglobin (closed circles) and sulfhemoglobin (open circles) in mg. per 100 ml. and as % of total hemoglobin in 26 cases of DHA. The shaded area indicates normal values of methemoglobin. Sulfhemoglobin is not found in normal blood.

renal failure or malignant disease, the systemic illness contributed significantly to the degree of anemia.

### Sulfhemoglobinemia, methemoglobinemia and Heinz bodies

All patients had sulfhemoglobinemia, since the presence of this abnormal pigment was the chief criterion of patient selection. The highest concentration was 1180 mg. per 100 ml. or 12.8% of total hemoglobin (Fig. 3).

Elevated levels of methemoglobin were less constant. The total amount of methemoglobin was increased (above 400 mg. per 100 ml.) in two patients; the highest level of 2400 mg. per 100 ml. (14% of the total hemoglobin) was observed in the patient who had ingested acetanilid as well as phenacetin. In five anemic patients the percentage methemoglobin exceeded the upper limit of normal (2.5%) by this method, although the total amount of methemoglobin was not increased. Elevated levels of methemoglobin usually reverted to normal within 24 hours after discontinuation of phenacetin.

The high percentage (84%) of patients with Heinz bodies in their red cells emphasizes the importance of this finding as an indicator of the presence of drug-induced hemolysis.

Fig. 4 shows the typical hematological course of a patient after the phenacetin-containing drug was discontinued. Concomitantly with the decrease in reticulocyte count there was a rise in hemoglobin concentration. Heinz bodies cleared within a week, but sulfhemoglobin persisted for more than two weeks.

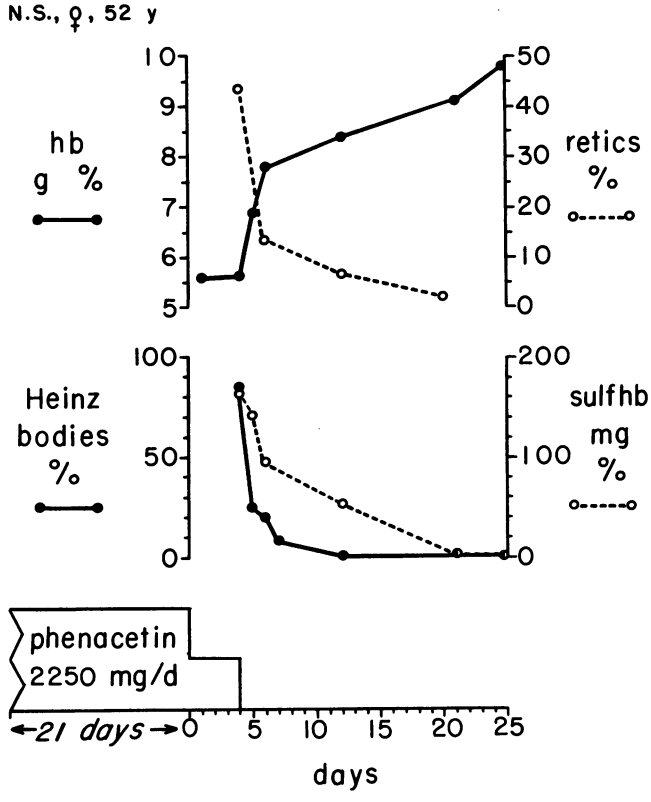


FIG. 4—Response of the Hb. concentration, reticulocyte count, Heinz body count and sulfhemoglobin concentration in a patient with chronic phenacetin abuse, after withdrawal of the drug.

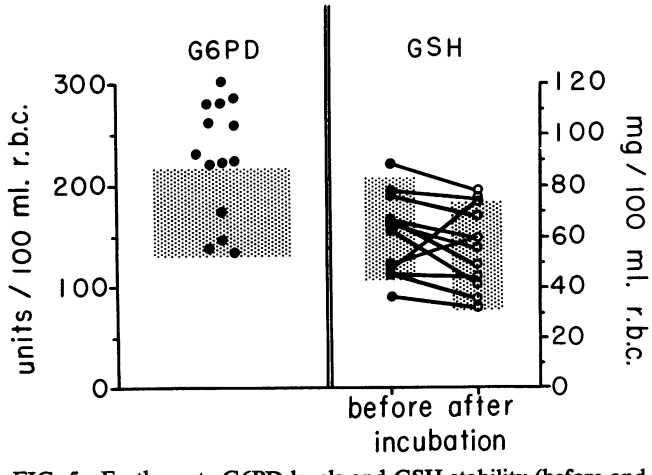


FIG. 5—Erythrocyte G6PD levels and GSH stability (before and after incubation of the blood with acetylphenylhydrazine) in 12 patients with DHA. The shaded areas indicate the normal values.

### G6PD, GSH and GSSG reductase

In 12 patients the erythrocyte G6PD level was normal or elevated and glutathione stability was normal, although in one patient the initial GSH level was slightly decreased (Fig. 5). In six other patients the G6PD spot test was normal. GSSG reductase activity (by spot test) was normal in an additional two patients. Hence in the patients examined there was no evidence of G6PD, glutathione reductase or GSH deficiency.

### Miscellaneous hematological data

Of 19 patients examined the direct Coombs' test was negative in 18 and slightly positive in one. Hemoglobin electrophoresis in nine patients was normal.

### Acute intoxication

One patient, in a suicidal attempt, ingested 100 tablets of an analgesic preparation, containing a total of 16 g. of phenacetin, 22 g. of acetylsalicylic acid and 3.2 g. of caffeine. The patient presented with cyanosis, diaphoresis, disorientation and transient obtundation. The maximal ASA blood level was 71.4 mg. per 100 ml. The subsequent course showed evidence of a hemolytic anemia with a drop in hemoglobin concentration from 16.1 to 10.7 g. per 100 ml., 5.6% reticulocytes, methemoglobinemia, and 21% Heinz bodies. Erythrocyte G6PD content and glutathione stability were normal.

### Incidence of recurrent phenacetin exposure

Phenacetin-containing compounds were prescribed for four patients subsequent to the recognition of phenacetin-induced hemolysis, even during the same hospital admission. Several other patients continued to be exposed because of habituation. Four patients were observed during more than one episode of hemolytic anemia.

### Treatment

In most patients the anemia responded rapidly to withdrawal of the offending drug, and to iron and folate supplementation if there were concomitant deficiencies of these substances. In patients with chronic renal failure or malignant disease normal hemoglobin levels were not achieved.

### Discussion

The recognition of drug-induced hemolysis may lead to the diagnosis of a clinical syndrome with gastrointestinal and renal manifestations which frequently runs a fatal

course. Hemolysis will often be the first indication that a patient is abusing an oxidant drug.

In the case of phenacetin, by arresting the abuse the development or progression of analgesic nephropathy may be prevented.

The single most valuable diagnostic procedure is careful examination of red cell morphology. The characteristic finding is the presence of "bitten out" and irregularly contracted red cells (Fig. 2). This suggests a drug-hemolytic process.[4] Other morphological abnormalities may appear in association with "bitten out" cells. Hypochromia may be present owing to iron deficiency as a result of chronic blood loss secondary to the gastrointestinal ulceration produced by salicylates. Folate deficiency with its attendant macro-ovalocytosis and hypersegmentation of the nuclei of granulocytes, is usually observed in patients who have had gastric surgery and is due to malnutrition or is part of a malabsorption syndrome. Burr cells and occasional spherocytes may be seen in azotemia. Hence the morphological picture is a composite, the most useful feature being the "bitten out" red cells.

Once abnormal morphology is detected, establishment of the diagnosis of DHA due to oxidant drugs requires the demonstration of sulfhemoglobinemia and Heinz bodies. In this series all patients had sulfhemoglobinemia and 84% had Heinz bodies. The latter were not detected in some patients, because their persistence following discontinuance of the drug is of much shorter duration than that of sulfhemoglobin (Fig. 4). The explanation for this disparity lies in the fact that sulfhemoglobin has no known effect on the structural stability of the red cell, whereas Heinz bodies alter the deformability of the erythrocyte and hence render it susceptible to splenic entrapment and destruction.[12] Red cell survival studies show that the hemolytic process in phenacetin abusers is due to intracorpuscular as well as extracorpuscular factors and that destruction of red cells takes place in the spleen.[17-19]

Although ingestion of small doses of phenacetin-containing analgesics for a limited period of time has not been shown to affect red cell survival or to cause Heinz body formation in normal subjects,[18] there is no doubt that massive doses of phenacetin-containing compounds can cause an acute hemolytic anemia in an individual without G6PD deficiency and without renal impairment, as is evident from the one case reported here. The hemolytic effect of phenacetin shows some correlation with the degree of renal impairment,[18] presumably because of impaired excretion of the metabolites. More than half of our patients had mild to moderate degrees of azotemia or evidence of urinary tract infection and may well have had impaired excretion of the toxic metabolites of phenacetin at doses considered to be in the therapeutic range. In many cases a time relationship between the onset of the hemolytic anemia and that of the renal disease could not be established. Frequently the presence of a drug-induced hemolytic anemia made the clinician aware of the true etiology of a hitherto obscure, chronic nephropathy.

Methemoglobin was not, or only transiently, elevated owing to rapid reduction by methemoglobin reductase. An exception was the marked methemoglobinemia observed in the patient who had ingested acetanilid, which is a well known, potent methemoglobin producer.[20]

Deficiencies of G6PD, GSSG reductase, glutathione peroxidase and GSH have been associated with increased susceptibility of the erythrocyte to the hemolytic effects of oxidant drugs.[9, 21] In the patients studied for these defects, G6PD levels were normal or elevated and GSH stability, a measure of GSSG reductase activity, was normal. These results confirm previous observations that this type of DHA frequently occurs in the absence of any demonstrable enzyme deficiency.[4]

The presence of an unstable hemoglobin or hemoglobin H renders the red cells sensitive to the action of oxidant drugs despite the presence of normal reducing mechanisms.[21] None of the patients examined in this study showed evidence of a hemoglobinopathy.

The direct Coombs' test was weakly positive in one patient, suggesting a possible immune process caused by phenacetin. However, the main cause of the red cell destruction in this patient was the oxidant effect of the drug, since 86% of the cells contained Heinz bodies.

Caffeine is an ingredient of many analgesic compounds. Dimitrov, Millar and Ziegra[22] have shown that caffeine inhibits the HMP shunt of polymorphonuclear leukocytes. The effect of this drug on the HMP shunt of erythrocytes and its role in the development of this type of drug-induced hemolytic anemia, deserve further investigation.

Approximately 50% of the patients in this series had reached the stage of the analgesic abuse syndrome. They were invariably heavy phenacetin ingesters (doses up to 2.5 g. per day) and often manifested psychiatric disturbances, peptic ulcer and its sequelae, and in some instances renal failure. It is the latter that finally threatens the patient's life. This complication can probably be avoided by early recognition of the other components of the syndrome, particularly the hemolytic anemia.

**Table III**
**List of analgesics which contain phenacetin**

| Commercial name: | Phenacetin (mg.) | Suggested dose (mg./day) | ASA (mg.) | Other substances Caffeine (mg.) | Other |
|---|---|---|---|---|---|
| Algiatran | 150 | 450-1200 | 230 | 30 | *† |
| Coricidin | 160 | 960 | 230 | 30 | † |
| Coriforte | 130 | 520-780 | — | 30 | † |
| Daprisal | 162 | 972 | 162 | — | † |
| Darvon Compound | 162 | 486-1296 | 227 | 32.4 | † |
| Empirin | 150 | 150-300 prn | 225 | 30 | |
| Fiorinal C | 130 | 780-1040 | 200 | 40 | *† |
| Neurasal | 195 | 195-390 prn | 325 | 11 | † |
| Norgesic | 160 | 480-960 | 225 | 30 | † |
| Novahistex APC + C | 200 | 1200 | 300 | 30 | *† |
| Percodan | 160 | 640 | 224 | 32 | † |
| Phenacodein | 150 | 150 prn | 210 | 30 | * |
| Phenalone | 45 | 90 | 210 | — | *† |
| Phenaphen | 194 | 1164-4656 | 162 | — | † |
| Robaxisal—PH | 97 | 776-1164 | 81 | — | † |
| Sinutab | 150 | 150-600 | — | — | † |
| Soma compound | 160 | 480-960 | — | 32 | † |

* = codeine
† = other substance

The fact that several patients were inadvertently prescribed phenacetin subsequent to the diagnosis of DHA demonstrates that sufficient awareness of the dangers of chronic phenacetin intake for the health of the patient still does not prevail in the medical community. This problem is compounded by the fact that phenacetin is still available in a wide variety of medications currently on the market (Table III). Although some drug companies have recognized the dangers of phenacetin and have recently discontinued its use, there is an obvious need for this recognition to become more widespread.

We wish to thank Mrs. S. Hutchison, Mrs. G. Charlow and Mrs. L. Brigden for excellent technical assistance.

**Résumé**

*Anémie hémolytique provoquée par la phénacétine*

Les auteurs exposent les caractéristiques hématologiques de l'anémie hémolytique provoquée par la phénacétine, en vue d'attirer l'attention du médecin sur des anomalies qui permettent de croire à l'emploi chronique de la phénacétine. Le signe avant-coureur le plus fréquent de cette hémopathie médicamenteuse est la présence d'érythrocytes fragmentés ou crénelés ("bitten out"). Ce diagnostic est confirmé par la découverte de corps d'Ehrlich-Heinz et de sulfhémoglobinémie. La reconnaissance précoce de cette forme d'abus de médicaments peut empêcher le développement ou l'évolution d'une néphropathie analgésique.

## References

1. GAULT MH, RUDWAL TC, ENGLES WD, et al: Syndrome associated with the abuse of analgesics. *Ann Intern Med* 68; 906, 1968
2. DAWBORN JK, FAIRLEY KF, KINCAID-SMITH P, et al: The association of peptic ulceration, chronic renal disease, and analgesic abuse. *Q J Med* 35: 69, 1966
3. SHELLEY JH: Phenacetin through the looking glass. *Clin Pharmacol Ther* 8: 427, 1967
4. DACIE JV: *The Haemolytic Anaemias IV*, second ed, London, J & A Churchill Ltd., 1967
5. DAUSSET J, CONTU L: A case of haemolytic anaemia due to phenacetin allergy. *Vox Sang* 9: 599, 1964
6. MACGIBBON BH, LOUGHRIDGE LW, HOURIHANE DO, et al: Autoimmune haemolytic anaemia with acute renal failure due to phenacetin and p-aminosalicylic acid. *Lancet* I: 7, 1960
7. MUIRHEAD EE, HALDEN ER, GROVES M: Drug-dependent Coombs (antiglobulin) test and anemia. *Arch Intern Med* 101: 87, 1958
8. JACOB HS, JANDL JH: Effects of sulfhydryl inhibition on red blood cells. III Glutathione in the regulation of the hexose monophosphate pathway. *J Biol Chem* 241: 4243, 1966
9. BEUTLER E: Drug-induced hemolytic anemia. *Pharmacol Rev* 21: 73, 1969
10. ALLEN DW, JANDL JH: Oxidative hemolysis and precipitation of hemoglobin. II Role of thiols in oxidant drug action. *J Clin Invest* 40: 454, 1961
11. JANDL JH, ENGLE LK, ALLEN DW: Oxidative hemolysis and precipitation of hemoglobin. I Heinz body anemias as an acceleration of red cell aging. *J Clin Invest* 39: 1818, 1960
12. RIFKIND RA: Destruction of injured red cells *in vivo*. *Am J Med* 41: 711, 1966
13. DE LEEUW NKM, SHAPIRO L, LOWENSTEIN L: Drug-induced hemolytic anemia, *Ann Intern Med* 58: 592, 1963
14. BEUTLER E, MITCHELL M: Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency. *Blood* 32: 816, 1968
15. EVELYN KA, MALLOY HT: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *J Biol Chem* 126: 655, 1938
16. KUNKEL HG, CEPPELLINI R, MÜLLER-EBERHARD U, et al: Observations on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassemia. *J Clin Invest* 36: 1615, 1956
17. LORENZEN I, SCHWARTZ M: Phenacetin-induced haemolysis in patients with chronic nephritis and phenacetin abuse. *Acta Med Scand* 168: 461, 1960
18. NISSEN NI, FRIIS T: Further study of the mechanism of reduction of erythrocyte survival time in phenacetin habitués and its relation to "phenacetin kidney disorders". *Acta Med Scand* 171: 125, 1962
19. FRIIS T, FOGH J, NISSEN NI: The mechanism of reduction of erythrocyte lifetime in phenacetin habitués with renal impairment. *Acta Med Scand* 168: 127, 1960
20. GOODMAN LS, GILMAN A: *Pharmacological Basis of Therapeutics*, third ed, New York, MacMillan, 1965, p 332
21. NECHELES TF, ALLEN DM: Heinz-body anemias. *N Engl J Med* 280: 203, 1969
22. DIMITROV NV, MILLAR J, ZIEGRA SR: The effects of caffeine on glucose metabolism of polymorphonuclear leukocytes. *J Pharmacol Exp Ther* 168: 240, 1969