The chronic use of procainamide is associated with a high incidence of drug-induced lupus and agranulocytosis. We have previously demonstrated that procainamide is metabolized in the liver to reactive hydroxylamine (PAHA) and nitroso (nitroso-PA) metabolites which covalently bind to protein and are toxic to lymphocytes. We proposed that these metabolites were responsible for the toxicities of procainamide. However, PAHA and nitroso-PA do not appear to escape the liver in significant concentrations. In this paper we describe the metabolism of procainamide to a reactive hydroxylamine by neutrophils and mononuclear leukocytes. Such metabolism only occurs if the cells have been stimulated to have a respiratory burst. These observations have obvious possible implications for the mechanism of procainamide-induced agranulocytosis (formation of a reactive metabolite by neutrophils) and procainamide-induced lupus (formation of a reactive metabolite by monocytes). The metabolism of drugs to reactive metabolites by monocytes may be a general mechanism for hypersensitivity reactions because monocytes play a key role in the processing of antigen and stimulation of antibody synthesis.

Protein hydroxyaminations are catalyzed by the enzyme hydroxylamine reductase. This enzyme is present in the liver and can be induced by a wide variety of drugs, including phenobarbital, tolbutamide, and phenytoin. When hydroxylamines are present in the liver, they are converted to reactive metabolites by a process known as phase II metabolism. These reactive metabolites then bind to protein and are eventually excreted in the urine. In the case of procainamide, the reactive metabolites are PAHA and nitroso-PA.

The liver is a major site of metabolism for procainamide. Most of the drug is metabolized in the liver to reactive hydroxylamine and nitroso compounds. These metabolites are then excreted in the urine. The metabolism of procainamide in the liver is important because it is responsible for the toxicities of the drug. The reactive metabolites that are formed in the liver can bind to protein and cause toxicity.

Materials and Methods

Leukocyte Isolation. After informed consent, blood was obtained from normal subjects by venipuncture and withdrawal into a heparinized syringe. The leukocytes were isolated by using a standard procedure (12). Briefly, dextran (2 mL of 6% w/v in normal saline for every 10 mL of blood, MW 500,000, Sigma Chemical Co., St. Louis, MO) was added to the blood and mixed. The mixture was allowed to stand for 45 min and the plasma layer was removed and layered on top of sodium diatrizoate-Ficoll (LSM, 2 mL for every 5 mL of plasma, Littton Bionetics, Charleston, SC). This was centrifuged at 500 g for 30 min. The polymorphonuclear leukocytes (PMN) which are mostly neutrophils were obtained from the pellet. The erythrocytes in the pellet were lysed with a buffered ammonium chloride solution (0.15 M ammonium chloride, 0.01 M potassium bicarbonate). Mononuclear leukocytes (MNl), which are a mixture of lymphocytes and monocytes, were obtained from the plasma/LSM interface. Mononuclear leukocytes (MNl), which are a mixture of lymphocytes and monocytes, were obtained from the plasma/LSM interface. Mononuclear leukocytes (MNl), which are a mixture of lymphocytes and monocytes, were obtained from the plasma/LSM interface. Mononuclear leukocytes (MNl), which are a mixture of lymphocytes and monocytes, were obtained from the plasma/LSM interface. Mononuclear leukocytes (MNl), which are a mixture of lymphocytes and monocytes, were obtained from the plasma/LSM interface.
µL of dimethyl sulfoxide, Sigma Chemical Co., St. Louis, MO) followed by procainamide (various concentrations in 10 µL of water, Sigma Chemical Co., St. Louis, MO) was added. The suspension was then incubated at 37 °C in a shaking water bath.

### Synthesis of Standards

The synthesis of 4-nitro-N-[2-(diethylamino)ethyl]benzamide hydrochloride (nitro-PA), 4-[(hydroxyamine)-N-[2-(diethylamino)ethyl]benzamide (PAHA), and 4-nitrosobenzene-2,3-dicarboxamide (nitroso-PA) were described previously (3). Procainamide N-oxide was synthesized in the same manner as had been done previously for N-acetylprocainamide N-oxide, and it also reacted in the same characteristic manner with bisulfite to give procainamide and desethylprocainamide (13).

### Analytical

After the incubation of procainamide with activated leukocytes was complete, the mixture was centrifuged at 13000g for 2 min. The supernatant (15 µL) was injected into the HPLC without further workup. HPLC was performed with a Beckman 110B pump (Berkeley, CA), a Waters C18, 5-mm i.d. Nova-Pak cartridge in a Z-module (Milford, MA), and a Beckman 160 UV absorbance detector at a wavelength of 254 nm. The solvent for procainamide and PAHA consisted of water, acetonitrile, acetate acid, and triethylamine (96:3:1:0.05, v/v). The flow rate of 1 mL/min, the retention times of PAHA, procainamide, nitroso-PA, and nitro-PA were 5.7 min and that of nitro-PA was 4.9 min. The solvent system for nitro-PA was the same except the ratio was 79:20:1:0.05 (v/v). With this solvent the retention times of nitro-PA was 1.8 min and that of nitro-PA was 4.9 min. The peak areas were integrated with a Shimadzu C-R3A Chromatopac (Tokyo, Japan). The standard curves for PAHA and nitro-PA were linear with correlation coefficients of 0.995 and 0.985, respectively.

The identity of the PAHA peak was confirmed by adding NaOH to increase the pH above 12, which converted the PAHA to nitro-PA as determined by HPLC. This is a characteristic reaction of arylhydroxylamines (3). The identity of the nitro-PA metabolite was confirmed by GC-MS. The instrument was a Hewlett Packard 5897A GC-MS system. The column was a DB-17 fused silica capillary column (30 m × 0.2 mm i.d., J & W, Folsom, CA). The carrier gas was helium at a pressure of 7.5 psi. The ionization was methane chemical ionization with the mass detector operating at 800 mTorr. The ionization was methan chemical ionization with a methane pressure of 300 mTorr. The instrument was operated in the negative ion mode. The only peak in the spectrum of authentic nitro-PA was at m/z 265. By use of selective ion monitoring at 265 the retention time was 22.3 min. The metabolic conversion also gave only one peak under these conditions at 22.3 min, and a mixture of authentic nitro-PA and metabolite also gave only one peak.

Evidence for nitroso-PA and procainamide N-oxide metabolites was sought with a different HPLC system consisting of a 15-cm, 5-µm, C18, Spherisorb column (Jones Chromatography, Llanbradach, U.K.) and a mobile phase consisting of water, acetonitrile, acetate acid, and triethylamine (80:20:1:0.05, v/v). With a flow rate of 1 mL/min, the retention times of PAHA, procainamide, procainamide N-oxide, nitro-PA, and nitroso-PA were 2.8, 3.8, 4.2, 12.7, and 14.1 min, respectively.

### Results

Analogous to our results with dapsone, procainamide was oxidized by activated PMN to nitro-PA. In the absence of PMA (which activates the cells) no oxidation occurred. In the presence of ascorbic acid, the PAHA intermediate was detected. This is presumably because the ascorbate prevented oxidation of PAHA to nitro-PA. Although hydrogen peroxide, by itself, did not oxidize procainamide to PAHA, it did oxidize PAHA to nitro-PA, (data not shown). The formation of nitro-PA and PAHA as a function of ascorbate concentration is shown in Figure 1. At a high ascorbate concentration (5 mM) neither of the metabolites were detected, presumably because the ascorbate reduced almost all of the hydrogen peroxide which is necessary for the metabolism. The oxidation of procainamide in the absence of ascorbic acid was roughly linear with time, but in the presence of ascorbic acid, although the production of observed metabolite was higher, it started to level off after about 10 min (Figure 2). The production of PAHA and nitro-PA as a function of PMN concentration and procainamide concentration is also shown in Figure 2. The metabolism was inhibited by azide and catalase (Figure 3) but not by inhibitors of prostaglandin synthetase (Table I).

Although procainamide oxidation by hydrogen peroxide alone was not detectable, the combination of purified myeloperoxidase (MPO) and hydrogen peroxide oxidizedprocainamide to PAHA and other reactive metabolites (data not shown). In contrast, horseradish peroxidase and hydrogen peroxide did not oxidize procainamide to PAHA or nitro-PA (data not shown). This will be the subject of a separate paper.

Similar results were obtained with MNL (Figures 4 and 5, Table II) except that no nitro-PA was produced even in the absence of ascorbate. However, ascorbate was added to the MNL incubations because it approximately doubled the yield of PAHA. These reactions are summarized in Scheme I.

Other possible metabolites, such as nitroso-PA and the N-oxide of the tertiary amine, were not detected. Nitroso-PA would not be expected because, in the absence of ascorbate, it would be rapidly oxidized to nitro-PA or covalently bind to protein, and, in the presence of ascorb-
Figure 2. Metabolism of procainamide by PMN in the presence and absence of ascorbic acid: (A) rate; (B) PMN concentration dependence; (C) substrate concentration dependence. Standard conditions: PMN concentration, 1.5 $\times$ 10$^6$ cells/mL; procainamide concentration, 0.1 mM; PMA concentration, 40 ng/mL; incubation time, 45 min. Ascorbic acid was added to incubations in which PAHA was determined (1 mM) but not to the incubations in which nitro-PA was determined. The values are the mean ± SE from four experiments.

Figure 3. Inhibition of PMN metabolism of procainamide: (A) inhibition by azide; (B) inhibition by catalase. Standard conditions: PMN concentration, 1.5 $\times$ 10$^6$ cells/mL; procainamide concentration, 0.1 mM; PMA concentration, 40 ng/mL; incubation time, 45 min. Ascorbic acid was added to incubations in which PAHA was determined (1 mM) but not to the incubations in which nitro-PA was determined. The values are the mean ± SE from four experiments.

Scheme I. Summary of Procainamide Metabolism by Leukocytes

1. Procainamide was oxidized by activated PMN to nitro-PA. This is a six-electron oxidation and is unlikely to occur in one step. The hydroxylamine and nitroso derivatives are the presumed two-electron oxidation intermediates. These intermediates are easily oxidized, and it is not surprising that they would not exist long in the oxidant environment of an activated PMN. We have previously shown that ascorbic acid reduces nitroso-PA to PAHA (4).

2. In the present work, ascorbate was able to prevent oxidation of PAHA to nitro-PA without reducing all the hydrogen peroxide which would prevent metabolism of procainamide. Since ascorbic acid and other reductants are

**Discussion**

Procainamide was oxidized by activated PMN to nitro-PA. This is a six-electron oxidation and is unlikely to
Metabolism of Procainamide by Leukocytes

The oxidation of procainamide to PAHA appears to be enzymatic since hydrogen peroxide or superoxide alone did not oxidize procainamide. However, further oxidation of PAHA to nitro-PA does occur with hydrogen peroxide alone. Since no metabolism occurred in the absence of activation of the cells and low concentrations of azide inhibited the metabolism, cytochrome P-450 must not contribute significantly to the metabolism. Lack of inhibition by aspirin and indomethacin rules out prostaglandin synthetase. It is known that MPO and hydrogen peroxide are released when PMN are activated. Since procainamide is also oxidized to PAHA by purified MPO in the presence of hydrogen peroxide, this is the likely explanation for the observed metabolism by PMN. This is supported by the observations that azide (which inhibits MPO) and catalase (which decomposes hydrogen peroxide) inhibit the metabolism. The mechanism appears to involve a two-electron oxidation because horseradish peroxidase (which is said to only catalyze one-electron oxidation) did not catalyze oxidation of procainamide to PAHA.

Procainamide was also metabolized by MNL (which are a mixture of monocytes and lymphocytes). As with PMN, the metabolism appears to be due to MPO since activation of the cells is required and metabolism is inhibited by azide. If this is true, the active cell must be the monocyte since lymphocytes do not contain MPO and are not capable of a respiratory burst. Unlike metabolism by PMN, MNL did not produce detectable concentrations of nitro-PA. PAHA was the only metabolite detected even in the absence of ascorbic acid. This may simply be due to the lesser amount of hydrogen peroxide present, both because monocytes do not produce as much as PMN and because only about $\frac{1}{4}$ of the MNL are monocytes.

As with dapsone and sulfadiazine, we propose that procainamide-induced agranulocytosis is due to the chemically reactive PAHA and/or nitroso-PA metabolites formed by activated PMN. The mechanism could involve direct bone marrow toxicity if myeloperoxidase-containing leukocytes were activated in the bone marrow. Alternatively, these reactive metabolites could be formed by activated peripheral neutrophils and bind to their cell membranes, causing the induction of autoantibodies which react with neutrophils. It is likely that a relatively high concentration of reactive metabolites will be present on the neutrophil surface since the hydrogen peroxide gen-
erating system is on the outer surface of the cell membrane, and the myeloperoxidase is released on the surface of the cell or into a phagosome which is formed by invagination of the cell membrane.

Likewise, reactive metabolites of procainamide are likely to bind to the cell membrane of activated monocytes. A key function of the monocyte and other macrophages is to process antigen and to present it, along with the class II major histocompatibility glycoprotein (MHC II), to lymphocytes (14). This is an important step in the induction of antibody synthesis. It has been suggested that binding of hapten to the membrane of monocytes may provide a strong stimulus for antibody synthesis (15). Furthermore, since it appears as if processed antigen may have to bind to MHC II (14), direct binding of a reactive metabolite to MHC II may obviate the need for processing haptenized protein by monocytes. Thus, the formation of reactive metabolites on the surface of activated monocytes where they can react with MHC is an attractive hypothesis for the mechanism of procainamide-induced lupus. This may represent a general mechanism for the induction of lupus and other hypersensitivity reactions since many of the drugs involved are aromatic amines or hydrazines (16). It also appears as if several other types of drugs can be metabolized to reactive metabolites by myeloperoxidase-/H2O2 (17-19). Some carcinogens can also be activated by this system (20).

Acknowledgment. This work was supported by a grant from the Medical Research Council of Canada (MA-9336).

References