2. The reduction or elimination of inhibition by morphine was considered to be a reduction of anxiety associated with anticipation of noxious stimuli. The results parallel in all essential details previous methodological work on man, and strongly suggest that the present procedure may be useful as a technic for the screening of possible analgesic drugs.

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## Observations on G-25671, A Phenylbutazone Analogue (4-(phenylthioethyl) -1,2-diphenyl 3,5-pyrazolidinedione).* (21263)

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Phenylbutazone (Butazolidin) has been shown to be a potent antirheumatic agent but it may produce such side effects as edema, gastric distress, skin reactions and, on rare occasions, gastrointestinal hemorrhage and bone marrow depression. In an attempt to develop a drug retaining the antirheumatic action of phenylbutazone but devoid of its side effects, a series of phenylbutazone analogues are being screened in animals and man for anti-inflammatory activity.† It is the purpose of this paper to report preliminary observations in man on one of the compounds (G-25671) which showed marked anti-inflammatory effects in the animal screening tests.

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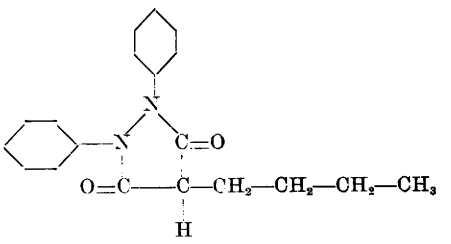
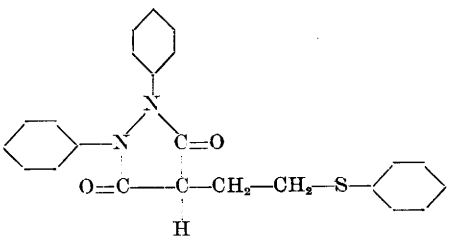
† The compounds, supplied by Geigy Pharmaceuticals, were synthesized by Haefliger, F., *et al.*, and screened in animals by Domenjoz, R., and Wilhelmi, G.

For comparison, the structures of phenylbutazone and G-25671 are shown:



Phenylbutazone



G-25671

*Materials and methods.* Concentration of G-25671 in plasma was determined by a

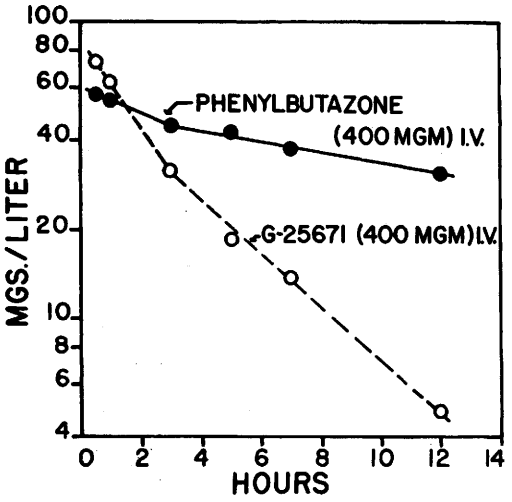



FIG. 1. Plasma levels of phenylbutazone and G-25671 after single intravenous dose of the drugs to same subject.

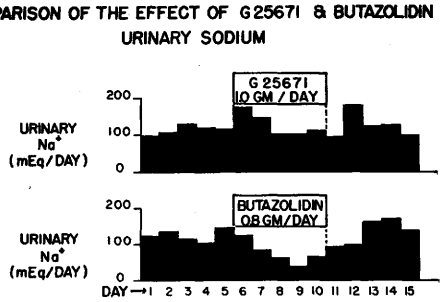
method essentially the same as that used for phenylbutazone(1), except that 3% isoamyl alcohol was added to the petroleum ether used for extraction of the drug and the spectrophotometric assay was made at 255 mu. The specificity of the method for the drug in plasma was established by counter-current distribution. Urinary sodium and potassium concentrations were determined with a flame photometer using lithium as an internal standard. Urinary chlorides were measured by the method of Wilson and Ball(2). Serum and urinary uric acid concentrations were estimated by a modification of the method of Buchanan, Block, and Christman(3), employing uricase, urea-cyanide-carbonate and arsenophosphotungstic acid. For investigation of the physiological disposition of the drug and its effect on electrolyte excretion, 12 non-arthritic patients without obvious heart, liver or kidney disease were used.

*Results. Rate of disappearance of G-25671 from plasma.* Five subjects received an intravenous injection of 400 mg of G-25671 over a 10-minute period and drug plasma levels were measured at various times thereafter. The levels declined at a rate averaging about 20%/hour (Fig. 1) after equilibrium between plasma and tissues had been achieved. Since only minimal amounts of the drug are excreted in the urine and since it is not extensively localized in tissues relative to plasma, this decline must represent its rate of biotransformation. The biotransformation of phenylbutazone, on the other hand, is much slower, averaging 20%/day (Fig. 1).

*Absorption of G-25671.* Information concerning the absorption of G-25671 from the gastrointestinal tract was obtained by comparing plasma levels of G-25671 in 2 subjects following oral and intravenous administration of single doses of 400 mg of the drug. Plasma levels obtained 3 hours after administration were approximately the same by either route, indicating that absorption was rapid and virtually complete.

*Plasma levels on repeated dosage.* Ten subjects received daily one g of G-25671 orally, in divided doses, for a period of 6 days. The drug plasma level 5 hours after the morning dose ranged from 30 to 60 mg/L. Within 24 hours after discontinuing therapy the drug was no longer detectable. By comparison, when patients received 800 mg of phenylbutazone daily, the plasma level ranged from about 80 to 160 mg/L 5 hours after discontinuing dosage and persisted in detectable quantity for a period of from 7 to 10 days.

*Effect on urinary excretion of water, electrolytes and phenolsulfonphthalein (PSP).* The effect of G-25671 and phenylbutazone on urinary excretion of fluid and electrolytes was




| | G25671 | BUTAZOLIDIN |
|---|---|---|
| MAXIMUM CHANGE IN HEMATOCRIT | 43→45 | 46→40 |
| MAXIMUM CHANGE IN BODY WEIGHT (kg) | 46.7→46.8 | 48.6→50.5 |
| URINARY $Cl^-$ | NO CHANGE | DECREASE SIMILAR TO $Na^+$ |
| URINARY $K^+$ | NO CHANGE | NO CHANGE |

FIG. 2. Effect of G-25671 and phenylbutazone in same subject on urinary electrolytes, body wt and hematocrit.

compared in 2 subjects on a controlled sodium intake of about 100 mEq per day. No demonstrable retention of fluid or electrolytes was observed during the administration of G-25671 in daily oral doses up to 1.5 g in contrast to the marked retention of sodium produced by phenylbutazone (Fig. 2). It will be noted that G-25671 produced no weight gain or fall in hematocrit such as was observed during phenylbutazone administration. The effect of G-25671 and phenylbutazone on the excretion of PSP was compared in 3 subjects. Both G-25671 and phenylbutazone produced a significant decrease in the rate of excretion of PSP (Table I).

*Effect on urinary urate excretion.* The drug was found to exert a marked uricosuric effect. The mean increase in urinary uric acid excretion in 4 gouty subjects given 1 g per day by mouth (in divided doses) and maintained on a constant low purine diet was 42% in the first 24 hours of medication, 91% in 48 hours and 65% in 72 hours. The mean decrease in serum uric acid concentration in nine gouty subjects including the 4 subjects in whom the urinary uric acid excretion was measured was 18% after 24 hours, 45% after 48 hours and 49% after 72 hours. A characteristic increase in urinary urate excretion with accompanying fall in serum urate level is shown in Fig. 3. The uricosuric effect ceased rapidly upon discontinuance of medication (Fig. 3). The uricosuric action of G-25671 is more marked than that observed with phenylbutazone(4).

*Anti-inflammatory effects in man.* Currie has already observed that G-25671 exerts an antirheumatic effect in subjects with rheumatoid arthritis(5). Preliminary trials were

TABLE I. Comparison of Effect of G-25671 and Phenylbutazone on Phenolsulfonphthalein (PSP) Excretion, in 3 Patients.

| % of dose of PSP excreted in 30 min. | | | |
|---|---|---|---|
| Effect of G-25671 | | Effect of phenylbutazone | |
| Control | After drug* | Control | After drug† |
| 33.0 | 8.5 | 35 | 15.0 |
| 40.5 | 26.0 | 50 | 29.5 |
| 30.0 | 21.5 | 35 | 22.5 |

* 1 g orally given 3 hr before inj. of PSP.
† .8 g *Idem*

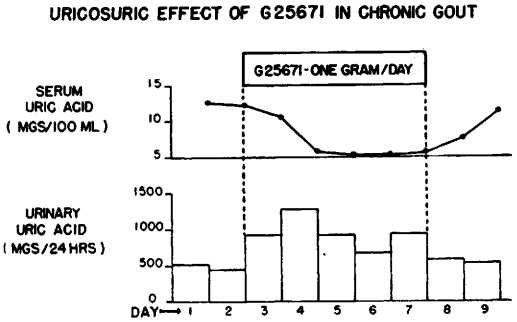



FIG. 3. Effect of G-25671 on serum and urinary uric acid in subject with chronic gout.

carried out by us in 10 patients with active rheumatoid arthritis and 10 with acute gouty arthritis. The drug was administered in doses of 1 to 1.6 g per day. A distinct anti-inflammatory effect was noted in most of the cases but the effects in general appeared to be less marked than those to be expected with phenylbutazone. The only overt side effect noted over periods of up to two weeks medication was a drug rash in one patieent, which rapidly subsided on stopping the drug. Long-term toxicity studies in animals are now in progress. Should further experience indicate that the drug has such low order of toxicity that long-term administration is feasible it may have clinical application, particularly in the treatment of chronic gouty arthritis because of its combined uricosuric and anti-inflammatory properties.

*Summary and Conclusions.* 1. Observations were made on the physiological behavior of a compound in which the butyl side chain of phenylbutazone was replaced by a phenylthioethyl group. 2. Rate of biotransformation of this compound in man was greatly accelerated, with a biologic half life of only 3 hours compared to 70 hours for phenylbutazone. Such rapid disappearance might have some advantage in minimizing toxic manifestations but would pose the problem of maintaining therapeutic levels since the drug would have to be given at relatively frequent levels. 3. Like phenylbutazone, G-25671 exerted distinct antirheumatic effects but produced little or no sodium retention and consequently no hemodilution. These observations show that the antirheumatic and sodium-retaining properties in the phenylbutazone

series may be dissociated. 4. The drug has a more marked uricosuric effect than phenylbutazone.

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## Phospholipid Studies of Different Serum Lipoproteins Employing $P_{32}$. (21264)

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The distribution of the lipoproteins of human serum into 2 main types, the $\alpha$ and the $\beta$ lipoproteins, is now well established as a result of chemical(1,2) and electrophoretic fractionation(3,4). Each of these contains phospholipid and the average normal serum shows approximately equal amounts of $\alpha$ and $\beta$ phospholipid. The two lipoproteins differ considerably in cholesterol and protein concentration(2,3,5). A number of studies have been directed toward an investigation of phospholipid turnover employing $P_{32}$ both in animals and humans (6-8). These have been mainly concerned with total serum phospholipid measurements. The purpose of the present report is to present data concerning phospholipid $P_{32}$ analyses in the individual types of lipoproteins separated electrophoretically.

*Material and Methods.* Six patients, 3 normal individuals and 3 patients with mild liver disease, were employed in this study. Each was given approximately 0.2 millicuries of $P_{32}$ in the form of $Na_2HPO_4$ orally. Bleedings were usually made at 6, 12, 24 and 48 hours after administration. Electrophoretic separation of the lipoproteins was carried out in a starch supporting medium as described previously(3,9). Broad starch blocks were usually employed, measuring 22 cm in width, 45 cm in length and 1.2 cm in thickness. This permitted the separation of 3 samples at the same time furnishing a direct comparison. Samples ranging from 2-10 cc were applied to the starch block and the separation carried out at 350V over a period of 18 hours in a cold room at 4°C. The starch block encased in wax paper was kept in a relatively dry state throughout the procedure. Following the separation, the starch block was cut up into ½ inch segments which were then thoroughly dried before a fan. These were then each extracted with 25 cc of alcohol-ether (Bloors reagent) and these extracts employed for phosphorus analyses and for radioactivity measurements. The alcohol-ether extracts were evaporated down to approximately 4 cc volume and transferred to planchettes for further evaporation to dryness followed by counting. Recoveries of serum lipid phosphorus in the fractions ranged from 85-102% by this procedure. An alternate procedure that was employed for purposes of verification was to obtain the aqueous protein solution directly from the undried starch segments by displacement filtration(9). The protein was then precipitated by $ZnSO_4$ and NaOH(10), washed, and extracted with alcohol-ether. Similar results were obtained by the two procedures although the latter was more time-consuming and recoveries were not as good as by direct extraction of the dry segments. Barbital buffer pH 8.6, $\Gamma/2$ 0.05 was used in these experiments. Phosphorus was converted to phospholipid by the factor 25.

*Results.* The distribution of radioactivity in the lipid extracts of the starch segments following electrophoretic separation of normal sera taken 12, 24 and 48 hours after oral ad-