that of human focal epilepsy of cortical origin than any other of the numerous clinical types.

Although transient hematuria was noted on 3 occasions in 2 monkeys when the highest dosage (35 mg/kg i.v.) of chlordiazepoxide was used this did not occur in 15 other trials of this dosage, or in 45 additional tests of smaller dosages. To our knowledge, hematuria has apparently not been reported in other experimental studies of chlordiazepoxide.

Tower(6) among others has noted the clinical limitations in the predictive value of laboratory tests for anticonvulsant drugs. Extensive batteries of tests have been required since no single test has proved suitable for all drugs. Our method based on ability of a potential anticonvulsant to prevent or alter analeptic-induced seizures in epileptic monkeys should prove a useful additional technic. Theoretically, the best clinical results might be expected when the potential anticonvulsant drug is similarly administered to patients with epilepsy resembling that of the experimental monkey.

*Summary.* 1) A method using chronic epileptic monkeys is described for testing potential anticonvulsant drugs based on their capacity to prevent or alter clinical seizures induced by analeptic agents. 2) Intravenous chlordiazepoxide in large doses prevented clinical and EEG convulsant effects of challenge doses of intramuscular pentamethylenetetrazole in epileptic and normal monkeys.

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## Metabolism of Chlorpromazine. III. Isolation and Identification of Chlorpromazine-N-Oxide.* (27264)

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There is now substantial evidence that chlorpromazine is transformed *in vivo* into an extensive series of metabolites(1-4) whose pattern is species-dependent(4). The observed alterations in the phenothiazine nucleus of chlorpromazine include hydroxylation and sulfoxidation, but there are no indications of sulfone formation(3). The dimethylaminopropyl side chain is subject to either partial or complete demethylation(3,4). This latter phenomenon is especially apparent in humans, who excrete nor$_2$chlorpromazine sulfoxide as the major nonpolar metabolite.

We wish to report here the identification of a new chlorpromazine metabolite (chlorpromazine-N-oxide) which is isomeric with chlorpromazine sulfoxide but represents oxidation of the side-chain nitrogen, rather than the nuclear sulfur atom. Reference is made to Fig. 1 for the structure of this compound (CPNO)† as well as of the other products that have been identified to date.

*Experimental.* Urine from patients on oral chlorpromazine medication was brought to pH 11:5 and extracted with ethylene dichloride (Eastman) to obtain the desired nonpolar metabolite fraction(4). The extract was reduced in volume, spotted on Whatman #1 paper and chromatographed

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† The following abbreviations are used: CP = chlorpromazine, CPNO = chlorpromazine-N-oxide, CPSO = chlorpromazine sulfoxide, nor$_1$ = desmonomethyl, nor$_2$ = desdimethyl. Also see Table II, footnote (*).

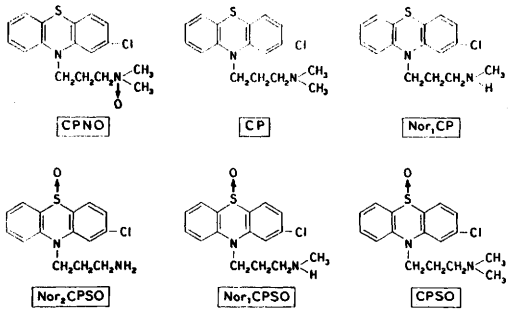


FIG. 1. Structures of chlorpromazine (CP) and its known metabolites. $Nor_1CP$ has been detected in urine of dogs but not humans. Relative excretion rates of these compounds are indicated in *Discussion.*

using our standard BzA solvent system at 22°C(3). Further chromatographic and electrophoretic studies were performed by cutting out the CPNO spot (spot #5, $R_f$ 0.64) and weaving it(5) onto fresh sheets of paper along with authentic CPNO. The chromatographic solvent systems which were used are listed in the footnote (*) to Table II. Paper electrophoresis was conducted between sheets of glass using 1% potassium bicarbonate (pH 8-8.5) as the buffer medium, running time 4 hr, 20 ma current. To achieve maximum movement and resolution, the samples were introduced at the anode end of the paper strips.

The various stains employed have been described(3). For the quantitative studies, CPNO was visualized on the BzA chromatograms with persulfate reagent and compared to a series of graded standards. It should be noted that the standards had been dissolved in normal urine, rather than water, before extraction and chromatography. Chlorpromazine was assayed after spot #6 had been rechromatographed in the AmMe (1:1) system.

*Results. Identification of CPNO.* Spot #5 yielded a positive Dragendorff reaction but negative primary amine (ninhydrin) and secondary amine (nitroprusside) tests. These results suggested that demethylation had not occurred in the side chain. The persulfate reagent gave a pink (rather than lavender) color, indicating the absence of hydroxyl groups in the phenothiazine nucleus. Physical evidence for the absence of hydroxyl, sulfoxide and sulfone groups was provided when the UV spectrum of the eluted material proved to be identical to chlorpromazine. It was deduced from these preliminary observations that compound #5 must be virtually identical to chlorpromazine except for some minor alteration in the side chain. Oxidation of the side-chain nitrogen atom was suspected when the metabolite was found to travel as a cation in an electric field; similar but more rapid movement was noted for the sulfoxides, whereas the unoxidized chlorpromazine molecule was stationary (Table I). Additional circumstantial evidence for the N-oxide structure was obtained from a study of the products of decomposition. The metabolite was eluted from urinary chromatograms and taken to dryness in a pyrex flask, forming a thin film on the inner glass surface. After standing in the desiccator for 6-12 weeks, it was found to have decomposed to yield 3 identifiable products. These were shown by paper chromatography to be CP, $Nor_1CP$ and a third substance which was similar in properties to chlorpromazine metabolite #7(3) ($R_f$ 0.87 in BzA, $R_f$ 0.09 in AmMe 1:1). The formation of CP and $Nor_1CP$ is compatible with the postulated CPNO precursor which, by analogy to other R–$N(CH_3)_2$ oxides, would expectedly decompose via demethylation to yield $Nor_1CP$, and via reduction by the liberated formaldehyde to yield CP(6,7).

At this juncture in our work, we were fortunate in obtaining samples of authentic CPNO for comparative studies. The standards were promptly tested and yielded the same color reactions, UV spectrum, electrophoretic pattern and chromatographic behavior (6 solvent systems) as compound #5. These observations served to validate our prior reasoning. Further evidence for the

TABLE I. Electrophoretic Migration Pattern of Some Chlorpromazine Derivatives.

| Compounds | Relative movement |
|---|---|
| CP<br>$Nor_1$ CP | Stationary |
| Compound #5<br>CPNO<br>$Nor_2$ CPSO | Intermediate |
| CPSO<br>$Nor_1$ CPSO<br>CPNO sulfoxide | Rapid |

TABLE II. Chromatographic Identification of Compound #5 as Chlorpromazine-N-Oxide.

| | $R_f$ | | | | |
|---|---|---|---|---|---|
| Solvent system* | Comp'd #5 | CPNO | Oxidation product of Comp'd #5 | Oxidation product of CPNO | CPNO sulfoxide |
| BzA | .62 | .62 | .14 | .14 | .14 |
| BuEt | .82 | .82 | .63 | .63 | .63 |
| AmMe 4:1 | .69 | .69 | .74 | .73 | .74 |
| AmMe 1:1 | .79 | .79 | .75 | .75 | .75 |
| EtBzF | † | † | (23 cm)‡ | (23 cm)‡ | (23 cm)‡ |
| BuA | .82 | .83 | | | |
| BuAmA | .75 | .75 | | | |

* BzA = benzene-acetic acid-water (2:2:1), descending.
BuEt = n-butanol-ethanol-water (15:2:5), ascending.
AmMe 4:1 = ammonia (58%)-methanol (4:1), ascending.
AmMe 1:1 = ammonia (58%)-methanol (1:1), ascending.
EtBzF = ethylene dichloride-benzene-formic acid-water (3:1:4:2), descending.
BuA = n-butanol-acetic acid-water (12:3:5), ascending.
BuAmA = n-butanol-ammonium acetate (1M, pH 7.0) (2:1), descending.

† Compounds fast moving, carried off paper behind solvent front.

‡ Distance travelled from origin in 45 hr.

CPNO structure was obtained by converting compound #5 and pure CPNO to a common derivative, *viz.*, CPNO sulfoxide. To effect this transformation, the filter paper strips containing both compounds were dipped in 3% hydrogen peroxide (90% alcoholic), placed in an oven at 40° for 15 minutes, and then exposed to the atmosphere for several hours to remove the last traces of peroxide. The 2 products formed now yielded a sulfoxide spectrum, with absorption peaks at 240, 275, 300 and 340 mμ. Their mutual identity was established by demonstrating similar electrophoretic and chromatographic behavior (Tables I, II) as well as similar color reactions. These properties, in turn, proved to be identical to those of authentic CPNO sulfoxide, which had been prepared in crystalline form from CPSO according to the directions of Kano and Fujimoto(8).

*Urinary CPNO excretion.* The excretion rate for CPNO was determined from 24-hr urine specimens which were processed in the usual manner (*Experimental*). Unchanged chlorpromazine was analyzed simultaneously for reference purposes. The 5 persons studied excreted approximately 0.6% of the administered chlorpromazine as CPNO. This figure appeared to be independent of the drug dosage in the range indicated (Table III). In experiments described earlier(4) it was calculated—but not reported—that the output of the then unidentified compound #5 was 0.9% (average, 2 male patients). On the basis of the 2 indicated average values, the weighted mean CPNO excretion by humans appears to be about 0.7%. CPNO excretion by dogs is several times higher, possibly 2 to 3.5% according to our unreported data based on 5-day urine collections from 2 female dogs. A more precise estimate cannot be given due to our lack of the appropriate standard (CPNO) at the time these analyses were made. It is of incidental interest that the dogs showed substantial daily variations in their relative excretion of CP to CPNO.

Only minute traces of unaltered CP (0.06%) could be detected in the present studies on humans. This compares to a higher figure (0.2%) reported earlier(4). The difference is not meaningful, but may be attributable either to our recent practice of using normal urine as a solvent for the CP

TABLE III. Daily Urinary Excretion Values for Chlorpromazine and Chlorpromazine-N-Oxide.

| Patient | Sex | CP dosage* | Avg % excretion† CP | Avg % excretion† CPNO |
|---|---|---|---|---|
| ES | ♀ | 900 | .072 | .69 |
| LB | ♀ | 900 | .046 | .43 |
| SE | ♂ | 900 | .054 | .50 |
| DO | ♂ | 1800 | .067 | .58 |
| LB | ♂ | 400 | .074 | .57 |
| Mean ± stand. dev. | | | .063 ±.012 | .55 ±.05 |

* mg chlorpromazine hydrochloride per diem.

† Each value represents the mean for two 24-hr urine specimens.

standard, or to our adoption of a second chromatographic system (AmMe) to afford further purification of the CP urine spot.

*Stability of CPNO.* Several urine specimens from patients on CP therapy were analyzed repeatedly for periods up to a week after their collection. During this time the CPNO and CP contents remained unchanged. The initial ethylene dichloride extracts also yielded constant values after refrigeration for a week. When CPNO or CP was added to normal urine there were no indications of any changes, either qualitative or quantitative, during storage. These observations suggest that CPNO does not arise spontaneously in urine from oxidation of CP or from some other source.

*Discussion.* If we base our judgment on its urinary output, chlorpromazine-N-oxide cannot be considered a major human metabolite of chlorpromazine. Nonetheless, it is pertinent that man excretes the N-oxide in somewhat larger amount (0.7%) than chlorpromazine sulfoxide (0.4%, ref. 4). The discovery of CPNO brings to 5 the number of components which we have identified in the human nonpolar fraction (Fraction *A*). Their relative urinary output is as follows: $Nor_2$-CPSO > $Nor_1$CPSO > CPNO > CPSO > CP. The sequence for dogs is: $Nor_1$CPSO = CPSO > CPNO = CP > $Nor_2$CPSO (> $Nor_1$CP ?).

No comprehensive information is available on distribution of CP and its metabolites in body depots. However, it seems unlikely that these compounds would bear the same quantitative relationship to each other in the tissues as they do in urine. As a consequence, a "minor" urinary excretion product might very well be retained in substantial amounts by the body and contribute significantly to the clinical action of CP. In this connection, recent pharmacological and behavioral tests on $Nor_1$CP and CPNO in animals indicate that these compounds approach CP in activity, whereas CPSO is markedly less active(9). Their tissue levels and specific effects on humans have not been determined. We cannot judge at present whether the many remaining metabolites have significant pharmacological activity, nor to what extent they contribute to the clinical and therapeutic action of CP.

The liver is undoubtedly implicated in the metabolism of CP. Consequently a study of the complex pattern of CP metabolism offers the biochemist a unique opportunity to evaluate competitive "detoxication" mechanisms *in vivo*. In this instance, CP may serve as a useful model substance to evaluate hepatic function in mental disorders. Studies along these lines are in progress and complement an earlier investigation on aromatic metabolism in schizophrenia(10).

The dimethylaminopropyl side chain is common to both chlorpromazine and imipramine. Accordingly, it might be anticipated that imipramine-N-oxide would result from the metabolism of imipramine. This has been confirmed recently in our laboratory. The presence of N-oxide group in other metabolites(11,12), in methaminodiazepoxide (Librium) and in pharmacological agents under development augurs a growing interest in its biological properties.

*Summary.* Chlorpromazine-N-oxide (CPNO) has been isolated from human urine and identified as a metabolite of chlorpromazine. Proof is based on the similarity between this product and authentic CPNO with respect to their ultraviolet spectra, color reactions, electrophoretic mobility, and paper chromatographic behavior. Further confirmation has been obtained by oxidation of the metabolite and authentic CPNO to form derivatives (CPNO sulfoxide) which are identical to each other as well as to authentic CPNO sulfoxide. CPNO is not a major human metabolite, but it is voided in somewhat larger amounts than chlorpromazine sulfoxide. Dogs surpass humans in their output of the N-oxide.

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## Potassium, Lethal and Tumor-Necrotizing Properties of Klebsiella Polysaccharide.* (27265)

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Endotoxins of gram-negative bacteria produce a variety of biologic responses including fever, hyperglycemia, shock, tumor-necrosis, death(1,2) and increased corticosteroidogenesis(3). The interrelationships and mechanisms of the varied biologic effects are uncertain. Some responses appear to oppose one another. For example, augmented corticosteroidogenesis, like exogenous glucocorticoids, may inhibit tumor-necrotizing(4,5), delayed lethal(5,6), and pyrogenic(7) properties of the endotoxins. Alterations in glycolysis and oxidative phosphorylation may provide a partial explanation for some of the biologic effects. Changes in liver glycogen, blood glucose and lactic acid levels of animals following injection of endotoxins(2,8) are accompanied by alterations in inorganic and organic phosphates, pyruvate and α ketoglutarate(9). Mitochondria from animals treated with endotoxin showed decreased esterification of inorganic phosphate(10) and the addition *in vitro* of non-lethal, haptenic bacterial polysaccharide to mitochondria from rat and mouse liver and guinea pig kidney decreased oxygen uptake and oxidative phosphorylation(11). In the present study, potassium (K), which plays an important but obscure role in cellular energy production and transfer mechanisms(12), has been found to markedly augment the tumor-necrotizing and delayed lethal effects of relatively non-toxic, haptenic bacterial polysaccharide.

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*Materials and methods.* The haptenic, capsular polysaccharide of Klebsiella pneumoniae, type B, was extracted with 0.1 N NaOH(13) and dissolved in either 5% glucose of 0.85% NaCl in 1, 10, 100, and 1,000 mg% concentration and injected intravenously, .01 to 10 mg/100 g body weight, into young male Holtzman rats, each 120-140 g, and both with and without Murphy-Sturm lymphosarcomas. The lymphosarcoma was transplanted by subcutaneous injection over lumbospinal muscles of approximately 300,000 tumor cells suspended in Earle's solution. Size of the tumor and weights of the rats were recorded daily. Control and other groups of tumor-bearing rats were observed for spontaneous regression—*i.e.*, reduction in size and disappearance of the transplantable tumors. Surviving rats in all tumor-bearing groups were observed for 6 or more weeks. The time of appearance of palpable tumors, rate of tumor growth and accompanying loss of body mass of rats in the present study were similar to those previously reported(14). Because the delayed lethal toxicity of the polysaccharide increased with tumor size, animals with tumors of 25 ± 2 mm mean diameter were used.

Before, during and after polysaccharide administration, KCl in either 5% glucose or in 0.85% NaCl, was injected intravenously, 0.001 to 0.5 meq K/l/100 g body weight. KCl solutions were injected within 3 to 5 seconds. Appropriate dilutions were made