thesis (5). The latter compound also has been shown to decrease prostaglandin E synthesis at the time that it decreases renal blood flow and the glomerular filtration rate (2). Since a prostaglandin synthesis reduction can reduce renal blood flow by removing prostaglandin E, an endogenous vasodilator (6), the reduction in renal blood flow by oxyphenbutazone could be due to such a mechanism.

A reduction in sodium and water excretion can occur as a result of such a hemodynamic change. Early and Schrier (7) showed that a decrease in renal blood flow can alter the distribution of blood flow or physical factors to enhance sodium and water reabsorption.

A decrease in the glomerular filtration rate would also be expected to reduce sodium and water excretion. Whether the decrease in the glomerular filtration rate is a consequence of the inhibition of prostaglandin synthesis, as is the decrease in renal blood flow and the increase in blood pressure (8), or a separate action is not clear. Previous studies with prostaglandin synthesis inhibitors showed variable effects on the glomerular filtration rate (2, 8).

Thus, these experiments demonstrate that oxyphenbutazone reduces renal blood flow and the glomerular filtration rate during the time that it decreases sodium and water excretion. A decrease in renal blood flow and the glomerular filtration rate could cause or contribute to the decrease in water and sodium excretion produced by oxyphenbutazone.

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Effects of Pyridoxine Hydrochloride (Vitamin B_6) on Chlorpromazine-Induced Serum Prolactin Rise in Male Rats

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Abstract □ To investigate if vitamin B_6 inhibits prolactin release and to compare this effect to that of bromocriptine, a known suppressor of prolactin release, a study was conducted in male rats. Animals were pretreated with pyridoxine hydrochloride, pyridoxal hydrochloride, saline, or bromocriptine 30 min prior to receiving varying doses of chlorpromazine hydrochloride. Blood samples were obtained 90 min later and analyzed for serum prolactin by a double-antibody radioimmunoassay. Another study involved pyridoxal hydrochloride and saline pretreatments 30 min prior to doses of chlorpromazine hydrochloride. Blood samples collected 60 min later were also analyzed for serum prolactin. Pyridoxine hydrochloride significantly suppressed the chlorpromazine-induced prolactin rise ($p < 0.01$). However, the suppression was significantly less than that produced by bromocriptine ($p < 0.01$). Pyridoxal hydrochloride, another natural form of vitamin B_6, failed to suppress prolactin under the conditions of both studies. This investigation may lend support to the concept that pyridoxine hydrochloride partially inhibits prolactin by a mechanism not involving dopamine.

Keyphrases □ Pyridoxine—effect on chlorpromazine-induced serum prolactin rise, rats □ Chlorpromazine—induction of serum prolactin rise, effect of pyridoxine, rats □ Prolactin—chlorpromazine-induced rise, effect of pyridoxine, rats

In 1973, Foukas (1) suggested that oral pyridoxine suppressed lactation within 7 days in 95% of postpartum women. This finding was supported by Marcus (2) but not by other investigators (3–6). Pyridoxine reduction of serum prolactin levels in the galactorrhea–amenorrhea syndrome has been reported (7, 8). Other studies showed that pyridoxine has no effect on elevated plasma prolactin levels due to various causes, including two subjects with chlorpromazine-induced hyperprolactinemia and galactorrhea (9, 10). However, Reiter and Root (11) observed a significant decrease in plasma prolactin in children following intravenous pyridoxine.

An *in vitro* investigation indicated that pyridoxine possessed some inhibitory effect on prolactin release from whole rat pituitary culture (12). Harris *et al.* (13) demonstrated that pyridoxine suppressed the plasma prolactin rise associated with proestrus and thyrotropin-releasing hormone stimulation in the rat. More recently, Husami *et al.* (14) presented evidence that high pyridoxine doses affected neither prolactin secretion nor lactation in humans, monkeys, and rats, including animals stimulated with thyrotropin-releasing hormone.

The present study was undertaken to determine the effects of pyridoxine and pyridoxal, two natural forms of vitamin B_6, on chlorpromazine-induced prolactin secretion in rats (15) and to compare the inhibition by these vitamins with the effects of bromocriptine, a known potent inhibitor of prolactin release (16).

EXPERIMENTAL

Two hundred and forty male Sprague–Dawley adult rats[1], 245–280 g, were divided into four equal groups. The rats were housed for 21 days prior to the study in a temperature-controlled (23 ± 3°) artificially illuminated (lights on from 7:00 am to 7:00 pm daily) room. The animals were given food[2] and water *ad libitum*. Each group was intraperitoneally in-

[1] Taconic Farms, Germantown, N.Y.
[2] Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.

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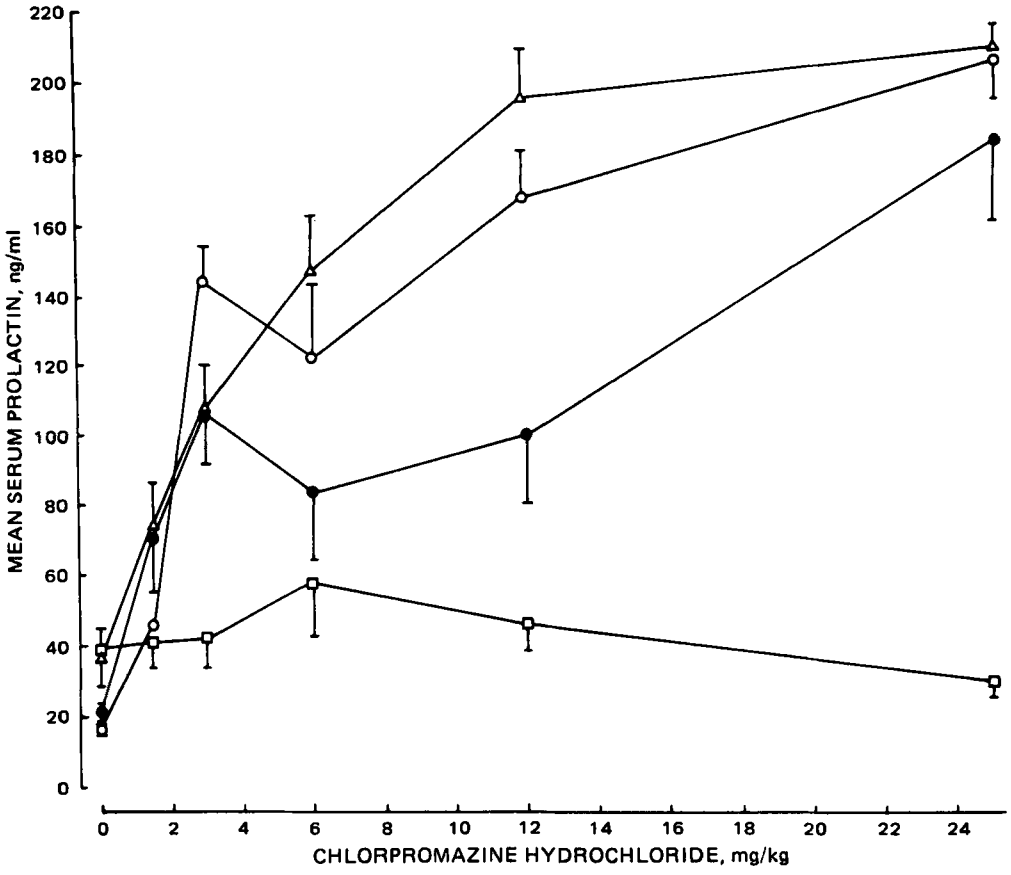

Figure 1—*Effects of intraperitoneal pyridoxine hydrochloride (75 mg, ●), pyridoxal hydrochloride (75 mg, ○), bromocriptine (1 mg, □), and saline (△) administered 30 min prior to intraperitoneal saline or chlorpromazine hydrochloride (1.25–25 mg/kg). Serum prolactin determinations were made on blood samples collected 90 min after chlorpromazine treatment. Each point represents the mean value for 10 animals. Vertical lines show standard errors of the mean.*

jected between 12:30 pm and 2:00 pm with 0.5 ml of aqueous solution containing 75 mg of pyridoxine hydrochloride[3], 75 mg of pyridoxal hydrochloride[3], 1 mg of bromocriptine[4], or normal saline[5].

Thirty minutes following pretreatment of each group, serial dilutions of chlorpromazine hydrochloride[6], ranging from 25 to 1.25 mg/kg, were administered intraperitoneally to five subgroups of 10 animals each; the remaining 10 animals received normal saline. Blood samples were obtained by decapitation from the trunk portion 90 min later, previously determined as the time of peak serum prolactin response to intraperitoneal chlorpromazine hydrochloride[7].

A further study under similar experimental conditions involved 12 animals, 245–280 g. Six animals received intraperitoneally 0.5 ml of an aqueous solution containing 75 mg of pyridoxal hydrochloride; the other six received an equal volume of normal saline. Thirty minutes later, five of the six animals from each group received one of five serial chlorpromazine hydrochloride dilutions ranging from 25 to 1.25 mg/kg ip, and the other animal received normal saline. Blood samples were obtained by decapitation from the trunk portion 60 min later.

Serum samples for both studies were separated and analyzed for prolactin by a double-antibody radioimmunoassay[8] based on the principle first described by Yallow and Berson (17). All results are expressed in terms of National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD)-rat prolactin. Each serum sample was assayed in duplicate, and the average was taken as representative of the true prolactin concentration. Statistical significance was determined by a two-way fixed analysis of variance, the Student t test, and linear trend analysis (18).

RESULTS

Figure 1 shows the effects of the different pretreatments on serum prolactin levels at 90 min following varied chlorpromazine hydrochloride doses. Replicate 2 × 6 analyses of variance to compare the drugs against normal saline showed that serum prolactin was significantly suppressed ($F_{1,108} = 9.799$, $p < 0.01$) by pyridoxine hydrochloride but not by pyridoxal hydrochloride ($F_{1,108} = 0.026$). In a 2 × 6 analysis of variance, bromocriptine suppressed prolactin ($F_{1,108} = 39.534$, $p < 0.01$) to a much greater extent than did pyridoxine hydrochloride. Trend analysis of pyridoxine hydrochloride and pyridoxal hydrochloride revealed that the effects of increasing drug concentrations were significantly linear ($F_{1,54} = 29.89$, $p < 0.01$, and $F_{1,54} = 118.24$, $p < 0.001$, respectively).

While pyridoxal hydrochloride was not significantly different from normal saline, it resembled the more effective pyridoxine hydrochloride in its concentration effects. In a further study to compare the effects of pyridoxal hydrochloride and normal saline pretreatments on serum prolactin levels at 60 min following varied chlorpromazine hydrochloride doses, the mean serum prolactin levels of both groups were not significantly different [135.4 ± 40.8 (*SE*) *versus* 72.7 ± 29.7 (*SE*) ng/ml, respectively; $t = 1.14$, $df = 10$].

DISCUSSION

Serum prolactin levels are largely controlled by a hypothalamic prolactin-inhibiting factor related to dopamine and, indeed, may be dopamine, and, to a lesser extent, by a prolactin-releasing factor also hypothalamic in origin (19). Pharmacological agents that reduce serum prolactin levels include dopamine agonists such as bromocriptine and dopamine-replenishing agents such as levodopa (20). Although both bromocriptine and levodopa also may transiently increase the prolactin clearance from plasma by stimulating its uptake into peripheral receptor sites, their exact inhibition mechanisms are not fully known (21).

Pyridoxine hydrochloride, in a pharmacological dose, exhibited some inhibitory effect on the serum prolactin rise induced by varied chlorpromazine hydrochloride doses. This suppression was significantly less than that produced by bromocriptine. On the other hand, its biologically equivalent form, pyridoxal hydrochloride, did not significantly alter prolactin secretion.

The manner by which pyridoxine hydrochloride exerts its prolactin inhibitory effect cannot be determined from the present data. It has been suggested that pyridoxine enhances hypothalamic dopamine synthesis through its metabolism to pyridoxal phosphate, the coenzyme for the conversion of dopa to dopamine by dopa decarboxylase (1, 2, 7, 8). This

[3] Sigma Chemical, St. Louis, Mo.
[4] Sandoz Laboratories, East Hanover, N.J.
[5] Abbott Laboratories, Chicago, Ill.
[6] Smith Kline & French Co., Carolina, Puerto Rico.
[7] Unpublished data.

theory is based on the widely quoted explanation of Duvoisin *et al.* (22) for their finding that pyridoxine reduces or abolishes the antiparkinsonian effect of levodopa. They suggested that pyridoxine, in the form of pyridoxal phosphate, increases decarboxylase activity so that more levodopa is converted to dopamine in the periphery and less is available to penetrate into the central nervous system. However, this mechanism was refuted by Johnson *et al.* (23), who found no evidence of decarboxylase facilitation in levodopa-treated Parkinsonian patients receiving pyridoxine. These investigators offered the formation of a Schiff's base complex between pyridoxal phosphate and dopamine (24, 25) and other mechanisms (26) as alternative explanations.

Harris *et al.* (13) suggested that pyridoxine hydrochloride directly inhibits prolactin release and that dopamine involvement is unlikely. The present results may support the concept of direct prolactin inhibition by pyridoxine hydrochloride since pyridoxal hydrochloride also can be readily metabolized to the active form of the vitamin that serves as the coenzyme in the conversion of dopa to dopamine. In addition, although studies with radioactive tracers showed that an equilibrium between all active vitamin B_6 forms is established in the mammalian organism, this equilibrium does not result when large doses are given (27, 28). Even though the effects of pyridoxal hydrochloride on chlorpromazine hydrochloride-induced prolactin secretion were tested at two different time intervals, a different sampling time may reveal a prolactin-suppressant effect for this form of the vitamin.

Clarification of the mechanism(s) by which pyridoxine hydrochloride partially inhibits prolactin secretion requires further study.

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Resolution of (±)-Propranolol

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Abstract □ Two improvements in propranolol resolution were developed. Both the (+)- and (−)-di-(*p*-toluoyl)tartaric acids were used as the resolving agents. This procedure reduced the number of crystallizations needed to obtain a pure product. Furthermore, synthesis of the resolving agent was improved.

Keyphrases □ Propranolol—racemic mixtures, resolution of enantiomers, (+)- and (−)-di-(*p*-toluoyl)tartaric acids used as resolving agents □ Enantiomers—propranolol, resolving agents

Propranolol (I) [1-isopropylamino-3-(1-naphthoxy)-2-propanol], the principal commercially available β-adrenergic blocking agent, is usually supplied as the racemic compound[1]. The (−)-isomer has as much as 60 times greater β-blocking activity than the (+)-isomer (1). Not only can there be significant differences in the pharmacological activity of the enantiomers, but, as has been shown for other drugs, there also can be significant metabolic differences (2, 3).

DISCUSSION

There is only one preliminary report on the metabolism of propranolol enantiomers (4). In preparation for a metabolic study in humans, two improvements in the overall procedure for resolution of these enantiomers were made. First, preparation of the resolving agent [di-(*p*-toluoyl)tartaric acid (II)] was modified. In contrast to the original report (5), the agent was found to be insoluble in pure benzene. Moreover, the customary spectral data were not reported and are included here.

Second, in the original report on racemic propranolol resolution (1), only the (−)-di-(*p*-toluoyl)tartaric acid was used to resolve both enan-

[1] Available as (±)-propranolol hydrochloride (Inderal).

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