endogenous corticosterone in tissues has doubled, and that the hormone has been displaced from cellular as well as plasma protein. With twice as large a pool of tissue corticosterone turning over at the same fractional rate, it follows that the rate of secretion from the adrenal gland must be doubled.

These interpretations are only conjectural. A definite cause and effect relationship between displacement of corticosteroids and antirheumatic action has not yet been established. It will be necessary to carry out kinetic studies with pure transcortin as well as with tissue proteins. However, the results do suggest that the action of some drugs may be mediated in part through a physical displacement of endogenous hormone.

An enormous amount of work has been done to elucidate the mechanism of action of the sulfonylurea drugs in diabetes. It is postulated that their primary action may be the freeing of endogenous insulin from protein-bound complexes in the pancreatic β cells, the plasma and the tissues. This could account for the proven increased release of insulin, the duration of which is uncertain, and for the increased insulin-like action on the liver and peripheral tissues. Much more evidence, however, is required to substantiate or refute this hypothesis (Duncan & Clarke 1965).

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Enzyme Stimulation and Inhibition in the Metabolism of Drugs

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Abstract
Studies in recent years have disclosed two types of drug interaction which may be important in drug therapy:

(1) Administration of one drug can speed up the metabolism of another drug. Animal experiments show that this results from the ability of drugs to induce the synthesis of drug-metabolizing enzymes in liver microsomes. This effect has considerable importance in pharmacologic and toxicologic studies carried out in animals, and recent work indicates that it may explain altered therapeutic responses observed in some patients when they receive several drugs at the same time. Substances present in the environment, such as the insecticides chlordane and DDT, have been shown in animals to stimulate drug-metabolizing enzymes in liver, but the significance of this observation for man is not known. Drugs which stimulate drug metabolism also enhance the hydroxylation of testosterone, estradiol, progesterone and cortisol by enzymes in liver microsomes. Further research is required to establish the physiological importance of this interaction of drugs in steroid metabolism.

(2) One drug may inhibit the metabolism of another drug and thus intensify and prolong its pharmacologic action. Although this effect is well documented in animals, recent reports suggest that this may also be important in man. For instance, the action of coumarin anticoagulants can be potentiated by administration of certain drugs which inhibit their metabolism. Monoamine oxidase inhibitors block the metabolism of certain sympathomimetic amines and this can lead to serious side-effects. Thus, hypertensive crises have been observed in patients receiving monoamine oxidase inhibitors who have eaten cheese with a high tyramine content.

It is a common practice for patients to be given several drugs at the same time, but sometimes one drug may reduce or intensify the pharmacological efficacy of another or the combination may result in an unexpected adverse effect. Our laboratory has been particularly interested in the ability of drugs to stimulate or inhibit the metabolism of other drugs and thereby alter their duration of action. These effects have been well studied in experimental animals and they now appear to have importance in understanding certain drug interactions in man (Conney & Burns 1962, Remmer 1962, Burns 1964, Fouts 1964).

Enzymes in liver microsomes, which metabolize many clinically useful drugs, are associated

with the smooth-surfaced endoplasmic reticulum in the liver cell. They are quite versatile in metabolizing drugs by various reactions, including N-dealkylation, ether cleavage, hydroxylation, deamination and glucuronide formation (Brodie *et al.* 1958). Administration of drugs can either stimulate or inhibit the metabolism of drugs by these enzymes, and it is the purpose of this paper to show how such effects may explain some interactions which occur in human drug therapy.

Stimulation of Drug Metabolism

The chronic administration of one drug can reduce the pharmacologic activity of another drug by stimulating its metabolic inactivation. Drugs exert this action by increasing the amount of drug-metabolizing enzymes in liver microsomes and this is referred to as enzyme induction

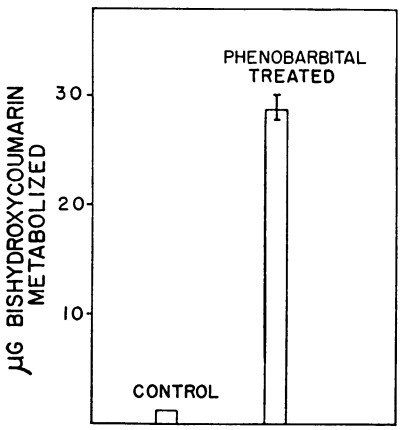

Fig 1 *Male rats weighing 40–45 g were injected intraperitoneally with 37 mg/kg of sodium phenobarbital twice daily for four days. A microsome-soluble fraction of liver was incubated with 50 μg of bishydroxycoumarin for thirty minutes in the presence of a TPNH-generating system. Disappearance of substrate was measured*

(Conney & Burns 1963). Electron microscopic studies have shown that the increase in enzyme activity is accompanied by a marked proliferation of smooth-surfaced endoplasmic reticulum of the liver cell (Fouts & Rogers 1965, Remmer & Merker 1963). Drug metabolism can be stimulated by different types of drugs, such as barbiturates and other hypnotics, analgesics, tranquilizers, antihistaminics, oral antidiabetics and uricosuric agents. The liver microsomal enzyme system that inactivates the muscle relaxant, zoxazolamine, and the hypnotic, hexobarbital, is a good example of a drug-metabolizing enzyme that is stimulated by drug administration. Treatment of rats with phenobarbital for several days enhances the metabolism of a subsequent dose of

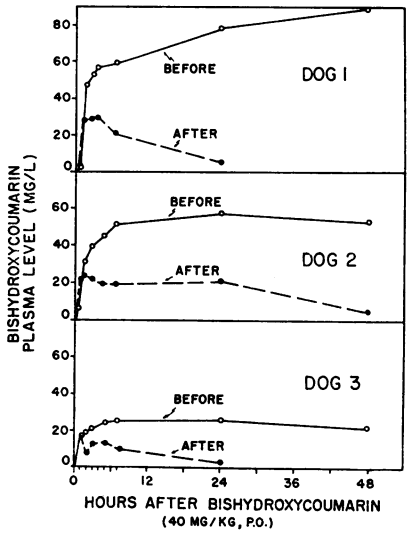

Fig 2 *Plasma levels of bishydroxycoumarin before and after treatment of dogs with 16 mg/kg of sodium phenobarbital per os daily for twenty-four days*

zoxazolamine or hexobarbital to such a marked degree as to abolish the pharmacologic effects of these agents almost completely (Conney *et al.* 1960).

A marked increase in the activity of the liver microsomal enzyme which metabolizes bishydroxycoumarin (Dicumarol) occurred in rats treated with phenobarbital (Cucinell *et al.* 1965). In accord with these *in vitro* results (Fig 1), administration of phenobarbital stimulated the rate of bishydroxycoumarin metabolism in the dog (Fig 2). Phenobarbital also enhanced the metabolism of bishydroxycoumarin in man, as was observed in a patient who was treated chronically with 75 mg/day of the anticoagulant (Fig 3). When he received one grain of phenobarbital daily for four weeks, in addition to bishydroxycoumarin, there was a substantial lowering of the plasma level of bishydroxy-

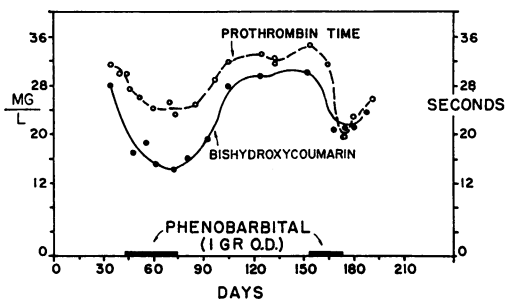

Fig 3 *Effect of phenobarbital on plasma levels of bishydroxycoumarin and on prothrombin time in a human subject treated chronically with 75 mg/day of bishydroxycoumarin*

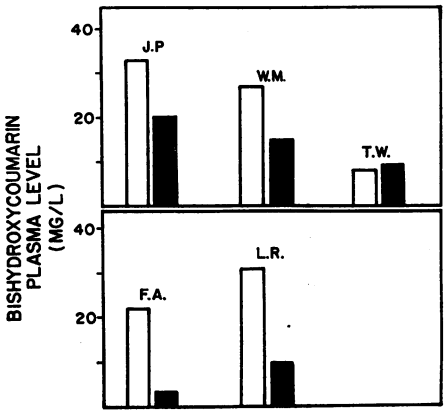

Fig 4 *Plasma level of bishydroxycoumarin forty-eight hours after oral administration of 500 mg of the drug to 5 human subjects. Bishydroxycoumarin was administered before (open bars) or after (solid bars) treatment with 120 mg of sodium phenobarbital daily for twenty-eight days*

coumarin and a decrease in the anticoagulant activity. Upon discontinuing phenobarbital, the plasma level of bishydroxycoumarin and the prothrombin time returned to their original values. The plasma levels of bishydroxycoumarin were compared before and after administration of phenobarbital to 5 other patients (Fig 4). The subjects were given a single 500 mg oral dose of bishydroxycoumarin and the plasma levels of the drug were determined at 24, 48, and 72 hours after the dose. After they had been treated with 120 mg of phenobarbital daily for three to four weeks, they were given a 500 mg dose of anticoagulant and the plasma levels were determined as before. After treatment with phenobarbital, 4 of these subjects had lower plasma levels of bishydroxycoumarin at 24, 48 and 72 hours, than before treatment. One of the patients who did not respond to phenobarbital treatment had low bishydroxycoumarin levels even before receiving phenobarbital. The plasma levels of bishydroxycoumarin obtained at 48 hours after the dose of the drug are given in Fig 4. The stimulatory effect of heptabarbital on the metabolism of certain coumarins has been reported and this accounts for the inhibitory action of the barbiturate on the anticoagulant activity of the coumarins (Dayton *et al.* 1961). These results can explain earlier reports that barbiturates decreased the anticoagulant activity of coumarin anticoagulants (Avellaneda 1955).

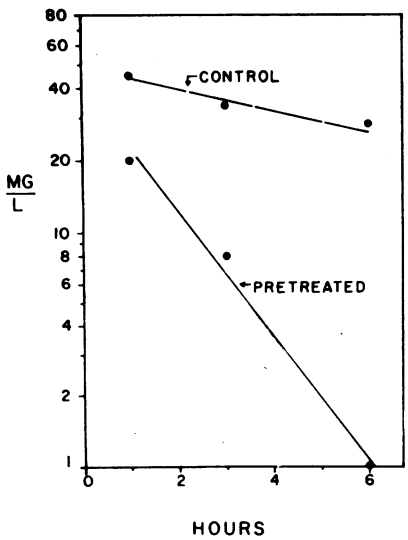

Fig 5 *Plasma level of diphenylhydantoin following an intravenous 50 mg/kg dose of diphenylhydantoin in a dog before and after oral treatment with 16 mg/kg of sodium phenobarbital daily for thirty days*

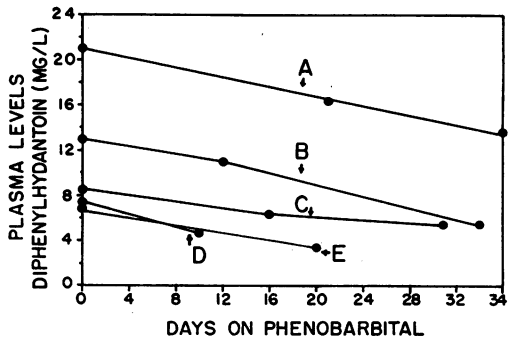

Fig 6 *Effect of phenobarbital (120 mg/day) on the plasma level of diphenylhydantoin (300 mg/day in 5 human subjects. Each subject received 300 mg/day of diphenylhydantoin chronically for at least two weeks before supplementing this therapy with 120 mg of phenobarbital daily from day zero*

Phenobarbital also stimulates the metabolism of other clinically useful drugs. Cucinell *et al.* (1963) and Cucinell *et al.* (1965) have shown that treatment of dogs with phenobarbital stimulated the metabolism of a subsequent dose of diphenylhydantoin (Dilantin)[1] (Fig 5). The plasma levels of diphenylhydantoin were compared in two groups of epileptic patients who received, for prolonged periods, either 300 mg of diphenylhydantoin daily or 300 mg of diphenylhydantoin plus 120 mg of phenobarbital. The plasma levels of diphenylhydantoin were significantly lower in those patients who were treated with phenobarbital. In order further to investigate whether phenobarbital stimulates the metabolism of diphenylhydantoin, 5 patients who were receiving diphenylhydantoin (300 mg/day) were supplemented daily with 120 mg of phenobarbital. The

[1]phenytoin sodium (Epanutin) in UK

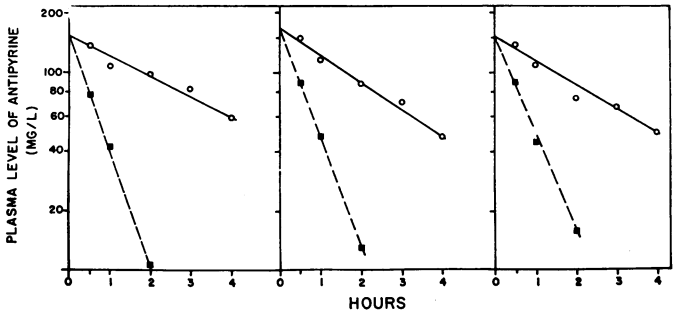

Fig 7 *Metabolism of antipyrine (100 mg/kg, intravenously) before and after daily treatment of 3 dogs with 16 mg/kg of phenobarbital orally for twenty-one days. The solid line represents plasma levels of antipyrine taken before treatment with phenobarbital and the broken line represents plasma levels of antipyrine taken after treatment*

plasma levels of diphenylhydantoin declined in these patients and this was particularly marked in those individuals with high initial levels of the drug (Fig 6). The ability of phenobarbital to stimulate the metabolism of diphenylhydantoin is apparently not a problem in the management of the epileptic patient since phenobarbital also possesses anticonvulsant activity. However, the simultaneous administration of drugs which would enhance the metabolism of diphenylhydantoin but lack anticonvulsant activity may present difficulty.

Treatment of rats with phenobarbital stimulated the metabolism of the antifungal drug, griseofulvin (Busfield *et al.* 1964). This effect also occurs in man since low blood levels of griseofulvin were obtained after human subjects received phenobarbital (Busfield *et al.* 1963). It is possible that the simultaneous administration of phenobarbital may reduce the antifungal activity of griseofulvin.

Treatment of dogs with phenobarbital also stimulated markedly the metabolism of antipyrine and this is shown by the results in Fig 7 (Cucinell *et al.* 1965). Phenobarbital treatment did not change the distribution of antipyrine which is known to be distributed evenly in body water. The possibility that phenobarbital administration may also stimulate the metabolism of antipyrine in man is under investigation.

A good example of enzyme induction in man is the ability of phenylbutazone (Butazolidin) to stimulate the metabolism of aminopyrine[1] (Chen *et al.* 1962). Six human subjects each received an intravenous dose of aminopyrine and plasma levels of the drug and its metabolite, 4-aminoantipyrine, were measured (Fig 8). After the subjects were treated with phenylbutazone, they again received the same intravenous dose of aminopyrine. In each case the plasma levels of aminopyrine after treatment with phenylbutazone were substantially lower than before treatment, whereas the plasma levels of 4-aminoantipyrine were either the same or higher than before treatment. In accord with these data, administration to man of various barbiturates accelerated the metabolism of the aminopyrine derivative, dipyrone (Remmer 1962).

Not only drugs, but also substances present in the environment, stimulate drug-metabolizing enzymes in liver microsomes. Exposure of rodents to insecticides, such as chlordane and DDT, stimulated drug-metabolizing enzyme activity and shortened the duration of action of hexobarbital (Hart *et al.* 1963). This effect was discovered accidentally in two different laboratories after animal quarters were sprayed with these insecticides. The results in Fig 9 show that treatment of dogs with small oral doses of chlordane

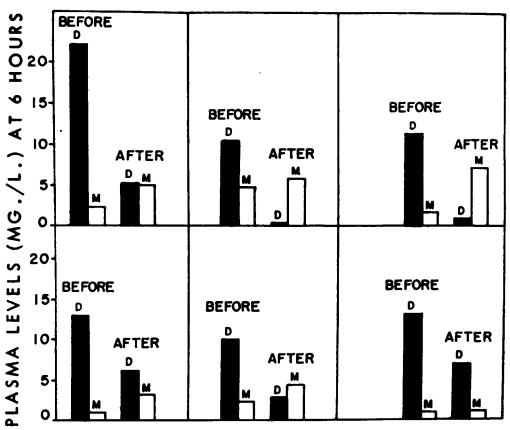

Fig 8 *Stimulatory effect of phenylbutazone on aminopyrine metabolism in man. Plasma levels of aminopyrine* (D) *and its metabolite, 4-aminoantipyrine* (M) *six hours after intravenous administration to human subjects of a 0·80 g dose of aminopyrine. Aminopyrine was administered before and after treatment with phenylbutazone (400 to 800 mg per day orally for seven to eleven days)*

[1]amidopyrine in UK

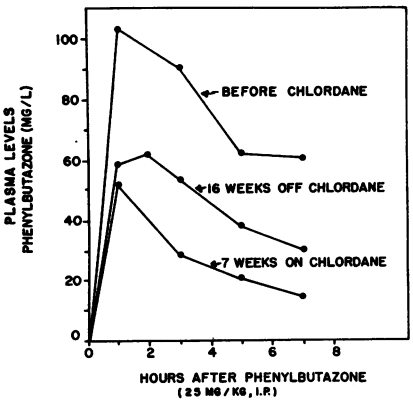

Fig 9 *Stimulatory effect of chlordane on phenylbutazone metabolism in the dog. Phenylbutazone (25 mg/kg intraperitoneally) was administered before, during or after stopping the oral administration of 5 mg/kg of chlordane three times a week*

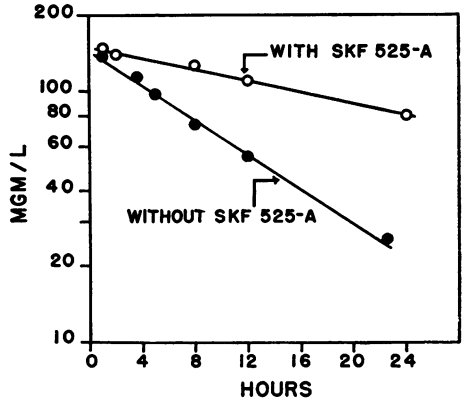

Fig 10 *Inhibitory effect of SKF 525-A on the metabolism of phenylbutazone. A dog received 50 mg/kg of phenylbutazone intravenously and plasma levels of phenylbutazone were measured at various time intervals. Two weeks later the procedure was repeated, except that the dog received 40 mg/kg of SKF 525-A intraperitoneally thirty minutes prior to administration of phenylbutazone*

for seven weeks markedly stimulated the metabolism of phenylbutazone, a drug which is metabolized by liver microsomes (Burns *et al.* 1965). This effect of chlordane is long-lasting, since dogs metabolized phenylbutazone at an accelerated rate even five months after chlordane administration had been discontinued. Although these observations, which show the potent effect of insecticides on drug metabolism, are important for animal experiments, their relevance to drug therapy in man remains to be determined.

Recent studies in our laboratory showed that drugs may also interact in the metabolism of a variety of steroids. Evidence has been found that steroid hormones are normal body substrates for oxidative drug-metabolizing enzymes in liver microsomes (Kuntzman *et al.* 1964) and, consequently, drugs that stimulate the microsomal oxidation of drugs also stimulate the microsomal hydroxylation of steroids (Conney, Schneidman, Jacobson & Kuntzman 1965).

Thus, treatment of rats with phenobarbital, chlorcyclizine,[1] phenylbutazone and other commonly used drugs stimulated several-fold the liver microsomal hydroxylation of testosterone, estradiol-17β, progesterone and desoxycorticosterone. Recently, it was found that treatment of human subjects with diphenylhydantoin markedly increased the extra-adrenal metabolism of cortisol to 6-hydroxycortisol which was excreted in the urine (Werk *et al.* 1964). An explanation for the stimulatory effect of diphenylhydantoin on cortisol metabolism in man comes from the finding that treatment of guinea-pigs with diphenylhydantoin for several days stimulated the formation of enzymes in liver microsomes that 6β-hydroxylates cortisol (Conney, Jacobson, Schneidman & Kuntzman 1965). The physiological importance of drug-induced stimulation of steroid hydroxylation in liver must await furthe investigation.

Inhibition of Drug Metabolism

Many examples are now known where drugs can inhibit the metabolic detoxification of other drugs and thus cause an increase in their duration and intensity of pharmacological action (Gillette 1963). Examples of inhibitors of drug metabolism in animals include SKF 525-A (β-diethylaminoethyl diphenylpropylacetate), iproniazid (Marsilid), β-phenylisopropylhydrazine (Catron), nialamid (Niamid), triparanol (MER/29), chloramphenicol, *p*-aminosalicylic acid and narcotics, such as meperidine[1] and morphine.

Two examples of inhibition of drug metabolism are: (1) Treatment of dogs with SKF 525-A considerably decreased the rate of metabolism of phenylbutazone, and this is shown in Fig 10 (Burns *et al.* 1955). (2) The metabolism of acetophenetidin[2] to N-acetyl-*p*-aminophenol was also markedly inhibited in rats which were pretreated with SKF 525-A (Burns *et al.* 1963). The animals which received SKF 525-A had considerably higher plasma levels of acetophenetidin three hours after a dose of the drug than did the control rats (Fig 11). In accord with these results, the plasma levels of the major metabolite of

[1]Di-Paralene, Histantin in UK

[1]pethidine in UK
[2]phenacetin in UK

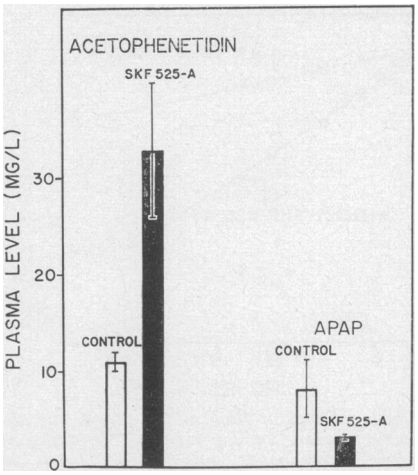

Fig 11 *Inhibitory effect of SKF 525-A on acetophenetidin metabolism. Plasma levels of acetophenetidin and N-acetyl-p-aminophenol (APAP) were determined three hours after an intraperitoneal injection of 200 mg/kg of acetophenetidin. Open bars represent values for control rats and solid bars represent values for rats injected intraperitoneally with 35 mg/kg of SKF 525-A forty minutes prior to acetophenetidin administration*

acetophenetidin, N-acetyl-*p*-aminophenol, were lower in the treated rats than in the control rats. Inhibition of acetophenetidin metabolism by SKF 525-A resulted in a significant prolongation of the drug's antipyretic action.

Although the ability of drugs to inhibit the metabolism of other drugs has been well documented in experimental animals, this effect has been studied only recently in man. For instance, drug inhibition of drug metabolism may explain why oxyphenbutazone (Tanderil) potentiates the anticoagulant action of coumadin[1] (Fox 1964). Recently, Weiner *et al.* (1965) have reported that the plasma levels of bishydroxycoumarin and the anticoagulant response to the drug were prolonged in human subjects treated with oxyphenbutazone. These investigators also reported that the anabolic steroid, methandrostenolone,[2] slowed the metabolism of oxyphenbutazone in man. This steroid apparently acted by inhibiting glucuronyl transferase, an enzyme needed for the conjugation of oxyphenbutazone with glucuronic acid.

Recent reports indicate that patients treated with monoamine oxidase inhibitors, such as tranylcypromine (Parnate) and iproniazid, are unusually sensitive to a subsequent dose of sympathomimetic amines which are metabolized by this enzyme. Unfortunately, severe hypertensive reactions have occurred when individuals treated with monoamine oxidase inhibitors ate cheese or other foods with a high tyramine content.

Some compounds which inhibit metabolism of certain drugs when given acutely to animals will stimulate the metabolism of the same drugs when given chronically (Serrone & Fujimoto 1962, Kato *et al.* 1964, Gillette 1963). For instance, an acute dose of SKF 525-A markedly inhibits the metabolism of hexobarbital and thus prolongs its hypnotic action. However, when SKF 525-A was given chronically to rats, the metabolism of the barbiturate was accelerated and its hypnotic action drastically reduced. Other examples of compounds which can either inhibit or stimulate the metabolism of drugs by enzymes in liver microsomes, depending whether they are given acutely or chronically, are chlorcyclizine, phenylbutazone and glutethimide (Doriden). Although this biphasic effect has been well described in experimental animals, further work is required to establish the importance of this effect in man.

[1]warfarin sodium (Marevan) in UK
[2]methandienone (Dianabol) in UK

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