_____Review Article_____

Synergism

With Special Reference to Central Nervous System Depressants

By G. VICTOR ROSSI

EXAMINATION OF THE *Papyrus Ebers* translation (126) suggests that therapists have long been intrigued by the interaction of drugs in combination. Traditionally, drugs have been prescribed in mixtures in attempts to surpass the effects of the separate components. Formulation of mixtures of active agents remained almost exclusively on an empirical basis for centuries. Indeed, the rational use of drug combinations remains a challenge to the present day medical practitioner (192).

Green (93) has aptly stated that "drug action must ultimately be explicable on a molecular basis, but owing to the complexity of living processes it is rarely possible to attribute a pharmacologic action, even qualitatively, to any precise chemical or biochemical reaction." Analyses of specific parameters are further complicated by superimposition of the action of one drug upon that of another. Nevertheless, advances are being made toward elucidation of the physicochemical bases of drug action and the fundamental mechanisms of drug interaction.

A seemingly infinite number of research publications embody, in title or text, the terms *synergism* or *potentiation*. On the assumption that certain general principles and problems relating to these phenomena might be illustrated by consideration of a selected group of pharmacologic agents, this review has been restricted primarily

to central nervous system depressants. To facilitate presentation, these have been arbitrarily subdivided into three categories: analgesics, anesthetics, and hypnotics.

Nomenclature.—The ambiguity of nomenclature relative to the phenomena of drug interaction is clearly evident upon perusal of the introductory chapters of modern textbooks of pharmacology. Semantic confusion has impeded progress in various disciplines. The International Committee for the Nomenclature of Blood Clotting Factors was established with the primary objective of clarifying the chaotic terminology in this field (41). Although the problems are not comparable in scope, a need to define precisely and establish a common meaning for terms applicable to the mutual modification of drugs in combination exists.

In his classical analysis of the subject, Veldstra (207) stated that the term potentiation means "to endow with power." It was further noted that the individual components of drug combinations possess an intrinsic "power" (*i.e.*, specific activity). Therefore, in synergic combinations, "power" is not conferred, but the effectiveness of the "power" originally present may be enhanced. Inasmuch as there is no evidence of potentiation, according to the proposed definition, Veldstra recommended that use of the term in this connection be discontinued. A plethora of scientific articles published subsequent to this indictment attests to the undiminished popularity of the de-

scriptive term "potentiation." In defense of this choice it may be noted that a more liberal definition is provided by another authority: potentiate—"to make potent or more effective" (210).

It is this reviewer's impression that the term "potentiation," as employed in the majority of cases, is intended to connote "that situation wherein one agent shows no appreciable effect on the biological system but exaggerates the response of the system to another substance" (213). However, the enhancement of response effected by a combination of an active and an inactive compound has also been designated as "sensitization" (169). Other investigators (78) have noted that a potentiator may exert its effect by impeding the metabolic transformation of the active drug or by sensitizing the organism to the drug. These authors have recommended that a compound of the first type be classified as a prolonging agent; the second, as a true potentiator.

The heuristic merit of the word "potentiation" is perhaps as acceptable as any other. Because of the diversity of current interpretations, however, the term will be meaningless unless the individual author clearly delineates the concepts implied by its use.

In this review the general term "synergism" will be used in reference to all situations wherein facilitation of a pharmacologic response is obtained by the combined action of two or more compounds. Although some authors have defined synergism in a more restricted sense, the term (Gk. *synergos*) literally means "working together" (210), cooperation. For clarity of expression, the concept of synergism as translated into molecular terms by Veldstra (207) warrants repetition: "the combination effects a certain response with a smaller number of molecules than that required for the most active compound separately, or in the range of suboptimal concentrations, the effect of a certain number of molecules of this compound is enhanced in the mixture."

ANALGESICS

The ideal of a potent nontoxic and nonaddictive analgesic awaits realization. New molecules have not evidenced satisfactory dissociation of the seemingly obligatory relationship between analgesia and toxicity (159). Thus, other means of compensating for the deficiencies inherent in currently available compounds have been explored.

Narcotic Antagonists.—One approach to the optimal utilization of analgesics has been directed toward the concomitant administration of a narcotic and a narcotic antagonist. In one sense, narcotic antagonists may be considered as "selective synergists." Nalorphine and levallorphan inhibit many (but not all) of the pharmacodynamic effects of morphine and other narcotic analgesics (150). Their remarkable antidotal effect on narcotic-induced respiratory depression suggested the possible clinical utility of narcotic: antagonist mixtures in the alleviation of severe pain. Despite early favorable reports, the use of morphine:nalorphine combinations has not gained wide acceptance. In controlled studies involving postoperative patients and healthy volunteers, a combination of 2 mg. of nalorphine and 10 mg. of morphine was found to produce analgesia and side-effects indistinguishable from those achieved by 10 mg. of morphine (10, 124). Houde and Wallenstein (99) observed that the incidence of side-effects increased in direct proportion to the relative concentration of nalorphine in morphine : nalorphine mixtures administered to hospitalized cancer patients. Analgesic:levallorphan combinations have been viewed with somewhat greater favor. Cullen and Santos (50) used a premixed solution of levorphan tartrate and levallorphan tartrate in an 8:1 or 10:1 ratio for the relief of chronic severe pain. The analgesic effectiveness of the combination was considered essentially equivalent to levorphan, whereas the respiratory depression was significantly less than that produced by the narcotic alone. Foldes, *et al.* (75), reported that levallorphan effectively blocked the respiratory depressant action of alphaprodine when these compounds were injected simultaneously for the purpose of obstetrical analgesia. Comparable results were obtained with oxymorphone:levallorphan mixtures (76).

The numerous clinical studies involving combinations of narcotics and narcotic antagonists have been critically reviewed by Eckenhoff and Oech (66). These authors hold that narcotic depression of respiration may be minimized by prior or simultaneous administration of a specific antagonist in the alleviation of acute painful conditions. In their opinion, however, such mixtures are not applicable to the treatment of chronic pain. This limited therapeutic efficacy of narcotic:antagonist combinations is not conceded by Telford and Keats (201).

Synergists.—Another approach to the goal of improving the efficacy of existing analgesic drugs has involved their use in combination with a variety of essentially unrelated compounds found, largely by "trial and error," to augment

analgesic activity. Both adrenergic (89, 102) and cholinergic (111, 112, 178) drugs have been reported to enhance the effectiveness of morphine. Veldstra (207) has documented the extensive literature relating to enhancement of the analgesic activity of narcotics by such diverse agents as suramin, sparteine, antispasmodic agents, and several methonium compounds. Recent studies have demonstrated that the activity of narcotic analgesics in experimental animals may also be augmented by captodiamine (113), carbetidine (143), chlortetracycline (145), mephenesin (170), mephenoxalone (92), methylquizolone (14), pentolinium (94), phenetamine (131), quinine (157), reserpine (51), tryptamine, 5-hydroxytryptamine, amphetamine and mescaline (187), and SU-8629 (2-amino-indane HCl) (216). Data supporting the clinical efficacy of these combinations is lacking, however.

SKF 525-A.—With the exception of a hypocholesterolemic effect demonstrated in several animal species (56), SKF 525-A (β-diethylamino-ethyl-diphenylpropylacetate HCl) possesses minimal direct pharmacologic activity (23). Primary interest in this compound has centered on its intriguing ability to act synergistically with drugs of diverse chemical structure and pharmacodynamic properties. Among the different types of drugs whose characteristic activities have been shown to be augmented by SKF 525-A are included: barbiturates and nonbarbiturate hypnotics (44), analgesics (45), antiepileptic drugs (195), spinal cord depressants and central nervous system stimulants (136), skeletal muscle relaxants (153), antihypertensive agents (90), and certain antibacterial and antiprotozoal drugs (202).

The key to the practicality of synergistic drug combinations is selectivity. Nonselective enhancement of the entire pharmacodynamic spectrum of a given drug provides a doubtful therapeutic advantage. Optimally, an augmentation of the therapeutically useful properties of the active component, accompanied by suppression of undesirable effects, is achieved. Exploration of the mechanisms of selective synergy remains to be exploited as a possible avenue to the objective of improved pharmacotherapy.

Cook, *et al.* (45, 46), showed that SKF 525-A enhanced the analgesic effect of various narcotics in rats without influencing either the LD$_{50}$ (of morphine or meperidine) or the respiratory suppressant action (of morphine) in the same animals. This clearly demonstrated that different facets of the narcotic spectrum could be influenced selectively. Although the synergistic agent in this case (SKF 525-A) has proven an

extremely useful tool in the investigation of pathways of drug metabolism, it remains restricted to experimental use.

It may be of interest that chronic administration of SKF 525-A is associated with hepatotoxicity in rats (204) and dogs (56). Holmes and Bentz (96) have postulated that interference with an important electron transfer coenzyme (coenzyme Q) may account for SKF 525-A-induced accumulation of lipid material in the livers of animals.

Phenothiazines (Animal Studies).—In the initial report on the subject Courvoisier and her associates (49) noted that chlorpromazine, although devoid of intrinsic analgesic activity, increased the intensity and prolonged the duration of action of morphine in mice. Subsequently, several investigators, using a variety of analgesimetric techniques, obtained comparable results in experimental animals with chlorpromazine and other phenothiazine derivatives (51, 55, 81, 152, 154, 162, 180, 205). The statement that the phenothiazines are not effective pain-relieving drugs, but that they potentiate the activity of specific analgesics, reflects the current consensus. Both of these views represent oversimplifications based largely on the original studies with promethazine and chlorpromazine.

Carter and David (38) reported that preinjection of chlorpromazine, thioperazine, and prochlorperazine effectively augmented the analgesic activity of morphine, meperidine, phenazocine, and raceoramide (SKF 5137) in rats. Of these three phenothiazine derivatives, chlorpromazine and thioperazine were found to diminish the degree of tolerance, but not necessarily the rate at which tolerance developed, when the narcotics were administered once daily during a 9-week period. Comparable enhancement by chlorpromazine of the analgesic activity of morphine in rats was observed by Mazurkiewicz and Lu (140). However, these investigators noted that chlorpromazine did not retard the development of tolerance in rats given a single daily injection of the narcotic during a period of 7 weeks.

Weiss and Rossi (212) reported that preinjection of a trifluorinated phenothiazine derivative (NDR-3680) enhanced the analgesic activity of codeine, dihydrocodeine, and morphine (d'-Amour-Smith analgesimetric method), and inhibited the constipating and respiratory depressant effect of codeine and morphine, but not dihydrocodeine, in rats. These investigators also found that phenothiazine pretreatment increased the extent and duration of protection provided by codeine, dihydrocodeine, and morphine against phenylquinone-induced writhing, reduced the

acute toxicity of codeine and dihydrocodeine, but increased the toxicity of morphine in mice.

Although there have been many differences in magnitude and a few differences in direction of the response, investigations performed with experimental animals have provided more consistent evidence of a synergistic relationship between the phenothiazines and the analgesic activity of narcotics than those studies performed in man.

Phenothiazines (Human Studies).—Numerous publications have appeared which describe the effects of phenothiazines, alone and in conjunction with barbiturates or analgesics, in surgical and obstetrical situations (82, 114, 137, 188, 191, 211), and in chronic pain of diverse origin (132, 174, 208). The lack of uniformity of rating systems, the paucity of control series, and the failure to provide statistical validation of the significance of the difference between treatments, mitigates against acceptance of facile claims of phenothiazine "potentiation" characteristic of many reports in this category. This reviewer is cognizant of the inordinate difficulties encountered in the evaluation of drugs intended for the amelioration of subjective phenomena in humans. However, these difficulties cannot always be accepted as an adequate excuse for poor experimental design. Controlled studies often reveal astonishing gaps between clinical impressions, theoretical assumptions, and established facts. Beecher (9), Wolf (217), and Modell and Houde (147) hold that there are no compelling reasons why quantitative methods cannot be utilized in the evaluation of subjective responses in humans.

Boulton (16) reported that the administration of chlorpromazine (25 mg. orally), prior to and following anesthesia, reduced the postoperative requirements of meperidine by 23% in females and by 6 to 7% in males who had undergone thoracic surgery. Dyrberg and Johansen (64) found no difference in the postoperative requirements for analgesic drugs among patients premedicated with 10 mg. of morphine or 50 mg. of chlorpromazine. Addition of 5 mg. of perphenazine to the standard premedication was found by Phillips, *et al.* (161), to reduce the incidence of requirements of analgesic drugs in the postoperative period by approximately 50%. Comparable results were obtained by Lear, *et al.* (128), with triflupromazine. The variables involved in such clinical situations make it extremely difficult to dissociate the effects of phenothiazines *per se* on anxiety reactions, and the possible enhancement of the analgesic activity of narcotics as distinct from prolongation of the action of general anesthetics.

Sadove, *et al.* (174), reported that narcotics and

sedatives, which had previously proven ineffective in 22 of 28 patients with malignant lesions, provided satisfactory relief of pain when given in conjunction with chlorpromazine. Wallis (208) concluded that chlorpromazine effectively augmented the action of narcotics in the majority of patients suffering chronic pain of diverse origin. Light, *et al.* (132), noted that promethazine enhanced and prolonged the effectiveness of meperidine and morphine in two patients suffering the pain of inoperable malignancy. In a controlled crossover study, Houde and Wallenstein (98) found that single doses of 25 mg. of chlorpromazine alone exhibited no significant analgesic effect in 34 hospitalized patients with chronic severe pain. Furthermore, the relief obtained with a combination of morphine sulfate (10 mg.) and chlorpromazine (25 mg.) was essentially the same as that provided by the narcotic alone in these patients.

Respiratory and Circulatory Effects.—In a discussion of combination therapy, Sollmann (189) stated that "synergism is utilized to secure the summation of the desirable effects of several drugs, while the side actions are not increased in proportion, or may even be neutralized." Several experimental animal studies noted in this review have demonstrated that the simultaneous use of two drugs may result in a selective reinforcement of certain pharmacodynamic properties with a resultant increase in the therapeutic index. However, few clinical data substantiate that such a fortuitous situation applies to the combined use of phenothiazines and narcotics in humans.

Divergent clinical reports indicate that phenothiazines suppress (127, 166), intensify (122), or do not significantly alter (68, 80, 108) the effects of narcotics on respiration. Obviously, the influence of phenothiazine derivatives on narcotic-induced respiratory depression in humans remains equivocal. Data provided in support of phenothiazine antagonism of the respiratory effects of narcotics are not particularly convincing, however.

The alterations of circulatory homeostasis by phenothiazine derivatives and by narcotic analgesics, separately, is well documented. Reduction of systemic arterial pressure by the phenothiazines is apparently due to a combination of factors, including depression of the vasomotor regulatory centers (198), adrenergic blockade (88), and direct relaxation of arterial smooth muscle (73). The hypotensive activity of narcotic analgesics may also be attributed to multiple mechanisms, which include inhibition of the vasomotor centers (70), and peripheral vasodilation

due to a direct effect on the blood vessels (179), or mediated *via* histamine liberation (72). Variations are considerable among the different members of each series; however, the phenothiazines generally manifest greater vasodepressor potency than the narcotics. The theoretical assumption was made by Burgi (207) that "in combining drugs with the same end effect, the resulting activity is additive when the sites of action of the components are identical and superadditive if they are different." Although this generalization is not universally applicable, the complex of mechanisms associated with the hypotensive effect of the phenothiazines and the narcotics would appear to provide an ample basis for synergistic activity. Data supporting this contention are lacking; however, Eckenhoff and Oech (66) consider that the combination of a narcotic with a phenothiazine derivative theoretically imposes a greater threat to the circulation than it does to the respiration.

Qualitative Differences.—The "drug explosion" (146) has added impetus to a pre-existing tendency to group structurally and/or pharmacodynamically related chemicals into a common category. The phenothiazines represent a case in point. Exaggerated emphasis has been placed on the phenothiazine nucleus as a determinant of biodynamic activity; the majority of published reports foster the impression that the various substitutions largely influence the properties of the molecule in a quantitative rather than a qualitative sense. Increasing sophistication has resulted in dissatisfaction with such generalizations.

The property of synergy has become identified indiscriminately with the phenothiazines as a group, although this association does not hold necessarily for all members of the series. Possible explanations for selectivity in regard to the enhancement of analgesic activity may be discerned in those studies which have demonstrated that the phenothiazines cannot be considered as a homogenous group with respect to their effect on pain.

Analgesic versus Antianalgesic Activity.— Hougs and Skouby (100), using the Hardy, Wolff, and Goodell radiant heat analgesimetric method, noted that chlorinated phenothiazine derivatives exerted a mild analgesic action which was not demonstrable with the nonchlorinated compounds. Boreus and Sandberg (15), who also employed a thermal technique for inducing pain in healthy human volunteers, concluded that although chlorpromazine and acetylpromazine reduced somewhat the sensitivity to noxious stimulation, neither significantly increased the

analgesic action of methadone. Mepazine, which evidenced no analgesic action when administered alone, antagonized the pain-relieving effect of methadone.

Methotrimeprazine was found to be equivalent (on a milligram basis) to morphine in providing relief of pain in postoperative and postpartum patients (125). Promazine, chlorpromazine, and trimeprazine evidenced modest effectiveness against experimentally induced pain in humans, whereas other phenothiazine derivatives manifested "slight" (prochlorperazine, perphenazine, trifluoperazine, triflupromazine) or "marked" (promethazine, mepazine) antianalgesic activity (149). Methdilazine, despite a marked sedative propensity, significantly increased the amount of meperidine required for the control of postoperative pain (200). Thus, contrasted to other investigators (133) who reported an augmentation of the activity of morphine and meperidine by methdilazine (radiant heat analgesimetric method in rats), Taylor, *et al.* (200), concluded that this phenothiazine derivative manifested a definite analgesic antagonism. It is possible that these apparently divergent conclusions are both valid, but that one is encountering the classical question of the relationship between the perceptual and psychic components of the pain phenomenon in man and the reflex reaction patterns to noxious stimuli in laboratory animals (109).

This dichotomous effect on patient sensitivity to pain may be reflected in the degree to which the action of the central nervous system depressants can be influenced by premedication with different phenothiazine derivatives. Dundee and Moore (62) found that, with the exception of those derivatives which evidenced a marked antianalgesic action, the phenothiazines under consideration significantly reduced the total dosage of barbiturate (methohexitone) required during the performance of a standard surgical procedure. The increased need for supplementary doses of barbiturate following premedication with promethazine and mepazine suggested the possibility of an antagonism between the antianalgesic phenothiazines and the anesthetic state.

Other evidence suggests that categorization of the various phenothiazine derivatives on the basis of an "analgesic" or "antianalgesic" effect may be artefactual. Clinical studies have indicated that barbiturates may have a diphasic effect on sensitivity to pain in man (4). Relatively high concentrations induce an anesthetic state *per se* or augment the analgesic or anesthetic activity of other agents. In contrast, a reduced threshold to pain may be experienced in the

presence of low levels of barbiturate in the central nervous system. A comparable diphasic action in regard to analgesia may be characteristic of other central depressants, *viz.*, phenothiazines (63). It is within the realm of possibility that, depending on dosage and/or time parameters, a particular phenothiazine might manifest either a synergistic or an antianalgesic effect.

ANESTHETICS

Using mice as the test subjects, Carson and Domino (37) found that relatively large doses of chlorpromazine (15 mg./Kg., intraperitoneally) significantly reduced the mean time required for loss of the righting reflex during exposure to ethyl ether, chloroform, or halothane. However, an equal dose of promethazine did not alter the time required for onset of anesthesia with these volatile agents. Brunaud, *et al.* (28), showed that propiomazine was considerably more effective than promethazine in its ability to prolong ether anesthesia in mice. Perhaps because of the technical difficulties associated with the quantitative administration of volatile anesthetics to small animals, most of the experimental animal studies in this field relate to effects obtained with fixed anesthetics (*i.e.*, barbiturates). Although the division is arbitrary, these reports will be considered in the section on *Hypnotics*.

Awareness of the potential application of phenothiazines in surgical anesthesia stemmed largely from the publications of Laborit and his coworkers (115, 116, 118–120), particularly in regard to promethazine and chlorpromazine. The role of this group in the development of the concept of "potentiated anesthesia" has been detailed by Laborit (117).

The versatility of phenothiazines in surgery is characterized by the reports of Sadove (173) and Dobkin (58), who describe the use of promethazine as a sedative preoperatively, as a supplement to anesthetics in producing the lighter planes of anesthesia and the hypothermic state, and as a means of combating emesis and hiccups. Taylor, *et al.* (199), observed that premedication with chlorpromazine alone permitted surgical anesthesia to be obtained with significantly lower concentrations of ethyl ether than with atropine alone. Carroll and Moir (35), and Adelman, *et al.* (1), reported that premedication with promethazine facilitated the induction and maintenance of general anesthesia and appeared to reduce the requirements of anesthetic agents in surgical and obstetrical cases. Stone (193) found that triflupromazine facilitated the action of general anesthetics in doses which did not

usually depress circulation. Thiopental anesthesia in man has been found to be augmented by a variety of central nervous system depressants, including antiemetics, antihistamines, and phenothiazine and nonphenothiazine ataraxics (60).

HYPNOTICS

Drowsiness associated with the clinical use of many antihistaminic drugs has been observed repeatedly. In experimental animals, however, it is ordinarily not possible to demonstrate sedation by the antihistamines when administered alone. In 1948, Winter (214), proceeding on the assumption that a simultaneous central excitatory action might mask a covert sedative effect, found that where antihistamines *per se* did not grossly manifest central depressant activity, they significantly prolonged the hypnotic action of hexobarbital in mice and guinea pigs. Although the basic technique had been previously employed by other investigators (34), Winter's report (214) catalyzed a surge of interest, which continues unabated in the potentialities of this tool.

Determination of the ability of a compound to prolong the duration of the loss of the righting reflex induced by a hypnotic dose of a barbiturate (generally hexobarbital) in laboratory animals (generally mice) remains a procedure widely used on the assumption that it provides a means of detecting subtle components of central nervous system depressant activity. The most frequently employed modification of this procedure is based on determination of the ability of a compound to convert a subhypnotic dose of a barbiturate to one which will induce a loss of the righting reflex (168, 206, 218).

There are few substances whose activity, based on either one or both of these criteria, has escaped examination.[1] Early studies relating to prolongation of barbiturate hypnosis, with particular reference to the antihistamines, disulfiram, carbohydrate metabolites, SKF 525-A, and alpha-tocopherol phosphate, have been previously reviewed (207). Within the past several years a representative grouping of compounds of diverse structure which have been reported to enhance the action of barbiturates and other hypnotics, includes: chlorpromazine, promazine, and their metabolites or "model" metabolites, monomethylchlorpromazine, chlorpromazine-N-oxide, 2-hydroxypromazine and 4-hydroxypromazine (163), other phenothiazine derivatives

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[1] Only selected reports concerning hypnotic drug synergism are cited in this review. A separate bibliography relative to the subject and containing in excess of 100 references is available on request.

(28, 133, 196), reserpine and other rauwolfia alkaloids (27), urethan (83), chloral hydrate (71), diphenylhydantoin and other anticonvulsant drugs (74), captodiamine (113), amphenidone, hydroxyzine and meprobamate (167), phenaglycodol and metaglycodol (209), emylcamate (141), metaxolone, carisoprodol, and benactyzine (36, 97), various thiaxanthene derivatives, including chlorprothixene (148), meperidine (139), morphine and other analgesics (54), and many other compounds evidencing overt central nervous system depressant activity. To this list of drugs, which would be expected to add to the depressant effect of barbiturates or other hypnotics, may be appended a heterogeneous group of compounds, all of which have been reported to augment sleeping time: N-acetyl-*p*-aminophenol (19), aminopyrine and phenylbutazone (67), octamethylpyrophosphoramide and other anticholinesterase agents (72), quinine (157), atropine and scopolamine (142), colchicine (8), chlortetracycline (145) and chloramphenicol (57), thyroxin (43), epinephrine, norepinephrine, ephedrine, and other adrenergic amines (144), nikethamide (168), and the antidepressant compounds, imipramine and amitriptyline (95), iproniazid and other amine oxidase inhibitors (69, 123).

Methods.—Assays based on (*A*) prolongation of sleeping time, and (*B*) conversion of a subthreshold dose of a hypnotic to one which will result in loss of the righting reflex, have previously been cited as those most commonly employed (with kaleidoscopic nuances) in studies of hypnotic drug synergism. Another perspective may be gained by a third procedure based on (*C*) re-induction of hypnosis after spontaneous arousal from anesthesia. The latter technique is used less frequently; therefore, relatively little data are available on which to base comparisons among the three methods.

The heterogeneity of molecules which have been found to extend the duration of barbiturate-induced sleep underscores the grossly nonspecific nature of this assay. Although it is of limited utility in attempts to discern qualitative differences, it is of value when used conjointly in the establishment of pharmacodynamic profiles, and it may be considered a versatile investigative tool. One ponders, nevertheless—to what extent has the magnificent simplicity of this "data machine" (major components: mouse, stopwatch) contributed to its ascendancy?

Interest in the ability of a compound to prolong the sleeping time induced by a hypnotic dose of a barbiturate (method *A*) resided initially on the supposition that the assay provided a rapid means

of detecting elusive central depressant activity, *e.g.*, in the evaluation of potential sedative or ataractic drugs. In a large number of cases the mechanism involves primarily (*a*) a summation of the central depressant effects of the drugs in question. It is apparent, however, that other factors, acting independently or conjointly, may also be responsible for the effect observed; these include: (*b*) increased rate of penetration into the central nervous system, (*c*) decreased binding to plasma or tissue components, *i.e.*, loss at "silent receptors," (*d*) reduced rate of biotransformation or (*e*) excretion, (*f*) contribution of the postictal depressant component of central stimulants, (*g*) "sensitization" to central depressant drugs as a consequence of alterations in acid-base balance or brain electrolyte patterns, etc. In the absence of other types of supporting data, it is obviously difficult to discern which of these factors (or conceivably other mechanisms not cited) are operative.

Some investigators (11) have used induction time of barbiturate hypnosis (*i.e.*, interval between injection and loss of righting reflex) as the criterion for alteration in the permeability of the "blood brain barrier." While this parameter may serve as a tentative indication of permeability changes, Child, *et al.* (39, 40), have shown that a decrease in induction time does not necessarily imply an increase in the rate of penetration of barbiturates into the brain.

Methods *B* and *C* are fundamentally related in that they both involve the ability of a compound to induce a loss of the righting reflex in the presence of a subhypnotic concentration of a barbiturate (or other hypnotic) in the blood. Method *C* constitutes the most rigorous challenge inasmuch as the second agent is not administered until after the brain concentration of barbiturate has receded below the anesthetic threshold, as evidenced by spontaneous arousal. Enhancement of barbiturate activity in all three situations depends not only on the inherent properties of the synergist but also on the dose administered, route of administration, and latency of action. These latter considerations are more critical in method *B* than in method *A*, and are most critical in method *C*, particularly in the presence of a hypnotic having a short biological half-life (*e.g.*, hexobarbital). Dismissal of these factors from consideration could conceivably lead to the adoption of erroneous conclusions regarding probable mechanisms of synergy.

The isobolometric studies of Loewe (134) provided discrete evidence of the dissociability of intensity of effect and duration of effect regarding barbiturate synergism. He found that the

HD$_{50}$ (median hypnotic dose) of pentobarbital was essentially unchanged by any dose of strychnine, whereas the HD$_{50}$ of butallylonal was diminished by approximately 50% by a wide range of strychnine doses in mice. Irrespective of the alteration of hypnotic threshold, strychnine prolonged the duration of sleeping time with both barbiturates.

Shagass (184) developed the concept of sedation threshold, a determination of the amount of barbiturate required to produce certain quantitative changes in the electroencephalogram, as a possible index of emotional tension in humans. In a related study of thiopental thresholds in rabbits, using "head drop" as the behavioral end point, Shagass, *et al.* (185), noted that chlorpromazine significantly diminished the amount of thiopental required to elicit the characteristic response. This procedure should be explored as a possibly sensitive and quantitative approach to the evaluation of barbiturate-synergist interactions.

Lessin (129) proposed a battery of three pharmacologic assays in mice to estimate inhibition of drug oxidation based on the assumption that a "nonspecific" liver oxidase system serves as the common denominator in: (a) prolongation of pentobarbital hypnosis, (b) intensification of chlorpromazine-induced hypothermia, and (c) reduction in the acute toxicity of octamethylpyrophosphoramide. Qualitatively similar results were obtained with isoniazid, iproniazid, and SKF 525-A in these three assays. It is an interesting approach; the validity of the hypothesis should be supported by examination of a larger series of compounds.

Variations in Response.—The many quantitative and occasional qualitative variations in response as they relate to species, sex, and age differences in the metabolism of drugs have been recently reviewed by Bousquet (17) and Brodie (22). Particularly relevant to this subject are the studies of Quinn, *et al.* (164), who demonstrated a striking relationship between hexobarbital sleeping time and the biological half-life of this barbiturate in several species. These investigators noted, however, that following administration of hexobarbital, mice, rats, and rabbits recovered the righting reflex at plasma levels of approximately 60 mcg. of barbiturate per ml., whereas hypnosis in dogs and man persisted until the level had declined to about 20 mcg. per ml. These data suggested that, in addition to differences in rates of drug metabolism, variations in the sensitivity of the central nervous system play a major role in species differences in response to depressant drugs. Other factors germane to

the interpretation of barbiturate synergism studies are the possible effects of hypothermia and hydration.

Hypothermia.—Studies conducted with a series of antihistamines demonstrated the absence of a relationship between histamine antagonism and synergic activity with barbiturates (2, 3). This conclusion was corroborated by the finding that histamine also prolonged barbiturate sleeping time. Although distinct quantitative differences were evident, Packman, *et al.* (158), found that histamine and each of 15 different antihistaminic compounds evaluated reduced the body temperature of mice. Subsequently, other investigators (71, 130, 218) reported that many drugs which prolong the action of hypnotics also lower body temperature. Conversely, β-tetrahydronaphthylamine, a potent pyretic agent, inhibited the prolonging action of 5-hydroxytryptamine on hexobarbital sleeping time in mice (160). There is little evidence of a strict causal relationship; nevertheless, the influence of a drug on body temperature is frequently a determinant of its effect on barbiturate hypnosis. Examination of the literature relative to barbiturate synergism suggests that not all investigators are aware of the importance of ambient temperature and the possible hypo- or hyperthermic effects of the compounds being studied on the response to hypnotic drugs.

Hydration and Dehydration.—Bhide (13) reported that hydration (intraperitoneal injection of water or 5% glucose solution) significantly increased hexobarbital sleeping time in mice. This finding was confirmed by Ramwell and Lester (165), who also observed that dehydration (resulting from deprivation of water or injection of various diuretics) markedly reduced the duration of hexobarbital hypnosis. The effect of water loading on the brain electrolyte pattern and electroshock seizure threshold has been detailed by Swinyard (195), for the experimental evaluation of anticonvulsant drugs. However, relatively little attention has been accorded the possible influence of alterations in the extracellular sodium concentration on the duration of barbiturate hypnosis. It is reasonable to speculate that changes in the electrolyte balance may constitute a critical factor in the observed effect of corticotropin and corticosteroids on sleeping time (215). The susceptibility of mice to dehydration should also be considered in the design of sleeping time experiments.

Dose and Time Factors.—Most investigators are cognizant of the limited value of data which relate the biological effect of a compound to a single dose evaluated at an arbitrarily selected

time interval after administration. Construction of dose-time-effect relationships requires serial determinations of response repeated in temporal sequence at various levels in the range from the minimum to the maximum effective concentration. However, practical considerations frequently restrict the completeness with which three-dimensional analyses are performed with individual compounds. Considering the additional complexities inherent in the pharmacologic evaluation of drug mixtures, it is not surprising that most studies in this area lack adequate perspective.

The observation of Shore, *et al.* (186), that relatively large doses (10 mg./Kg.) of lysergic acid diethylamide (LSD) antagonized a central action of 5-hydroxytryptamine (5-HT) in the intact animal, provided a basis for speculation as to the role of this amine in normal and disturbed mental processes and the mechanisms of action of psychosomimetic and psychosoplegic drugs. It is not within the scope of this review to consider the controversies in these areas; however, it is relevant to note certain apparently dose-related inconsistencies which have necessitated a re-evaluation of earlier concepts. LSD and 2-brom-*d*-lysergic acid diethylamide (BOL), in doses (2 to 5 mcg./Kg.) which did not alter the duration of hexobarbital hypnosis in mice, were found to enhance further the prolonging effect of 5-HT (but not reserpine) on hexobarbital sleeping time (175). Larger doses (2.5 to 80 mg./Kg.) of LSD, which have been claimed both to prolong (33, 219) and have no effect (27) on barbiturate hypnosis, and larger doses of BOL, having no effect (33) in this regard, blocked the prolongation of sleeping time induced by either 5-HT or reserpine (176) but not iproniazid (27).

In certain cases, either a synergistic or an antagonistic relationship may be effected, depending upon the time interval separating administration of two drugs. Prolongation of barbiturate-induced hypnosis by chlorpromazine, administered simultaneously or several hours prior to the hypnotic, is well documented. In contrast, Kato (105) showed that sleeping time was significantly reduced when pentobarbital or hexobarbital was administered 48 hours after chlorpromazine (15 mg./Kg., intraperitoneally) in rats. A comparable diphasic effect on the duration of hexobarbital action has been reported to occur with urethan (83), SKF 525-A, N-ethyl-3-piperidylbenzilate HCl, nikethamide, and iproniazid (183). Other studies have demonstrated a marked reduction in the duration of action of pentobarbital, hexobarbital, meprobamate, and zoxazolamine in rats injected 24 hours or more in advance with a variety of drugs and chemical carcinogens (17, 20, 42). The decreased sensitivity is explained by an accelerated *in vivo* biotransformation, subsequent to the increased activity of hepatic microsomal drug-metabolizing enzymes induced by a host of foreign compounds (85).

Recent evidence suggests that SKF 525-A and Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyl diethylamine HBr) may have an "immediate" inhibitory and a "late" inducing effect on the same enzyme systems (106, 107). The interesting hypothesis has been formulated that the initial inhibitory action may be the factor responsible for stimulating the compensatory increase in *de novo* biosynthesis of microsomal drug-metabolizing enzymes (107). Further studies are needed on the relationships between the inhibitory action and enzyme-inducing action of other compounds; in some cases, synergism and antagonism may be different sides of the same coin.

MECHANISMS

The number of different types of drug combinations for which a synergistic relationship has been claimed greatly exceeds the plausible explanations of the mechanisms involved. At present no unifying concept appears adequate to account for all the diverse forms of molecular interaction encompassed by the term "synergism." Veldstra (207) suggested that a large proportion of the cases may be explained on the basis of a competition between the active compound and the synergist at various "sites of loss" for the former. These sites of loss have been identified as loci of nonspecific adsorption (*i.e.*, storage at "silent receptors"), as enzyme surfaces functioning in the metabolism or detoxication of the active compound, or as excretion mechanisms. Although this pregnant concept provides a valuable frame of reference, it is obvious that detailed analyses are required to elucidate specific mechanisms of synergic activity.

Structure-Activity Relationships (Phenothiazines).—Adequate data are not available to establish clear relationships between chemical structure and synergic activity of the various phenothiazines, although some efforts in this direction have been reported. Dobkin (59) evaluated several phenothiazines on the basis of their ability to prolong thiopental anesthesia in the dog. Quantitative relationships could not be derived inasmuch as different doses of the various derivatives were used. Nevertheless, in the doses employed, promazine and propiomazine were most effective in prolonging the anesthetic

effect of thiopental; levomepromazine and meth-dilazine were somewhat less active, whereas mepazine, prochlorperazine, and trifluoperazine did not influence the duration of thiopental anesthesia.

A comparative study of seven phenothiazine derivatives revealed the lack of a definite relationship based on their ability to prolong barbiturate hypnosis in mice, and impair locomotor activity in rats, and climbing dexterity in mice (177). The outstanding exception in this case, perphenazine, exhibited the greatest activity in all three assays. Jindal, *et al.* (103), compared the effects of uniform doses (on a milligram basis) of a series of phenothiazine derivatives on the duration of pentobarbital-induced hypnosis in mice. The interesting aspect of this study is that compounds which prolonged sleeping time also evidenced antidiuretic effects in saline-loaded rats, whereas those compounds which either reduced or did not alter the duration of pentobarbital hypnosis had a diuretic effect in such rats. The authors suggested that a parallelism may exist between these two phenomena. Domino (61) stated that it is unlikely that antidiuretic hormone release is the only factor involved in the enhancement of pentobarbital anesthesia by phenothiazines, but it may be contributory.

Biochemical Mechanisms (Phenothiazines).—Bain and Mayer (7), and Laborit, *et al.* (121), have prepared comprehensive analyses of researches relating to the effects of chlorpromazine and certain other phenothiazines on various enzyme systems. There is a progressively increasing literature documenting the action of phenothiazines as relatively nonspecific inhibitors of many biochemical reactions *in vitro*. However, inferences are frequently drawn from *in vitro* experiments regarding the effects of drugs on enzymatic activity, with little consideration given to the influence of normal cellular architecture on biochemical processes *in vivo*. At present it is difficult to relate the pharmacodynamic actions of the phenothiazines to their currently established biochemical effects. Despite the relatively low potency evidenced in many of the *in vitro* systems, it is nevertheless tempting to speculate that inhibition of drug metabolism constitutes at least one aspect of the mechanism of phenothiazine synergy.

Decsi (53) compared a variety of central nervous system depressants, including eight phenothiazine derivatives, on the basis of their ability to inhibit a conditioned avoidance response in the rat, augment the hypnotic action of hexobarbital in the mouse, and inhibit oxidative phosphorylation and adenosine triphosphatase activity in rat

brain homogenates. The investigator concluded that there exists a quantitative relationship among the biochemical and pharmacologic effects described, thus enabling prediction of the *in vivo* potency of a drug solely on the basis of its *in vitro* activity. Inspection of the data failed to reveal adequate justification for this confidence.

Chlorpromazine was found, in healthy humans (194) and in rabbits (203), to elevate the blood alcohol concentration significantly above the levels detected in control series after adminstration of a standardized dose of ethanol. Although the study of Zirkle, *et al.* (220), did not substantiate the effect of chlorpromazine on the blood alcohol level, it demonstrated that chlorpromazine markedly increased the impairment of neuromuscular coordination following a standard dose of ethanol in man. This apparent discrepancy may be related to the indirect estimation of blood alcohol volume by means of a "Breathalyzer" in the latter study. Tipton, *et al.* (203), concluded that the elevation of the blood alcohol level by chlorpromazine was not due to an increase in the rate of absorption, but that it may be attributable to an inhibition of ethanol metabolism. In support of this premise, Khouw, *et al.* (110), have demonstrated that relatively low concentrations of chlorpromazine inhibit the activity of rabbit liver alcohol dehydrogenase.

Kaplan, *et al.* (104), demonstrated that large doses of nicotinamide markedly increased the levels of diphosphopyridine nucleotide (DPN) in mouse liver. These transiently elevated DPN levels were maintained for a prolonged period when chlorpromazine was administered prior to the injection of nicotinamide (31). Burton, *et al.* (30), observed that nicotinamide, in concentrations which did not influence the duration of pentobarbital-induced hypnosis in mice, significantly extended the effect of chlorpromazine on pentobarbital sleeping time. In a subsequent study, Burton, *et al.* (32), found that "nontranquillizing" derivatives of phenothiazine (*e.g.*, thioperazine) were ineffective in maintaining elevated DPN levels. It would be interesting to determine whether a relationship exists between the synergistic activity of various phenothiazine derivatives and their ability to maintain nicotinamide-induced levels of DPN.

Inhibition of drug inactivating mechanisms is not held accountable for phenothiazine synergy by all investigators. Martin, *et al.* (138), observed that chlorpromazine and chlorpromazine sulfoxide enhanced the magnitude and prolonged the duration of the chronotropic action of epinephrine and norepinephrine in the spinal vagotomized cat. Although the factors in this phe-

nomenon were not elucidated, it was emphasized that chlorpromazine did not alter the metabolism of these catecholamines by either blood or liver.

Brodie, *et al.* (25) observed that mice which had just recovered from hypnosis induced by hexobarbital, reverted immediately to the hypnotic state when injected with chlorpromazine. In contrast, mice were not visibly affected by SKF 525-A or amine oxidase inhibitors of the hydrazine type administered after return of the righting reflex (123). These researchers concluded that SKF 525-A and the amine oxidase inhibitors prolong anesthesia by interferring with hexobarbital metabolism, whereas chlorpromazine increases the sensitivity of the central nervous system to depressant drugs. The precise nature of this "sensitization" remains to be elucidated; the extent to which it may involve the central adrenergic blocking activity of chlorpromazine (26) or the postulated alteration of the "trophotropic-ergotropic" equilibrium (24) has not received attention.

Several types of evidence have been cited in support of the concept that a reduction or stabilization of the permeability of certain biological membranes may constitute the primary action of chlorpromazine. Spirtes and Guth (190) observed that concentrations of chlorpromazine less than required to influence oxidative phosphorylation diminished the water imbibition of mitochondria induced by thyroxin and other swelling agents. Gey and Pletscher (87) have summarized other findings in support of this view and have suggested that a reduction of membrane permeability *in vivo* may account for their observation of interference by chlorpromazine with the metabolism of aromatic amino acids in rat brain. In a report on the influence of chlorpromazine on the activity of monoamine oxidase inhibitors and monoamine releasers in rat brain Schwartz, *et al.* (181), similarly concluded that the phenothiazine probably interferes with the permeation of monoamines or their precursors through brain membranes. There are no data which relate this concept to the possible mechanisms of phenothiazine synergy; nevertheless, it is conceivable that biotransformation processes may be impeded by reduced accessibility to intracellular sites of metabolism in the absence of direct inhibition of the enzymes involved.

Biochemical Mechanisms (SKF 525-A and Other Synergists).—The polyvalent nature of the synergistic activity of SKF 525-A has been attributed largely to an interference with the biotransformation of active drugs. This hypo-

thesis is supported generally by the many reports which have established the prolongation of the action of various drugs by SKF 525-A, and specifically by those studies which have elucidated the effects of SKF 525-A on discrete metabolic pathways (17, 21, 42, 207).

SKF 525-A has been shown to inhibit the conversion of mephenesin to *o*-toloxylactic acid (135), the side-chain oxidation of hexobarbital, pentobarbital, and secobarbital (6, 48), the deamination of amphetamine, the formation of morphine glucosiduronide, the O-dealkylation of codeine (5, 48), the N-demethylation of aminopyrine, meperidine (48, 86), and the opiates (197), and the oxidation of phenylbutazone (29). Fouts and Brodie concluded that inhibition of drug-metabolizing enzyme systems present in liver microsomes (77) and in the soluble fraction of the liver cell (79) constituted a major factor in the synergistic activity of SKF 525-A.

The activity and/or toxicity of certain compounds is antagonized, rather than synergized, by SKF 525-A. This paradoxical effect has been explained on the basis of inhibition of enzyme systems required for the formation of active metabolites of the parent compound. For example, SKF 525-A diminished the prophylactic action of mepacrine against equine encephalomyelitis in mice (101, 202). This observation suggests, but does not prove, that SKF 525-A retards the conversion of mepacrine to an active antiviral metabolite. Organophosphates of the phosphorodiamidate and phosphorothionate classes are not toxic directly, but are converted to potent cholinesterase inhibitors by an oxidative reaction which, in mammals, is accomplished almost exclusively by liver microsomes (52). When added to mammalian liver preparations *in vitro*, SKF 525-A blocks the "activation" of schradan (tetramethylphosphordiamide anhydride), Guthion (dimethoxy ester of benzotriazine dithiophosphoric acid), and parathion (diethyl *p*-nitrophenyl phosphorothionate) (52, 151). SKF 525-A has been shown to be an effective antagonist of poisoning by schradan and Guthion, but not parathion in mice (155, 156). Presumably parathion possesses a greater affinity than the other organophosphates for the activating system *in vivo*, and therefore is not significantly altered by the presence of SKF 525-A (156). In summary, there have been cited multifaceted evidences that SKF 525-A influences enzymatic pathways involved in the detoxication of various drugs, and the conversion of certain latent species to biologically active molecules.

Interference with drug metabolism has been proposed as the basis for the synergistic activity

demonstrated with many compounds other than SKF 525-A. In the case of Lilly 18947 (77), iproniazid (78), JB 516 (123), captodiamine (65), chloramphenicol (57), and thyroxin (43), prolongation of hexobarbital sleeping time has been correlated with elevation of whole body concentrations of barbiturate and reduction of the rate of hexobarbital oxidation in an *in vitro* system. In studies of JB 516, N-ethyl-3-piperidyl benzilate and N-methyl-3-piperidyl diphenylcarbamate, Fujimoto, *et al.* (84), and Serrone and Fujimoto (182) related extension of hexobarbital hypnosis to whole body concentrations of the hypnotic, rates of disappearance from an isolated liver perfusion system, alteration of bromsulfalein retention, and inhibition of hexobarbital metabolism *in vitro*. Robison and Schueler (171) performed comparable studies with α-benzoylamine-β-(4-pyridyl)acrylic acid piperidide. Results of these investigations provided objective support of the proposed mechanism of action of the compounds in question. Such multidimensional analyses designed to elucidate fundamental synergic mechanisms are, unfortunately, the exception rather than the rule.

Data reported by several investigators suggest that the mechanism of action of SKF 525-A and certain other compounds which manifest synergic activity cannot be explained solely on the basis of retardation of drug metabolism. Several phenomena indicate that additional factors, not fully revealed at present, may also be operative. The phenomena include: (*a*) discriminative enhancement of certain properties of a drug, (*b*) reinstitution of a characteristic response by administration of the synergist after initial response to the active drug had terminated, (*c*) manifestation of a response by administration of a subthreshold dose of an active drug in combination with a synergist.

Corresponding specific observations involving SKF 525-A which do not conform totally to the "drug metabolism hypothesis," may be cited: (*a*) SKF 525-A prolonged hexobarbital-induced hypnosis without significantly altering the LD_{50} of the barbiturate (47) and enhanced the analgesic activity but not the respiratory depressant effect or LD_{50} of morphine (45). Similarly, the anticonvulsant activity and neurotoxicity of several central nervous system depressants were not uniformly increased by SKF 525-A (195). (*b*) Injection of SKF 525-A in the sciatic nerve-gastrocnemius muscle preparation of the chloralosed dog, after the muscle relaxant action of succinylcholine has ceased, resulted in a renewed and substantially increased curarizing effect (12, 18). These investigators

proposed that the synergist displaced the muscle relaxant compound fixed in an inactive form at nonspecific receptor sites. A renewed and sharply defined depressor response was elicited by the administration of SKF 525-A after the blood pressure reducing effect of several different hypotensive drugs had largely dissipated in unanesthetized rats and dogs (90, 91). (*c*) These authors also observed that subthreshold doses of the hypotensive drugs evoked a significant decrease in blood pressure when injected after the administration of nondepressor doses of SKF 525-A. Such experiments lend support, albeit indirectly, to the concept of displacement of the active molecule from loci of nonspecific adsorption by the synergic agent.

Attention directed toward the role of biotransformation mechanisms in synergic phenomena, while most revealing, has tended to subjugate consideration of other basic aspects of the drug activity spectrum, *i.e.*, absorption, binding, cellular and subcellular distribution and localization, and elimination. A perspective of the kinetics of drug synergy must await investigations comparable to those reviewed in this section performed in conjunction with analyses of factors other than drug biotransformation which may influence the pharmacologic response. Parallel studies, utilizing both biodynamic and biochemical parameters, may clarify mechanisms of synergy in relationship to the total metabolic sojourn of the active drug.

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