SYNERGISM AND POTENTIATION

WITH SPECIAL REFERENCE TO THE COMBINATION OF STRUCTURAL ANALOGUES

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I. INTRODUCTION

The practical interest in the effect of drug combinations dates back to the very beginning of pharmacology, since in combining drugs it has always been attempted to surpass the effects of the separate components.

Serious analysis of these phenomena was not, however, attempted before 1910, when Bürgi (53–62), who was the first to devote closer study to the effect of drug combinations, especially of those with similar activity, formulated the following law: "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 these are different".

For a long time this statement remained the central point of discussion concerning the augmentation of the effect of one of the components by the other (which Bürgi called potentiation). The material for such discussions increased steadily (25, 43, 46, 47, 65, 114, 115, 120, 139, 141, 162, 163, 182, 209, 212, 229, 231, 245, 299, 326, 327) and was analysed by Storm van Leeuwen (316, 317, 321, 322, 323) and especially by Loewe and his school (174–178, 218–224, 306) in great detail, graphic representations being used as a basis for the classification of the different types of mutual influence (267, 268, 269).

As early as 1913, Frei (110) had, in fact, applied a comparable method in studying combinations of disinfectants.

Unfortunately, the terms synergism, potentiation, antagonism, etc., used in this connection did not have the same meaning with different authors, mainly because some used the end-effect of the combination as a measure of comparison, while others used the individual activities of the components.

Rentz (282) tried to terminate the confusion, caused in this way, by an important comparative study of systematics and nomenclature concerning the effect of drug combinations, which resulted in an extensive scheme of the different forms of end-effects (282, p. 358; 283).

However, these graphs and schemes have not been factually substantiated in such a way as to provide a basis for further elucidation of these problems. Thus they have remained formally descriptive only. It is questionable whether we can expect anything more; even if the concentration-activity curves of the components are known in every detail, a combination set down in writing can indicate at most what may occur, but cannot predict what actually will occur when the combination is administered, quite apart from the fact that difficulties may arise in the interpretation of responses and effects observed with different test methods (133, 221).

Apparently the mutual influence of the components, which determines the final result, is generally too complex to be understood on the basis of measured individual *activities* (effects); for this reason it is necessary to substitute a study of their *modes of action.*

In this connection, it is significant that, in modern pharmacology textbooks, very limited attention is paid to the effect of drug combinations, and that the interpretation reached in the majority of discussions relative to the subject of this review may be exemplified by the following quotation: "By synergistic action is meant the production of an effect, which is more intense or more prolonged than would be obtained by either drug alone. This term is also used in a more limited way to designate those instances in which the effect is more than additive, *i.e.*, the response is greater than the algebraic summation of the effects of the drugs, when used alone. This latter definition also applies to the term potentiation" (250).

Synergism literally means working with, co-operation. This joint action of two or more compounds might result in an addition only, but the term has acquired the implication of denoting something extra, in a positive sense, with respect to the effect measured. ("Synergism is the co-operative action of discrete agencies, such that the total effect is greater than the sum of the two effects taken independently" (348).)

We shall use the word synergism in this sense, translated into molecular terms: 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.

In the schemes of Junkmann and Rentz (282) synergism in the above-mentioned sense, when found with a combination of an active and an inactive compound, is designated as sensitization, whereas in the case of both compounds possessing the same type of activity the term potentiation is used.

As this review will endeavour to make clear, there is no fundamental difference between these two forms of synergism and, moreover, no evidence of a true sensitization is generally present in cases of synergism. This nomenclature should therefore no longer be used.

There is a further reason for dropping the term "potentiation." Potentiation means "to endow with power". Now in drug combinations the components do possess a certain "power", *viz.* specific activity. In the combination, showing synergism, this power is not altered, but the effectiveness of the power already present is enhanced. The different ways in which this may be effected will be analysed in this review. No real potentiation occurring, the use of the term in this connection should be discontinued.

From this point onwards the true title of this review should be "synergism" alone.

II. DISTRIBUTION OF THE ACTIVE MOLECULES IN A BIOLOGICAL SYSTEM (TEST OBJECT)

1. Compounds having the same site of action. In analyzing the different possibilities of mutual influence by the components of a mixture with respect to the end-effect measured, it is extremely useful to visualize the "localization" of the molecules.

The response, which constitutes the measure of the physiological or pharmacological activity, is generally considered to result from an interaction between the active compound and the respective (often unknown) site of primary action (receptor). For a given concentration the intensity of this response is determined by the "quality" of the interaction, *i.e.*, by the intrinsic activity of the compound, and by its affinity to the site, on which the frequency of effective encounters is dependent (142).

The distinction between activity of a compound, once adsorbed on to the receptors, and its affinity to the same, has been made in plant physiology by Åberg (2), during investigations on auxins and antiauxins. Linser (214, 215), using different expressions, in fact analyzed his material on growth substances and inhibitors in a related way. The data thus obtained were subjected to mathematical treatment by Hellström (144) and Kaindl (179, 180, 181), respectively. Ariëns (10, 11) applied the same method of analysis to pharmacological investigations, working the concept of intrinsic activity into the Michaelis-Menten equation (184).

If we compare two compounds with different intrinsic activities, there is no possibility of enhancing the weak response of the compound with the low intrinsic activity to the level of that with the higher activity. If a low affinity for the receptor is the main cause of a weak effect, it may be possible to equal the effect of a compound with a higher affinity by means of increasing the concentration.

Considering now two compounds with the same type and identical site of action, a synergistic effect of the combination cannot be imagined.

The result of combining the compounds will depend on the relationship between affinities and intrinsic activities of the components. An additive effect

may occur if the activities are of the same order of magnitude. If one of the components is less active because of smaller intrinsic activity, its participation in the interaction implies a relatively decreased frequency of more effective encounters of the other compound with the receptor. In this way the less active compound, competing with the more active one for the sites of action, may behave to a certain extent like an antagonist. For this reason, Bürgi's law is certainly too generalized. The antagonistic character increases with decreasing intrinsic activity (assuming the affinity to remain fairly constant), and a compound (analogue of the more active component) with zero intrinsic activity is in fact a competitive antagonist. This type has been investigated extensively in the domain of metabolite antagonists (359).

Summarizing, we may say that the effect of a combined action of two compounds at the same site of primary action will not result in a synergism, but will, generally, even be unfavourable. The competition for the receptor will usually decrease the frequency of the best interactions, and with decreasing intrinsic activity of one of the components the combined action will more and more take the form of a competitive antagonism.

2. Compounds having different sites of action. The sites of action for two compounds having the same type of activity may be different. This is the case when, *e.g.*, the effect can be caused either by a direct stimulation or by the annihilation of an inhibition.

Competitive antagonists for different intermediates in a bio-synthetic chain fall in the same category, if the inhibition of the synthesis of the end-product is taken as their effect.

In both cases the combination of two compounds, linked in parallel or in series, as it were, may well result in a synergistic effect (*cf.* second part of Bürgi's law).

When the components of a combination possess different sites of action and different types of activity, no plausible prediction about the possibility of synergism can be made, unless their mode of action is well known.

Here "different type of activity" means that, if we take the activity of one component as a measure, the activity of the other one cannot be expressed in terms thereof, *i.e.*, with respect to the measured activity the situation is the same as with a combination of an active and an inactive compound. In our opinion this group can be analyzed in the best way from this point of view.

In thus considering the sites of primary action only, it has tacitly been assumed that they are freely accessible to the compounds, without the interference of secondary factors. This ideal situation does not occur, however, in the complex biological system of the normal test object used in studying physiological and pharmacological effects. To approximate more closely to the factual situation, the following section will also take into account these secondary factors.

3. Secondary factors. "Sites of loss" and their importance for synergism in combinations. The following considerations on secondary factors, influencing the end-effect of an active compound in a biological system, were developed during investigations on plant growth substances (343) with respect to transport and distribution factors. Afterwards they were applied more generally to physio-

logical and pharmacological actions (237, 340, 341, 342), especially in analyzing the conditions for synergism in combinations. They may be given here, preceding a discussion of the publications relative to this field, in which, independently, several authors have applied similar concepts to separate aspects of the problem.

It may plausibly be assumed that, if molecules of an active compound are functioning in a complex biological system, not all of them will reach the site of primary action unhindered. Some may be interacting at sites of secondary importance to the total action, while others may be adsorbed elsewhere in a harmless way or cause toxic effects in the system. Another part may be "trapped" on enzyme surfaces, being inactivated by decomposition or by coupling with other compounds and possibly excreted from the system. Moreover, further types of transport barriers may be present (lack of penetration). All of these events imply a waste or loss with respect to the primary action, and the loci of these secondary actions may be termed "sites of loss".

It may be mentioned here that, as early as 1925, Storm van Leeuwen (318, 319) distinguished between combination of the drug with dominant receptors, responsible for the primary pharmacological action, and "with other substances in the body, where no 'site of action' exists". These substances were called secondary receptors. The distribution between the two kinds of receptors was considered to determine the end-effect of the compound.

Sobotka and Glick (308) observed that the rate of hydrolysis of tributyrin by liver lipase was increased by addition of octyl alcohol in low concentrations, whereas higher concentrations were inhibitory. This was explained as follows: "The surface-active alcohol competes with the substrate not only for the active area on the enzyme, but also for the inactive 'dead spots'. Thus the presence of octyl alcohol actually diminishes the amount of tributyrin taken up by these inactive enzyme areas".

In an extensive review on drug-protein interactions, Goldstein (124) analyzed the distribution of a drug in the human body in more detail, considering the same "side tracks" as we did (*e.g.*, "silent" combinations which, from the standpoint of therapeutic effort, seem irrelevant). A very instructive scheme illustrates the influences of secondary interactions upon the primary action. A somewhat more generalized form (Fig. 1) will serve here.

Under II,1 it was indicated that a compound may be less active than a related one either because of a lower intrinsic activity or because of a slighter affinity to the site of action. Now a compound also may be less active (when compared with another on the basis of the concentration required to attain a certain level of activity) if its affinity for sites of loss is higher, in which case its molecules will reach the site of primary action to a lesser extent. If the affinity for the sites of loss becomes very high, they may not reach the critical concentration required for activity at the sites of action and will be completely inactive. Such compounds will generally be structurally related to the highly active compounds, since the common affinity to the sites of loss (differing quantitatively) depends upon this relationship.

If an analogue, less active or inactive for the above-mentioned reason, is

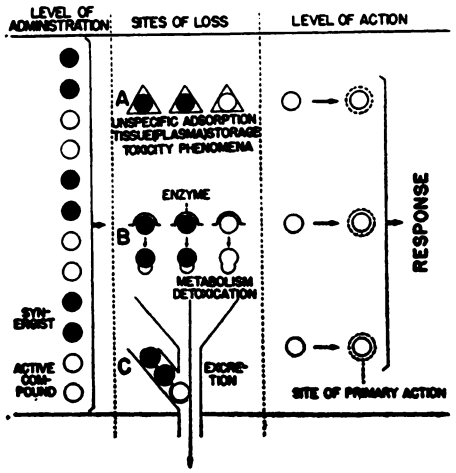


FIG. 1. Schematic representation of secondary factors interfering with the occupation of sites of primary action by the active compound.

Left: When the active compound is administered alone, part of it is excluded from action by adsorption on to sites of loss of various types.

Right: Together with a synergist, which presumably is preferentially adsorbed on to the sites of loss, the same degree of occupation of primary sites of action (*i.e.*, the same response) may be realized by a smaller number of molecules of the active compound.

applied together with the active compound, it will compete successfully with the latter for the sites of loss. Consequently, a higher percentage of the active compound will become available for its essential action. If the effect was not yet maximal, it will be enhanced, or otherwise the same activity may be attained with a smaller number of molecules, provided that those omitted be replaced by an analogue of the type indicated.

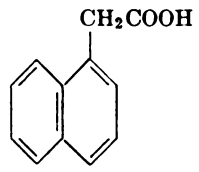
Thus a synergistic effect of the combination with respect to the primary activity is based on a competition between active compound and less active or inactive analogue for the sites of loss. Depending on the nature of the site of loss (Fig. 1), the exact mechanism of action of various synergists will be different. But in all cases the result is enhanced efficiency of the amount of active compound administered to the system, resulting in either a prolonged effect of a given amount (sparing action) or the production of the same effect with less of the active compound. Even the total amount of "work" (occupation of sites of loss and sites of primary action) is done by the combination with a smaller number of molecules than if the active compound were used alone. With increasing affinity of the synergist for the sites of loss, a proportionately smaller amount is required to replace the part of the active compound which is omitted. Consequently, the same occupation of both the sites of action and the sites of loss is realized with a smaller overall concentration.

Thus, the co-operation of the compounds in obtaining the desired end-effect is not a direct contribution to the primary activity, but the prevention of loss of the same.

It seems most likely that a very large number of synergistic activities will be based on the principle set forth in this section, *viz.*, on competition of the components for sites of loss.

III. PLANT GROWTH SUBSTANCES

The analysis leading to this concept of synergism was originally applied when investigating the inactivity or weak activity of certain analogues of growth substances. Since the comparisons drawn by this test method are relatively simple and very suitable for illustration, they may serve as an introduction to the subsequent discussion of synergism between drugs and other substances.



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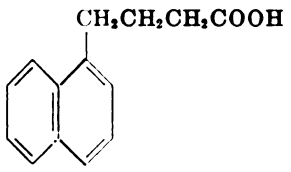

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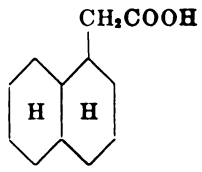

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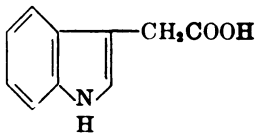

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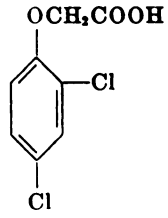

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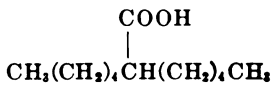

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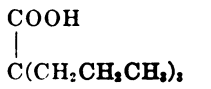

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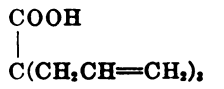

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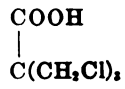

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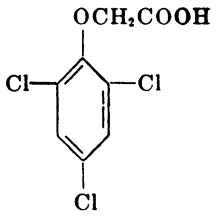

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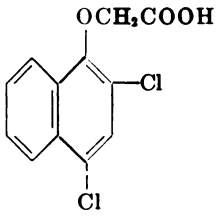

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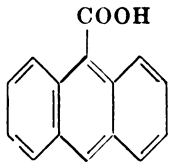

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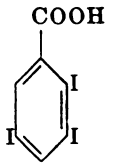

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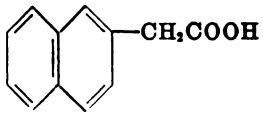

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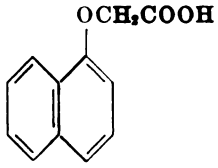


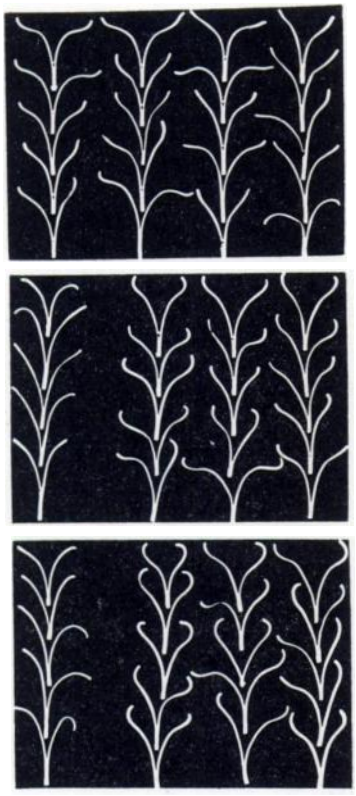
XV

The activity of naphthaleneacetic acid (I) was compared with that of its homologues and hydrogenated derivatives in the pea test (350). It was considered possible that the weak activity of γ-(1-naphthalene)-butyric acid (II) and the inactivity of decahydronaphthaleneacetic acid (III) were caused by increasing affinity for sites of loss, in which the shift of the hydrophilic/lipophilic (H/L) balance to the lipophilic side might play a part.

The results of tests with mixtures of naphthaleneacetic acid and both II and III were in accordance with expectations.

Submaximally active concentrations of I become maximally active by addition of the less active II or of the completely inactive III, in amounts which possess only very weak activity or none at all when used alone (Fig. 2).

In a combination of naphthaleneacetic acid with its fully-hydrogenated derivative III, even 2–3 % of what is required for maximal activity of the active acid, when administered alone, is sufficient to attain the maximum level. When comparing solutions of the mixture and of naphthaleneacetic acid, having the same maximal activity, the total number of molecules, per unit of volume, in the



a From left to right:
I:4, 1, 0.4, 0.1 × 10^{-5} mol/l

b I:- 1 0.4 0.1
III:2 2 2 2 × 10^{-5} mol/l

c I:- 1 0.4 0.1
VI:4 4 4 4 × 10^{-5} mol/l

FIG. 2. Pea test. The inward curvature of the longitudinally sectioned pea stem is the measure of activity. Naphthaleneacetic acid (I) was examined alone and together with either decahydronaphthaleneacetic acid (III) or di-*n*-amylacetic acid (VI). The figures refer to the concentration of the test substances.

former is only half of that in the latter. The synergistic effect is, therefore, very pronounced, and in this way it can be seen how small a fraction of the molecules of the active compound are actually performing their primary function.

The same results are obtained when these synergists are combined with indole-3-acetic acid (IV) or with 2,4-dichlorophenoxyacetic acid (V).

Normal fatty acids, corresponding to naphthaleneacetic acid in molecular size, are also synergists, although weaker ones. In this series the synergistic action increases if the carboxyl group is displaced towards the centre of the carbon chain, maximal activity being attained with di-*n*-amylacetic acid (VI), which may be considered as an open analogue of III. Its branched-chain isomers are equally active, whereas either lengthening or shortening of the C_5-chains results in a decreased activity (340, 344).

A comparable effect is shown by tri-*n*-propylacetic acid (VII); triallylacetic acid (VIII) is weaker and tri-chloromethylacetic acid (IX) is even less active (342).

All of these synergists have several features in common with the active growth substances, the main difference being that in the synergists the H/L balance, as compared with that of the most active growth substances, has shifted to the lipophilic side.

It seems that, by such modifications of a growth substance, synergists may be obtained rather generally, *e.g.*, 2,4,6-trichlorophenoxyacetic acid (X) and 2,4-dichloronaphthoxyacetic acid (XI) (both inactive as a growth substance) are synergists for naphthaleneacetic acid in the pea test. Furthermore, Thimann (336) showed 9-anthroic acid (XIII) and its 9,10-dihydro derivative to be active in the same way. The two may be considered to be derived from the (primary active) 1-naphthoic acid and its hydrogenated derivative by adding an extra benzene ring. Thimann and Bonner (337) observed that 2,3,5-triiodobenzoic acid (XIII; inactive in the pea test), when combined with low concentrations of indoleacetic acid, enables the latter to bring about a much greater increase in growth. In the pea test, 2,3,5-tribromobenzoic acid is a weaker synergist than its triiodo analogue, whereas the trichloro analogue is only very slightly active (342). Similarly, the lipophilic character of the acids decreases, as is shown by their effects in the beet test (343) and their interactions with the oleate coacervate (343, 344).

With respect to the high synergistic activity of di-*n*-amylacetic acid, which has the structure of a wetting agent, it is interesting to note that several highly-active wetting agents and the amino analogue of di-amylacetic acid (6-amino-undecane) are inactive as synergists. Since the molecular size of the compound and the presence of the carboxyl group as well as its position are apparently of importance, it seems, therefore, that this synergistic action implies more than an unspecific wetting or penetrant action. Very probably the molecules of the synergist "take over" the role of the growth substance at sites other than the primary and where the active compound would otherwise be wasted. In these cases, however, the character of the site of loss *viz.*, the mechanism of the action of the synergist, is unknown.

A synergist of the above-mentioned type will be effective in combination with a weakly-active growth substance if this lowered activity is caused by secondary factors. No effect can be expected, of course, if the low activity is due to "failing" at the site of action. It is interesting, therefore, that in contrast with the strong synergistic effect of di-*n*-amylacetic acid with naphthalene-1- and naphthalene-2-butyric acids, no such effect is observed with the naphthalene-propionic acids. Nor can the low activity of naphthalene-2-acetic acid (XIV) or of naphthoxy-1-acetic acid (XV) be enhanced in this way. Though synergism with another compound, *via* another site of loss, might still be possible, it seems very probable that this absence of effect with di-*n*-amylacetic acid indicates a real (primary) inactivity of these acids. Support for this view has now been obtained in the case of naphthoxy-1-acetic acid, since this compound has been proved to be an anti-auxin (1).

In an investigation on the rooting of petioles of *Ageratum houstonianum* Mill, induced by indoleacetic acid, van Raalte (275) showed indole to be synergistically active.

As indole proved to be inhibitory to an indoleacetic acid oxidase from etiolated pea seedlings (332, 333), it was suggested that the synergistic activity was based on this effect, assuming that an indoleacetic acid oxidase is present in *Ageratum* too. Though this occurrence has still to be proved, the suggestion is a very plausible one, since in the same rooting test indole is not a synergist for naphthaleneacetic acid, which is not affected by the enzyme. So that, in this case, the site of loss, at which the synergist competes with the active compound, is very probably known.

IV. INSECTICIDES

In the field of economic poisons the interest in combinations (discussed by Bliss (41)) has been stimulated to a large extent by the importance of transport factors in many cases. The penetration of an insecticide into the cells will, for instance, be determined mainly by its capacity for permeating through the cuticle. In a study on surface chemistry in relation to biology, the relevant problems were indicated in a general sense by Rideal (286), when discussing the factors which regulate the penetration of a compound into a living cell: "The diffusing molecules must be free to move under the osmotic gradient and for this purpose it is essential that neither their polar heads nor their non-polar chains should be anchored too firmly to immobile groups of the cell wall. It would appear that the function of the so-called adjuvants is, in many cases at least, to be preferentially adsorbed onto, and thus block up, the possible retarding groups".

Hurst (159, 160) analyzed this question, as it applies to insecticides, in great detail. He distinguished between insecticides which regulate their own access to the site of primary action in the cell and those which gain access by means of a carrier, which may itself lack primary activity. Mixtures of ethyl alcohol and octyl alcohol, acting on larvae of *Calliphora erythrocephala*, exhibit a distinct synergistic effect: "Selective access of one compound (ethyl alcohol) into the

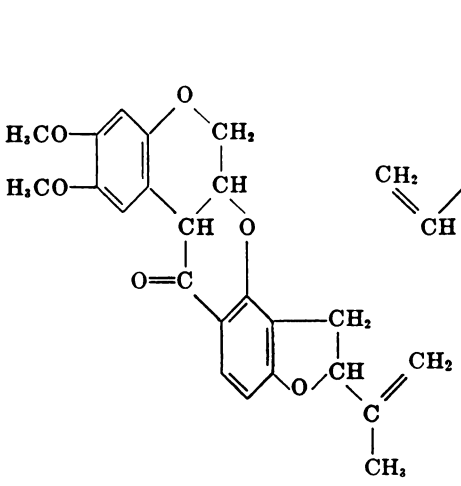
internal biophases of the biological system is induced by selective carrier action of another component (octyl alcohol) at a biophase, the cuticle, which is remote from the ultimate site of drug interaction".

In such effective combinations of insecticide and carrier, the latter often possesses fat solvent property. It will be clear that in this type of synergism (overcoming transport barriers) a close structural analogy between active compound and synergist is not as essential as in the cases where a competition for sites of loss is the underlying principle of the synergistic action.

The latter type of synergism plays an important part in other kinds of insecticide preparations, a possibility already indicated in discussing the formal structural analogy between rotenone (XVI), pyrethrins (XVII) and several highly active, empirically developed synergists which possess no insecticidal activity of their own (340).

These synergists, *e.g.*, piperonyl butoxide (XVIII), piperonyl cyclonene (XIX), propyl isome (XX) and N-isobutylundecylenamide (XXI) (45, 64, 265, 273, 274, 346) were developed after Haller *et al.* (136, 137, 138) had shown that the synergistic effect of sesame oil in combination with pyrethrins and rotenone (100, 101) was also exhibited by pure components of the oil, *e.g.*, sesamin (XXII). Recently Beroza (27, 28, cf. 68) reported that another constituent of the oil, *viz.*, sesamolin (XXIII) is still more effective as a synergist.

The formal arrangements of insecticides and synergists given here are chosen in order to illustrate their structural analogy and represent, of course, but one possible selection from the various spatial forms, in which the compounds may occur. The selection indicates, however, that these insecticides and synergists may take forms which are closely enough related to result in competition for a receptor.



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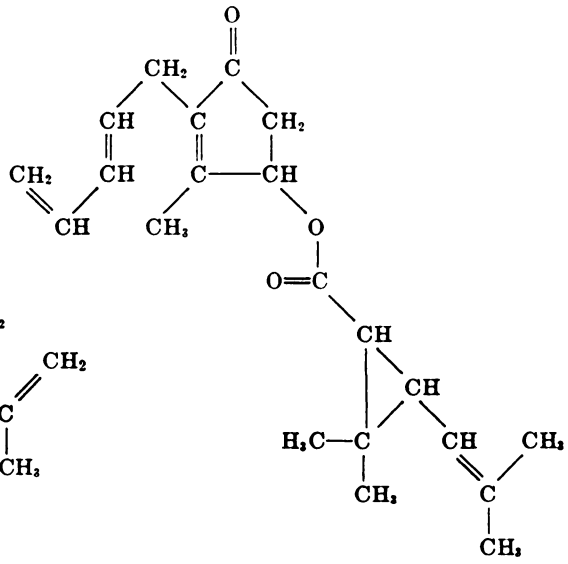

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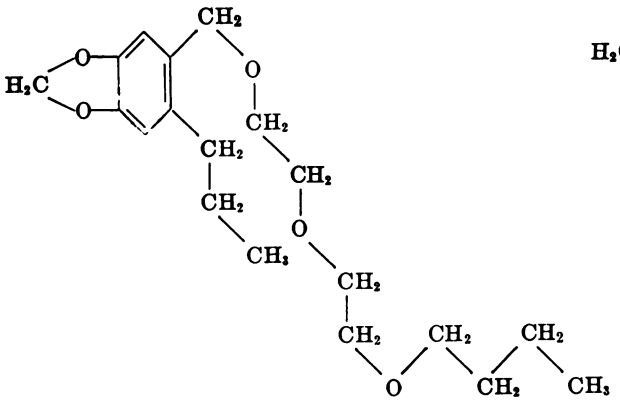

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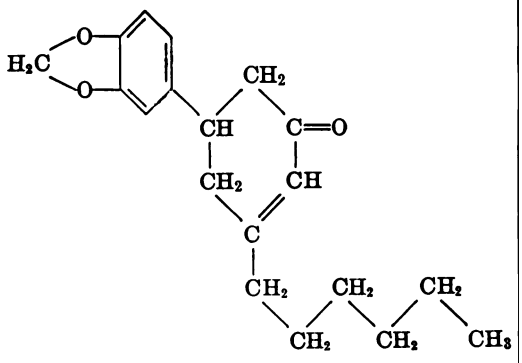

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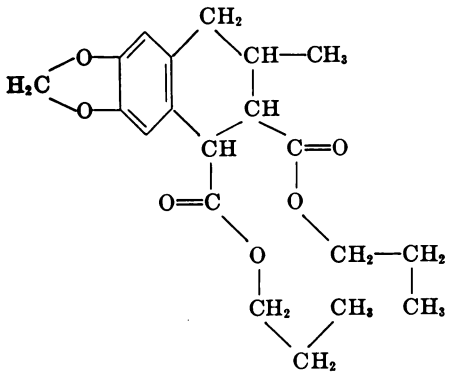

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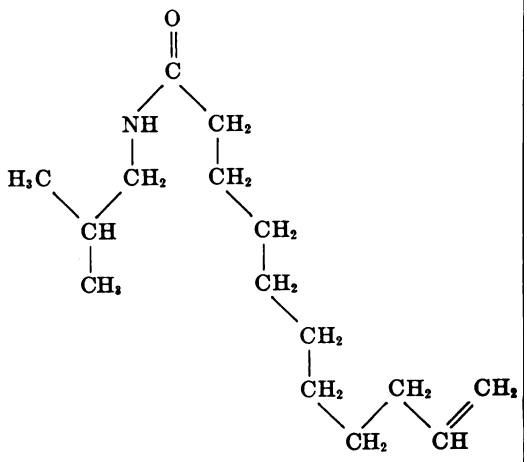

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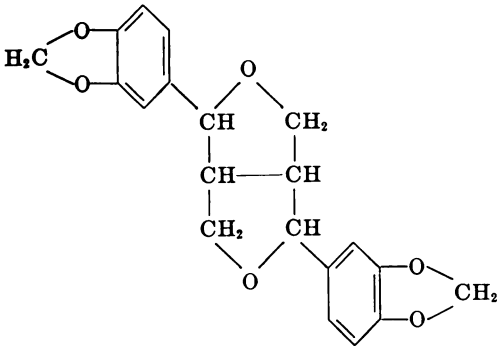

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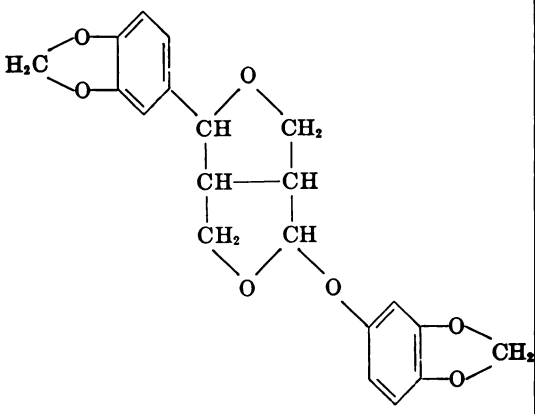

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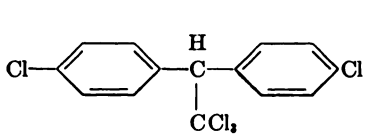

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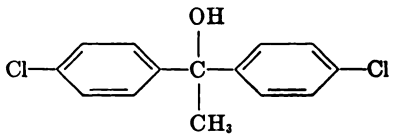

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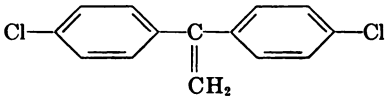

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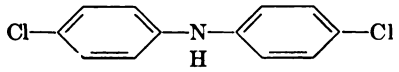

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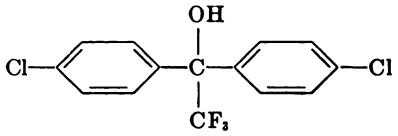

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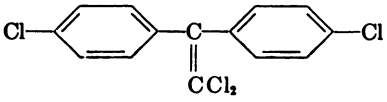

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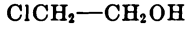

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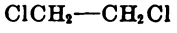


XXXII

Several tentative explanations have been offered with regard to the mode of action of pyrethrin synergists (90, 98, 213, 257, 258), including not only facilitation of the passage through the insect cuticle but also influence on the orientation of the pyrethrin molecules at the nerve sheath interface, which would lead to an enhanced accumulation of the insecticide at the site of action.

The importance of a possible structural analogy, which is not entirely clear in these cases, would be more evident if the views put forward by Wilson (352) and by Chamberlain (70) could be substantiated in detail. Wilson studied the effect of piperonyl butoxide and piperonyl cyclonene, in combination with pyrethrum, on house flies and obtained indications that the synergists interfere with detoxication processes. Chamberlain, in analyzing the mode of action of piperonyl butoxide, showed that the enzyme lipase very probably plays a part in the metabolism of the pyrethrins and that the action of the enzyme (from roach and house fly extracts) is inhibited to some extent by the synergist.

Matsubara (233) studied the detoxication of pyrethrum emulsions by ground tissue of the house fly (tested with mosquito larvae) and found this to be notably reduced by piperonyl butoxide.

More clear-cut results have been obtained in studying synergists for the DDT type (XXIV) of insecticides. The search for synergists has been especially stimulated in this case by the development of resistance towards DDT by the house fly. Accordingly, an attempt was made to determine whether the susceptibility of the resistant forms to the insecticide could be raised to the normal level again.

A large number of compounds were screened for these properties (24, 232, 259, 260, 309, 310, 311, 328, 329), and it was proved that the most effective synergists were to be found among structural analogues of DDT (XXV–XXIX). In this respect it is somewhat surprising that piperonyl cyclonene (XIX), which bears no apparent structural analogy to DDT, also shows synergistic activity with this insecticide, while all other pyrethrin synergists are without effect.

In the DMC (XXV) group, very small variations in the structure cause a strong decrease of synergistic activity, whereas in the diphenylamine derivatives and in the series of sulfonanilides the structural requirements are less stringent. The above-mentioned synergists are active in combination with DDT and related compounds, but not in admixture with other types of insecticides.

A clue to the mechanism of the action of the synergists was obtained by combining the results of different investigations. Sternburg *et al.* (315, *cf.* 354) showed that DDT is metabolized to the practically inactive DDE (XXX) by DDT-resistant flies, whereas the susceptible flies are unable to do so or to a far lesser extent.

Subsequently it was found that piperonyl cyclonene (259, 260, 354, 355) and DMC (261)) inhibit DDE formation in the resistant flies, thereby demonstrating in the latter instance a connection between the synergistic activity and the degree of inhibition. Perry *et al.* (259, 261) and Speroni *et al.* (309, 310, 311) then made the plausible suggestion that the synergists would act by preventing the enzymatic degradation of the insecticide in the resistant insects (135).

Thus, insight into the problem of development of resistance (241), simultaneously provides information about the mode of action of the synergists, implying a competition for the site of loss, which constitutes the detoxifying enzyme system. Though an enzyme may be inhibited by compounds other than substrate analogues (*cf.*, piperonyl cyclonene), the high synergistic effectiveness of structural analogues of the insecticide is comprehensible in this way.

It might also be worthwhile to evaluate the effect of mixtures, *e.g.*, the most active compound of a series combined with a less active or inactive one from the same series, in other groups of insecticides.

With other poisons of economic importance, *e.g.*, fungicides, the application of synergists might, in certain cases, be of equal importance but until now relatively little attention has been paid to possibilities in this field, and material for discussion is hardly available (96, 155).

In another application of chemicals for agricultural purposes, *viz.*, the use of ethylene chlorohydrin (XXXI) to interrupt the rest period of potatoes and thus hasten their germination, the addition of the structurally related, more lipophilic, ethylene dichloride (XXXII), results, according to Denny (93), in a synergism, the mechanism of which is as yet obscure.

V. ACETYLCHOLINE AND RELATED COMPOUNDS

During his investigations on synergism of drugs, Fühner (115), as early as 1918, found that physostigmine (eserine) enormously enhanced the action of acetylcholine on the leech muscle. He suggested that this synergism might be

explained by a protecting action of physostigmine with regard to the decomposition of acetylcholine, the instability of which, especially in alkaline medium, was well-known. Führer stated clearly, however, that the relations might be more complex, since, with the same test object, physostigmine also enhanced the action of the stable barium chloride, but failed to do so with other objects.

Though at present much more detailed information is available about the character and the function of acetylcholine decomposition by enzymes (cholinesterases) and the effects of anticholinesterases on this mechanism (52, 92, 191), the complex situation with respect to synergism in these processes has been unravelled only to a small extent.

Several authors (69, 225, 280, 281) have suggested that the enhancement of the action of acetylcholine and related acylcholines by known cholinesterase inhibitors (*e.g.*, eserine) might be due to a retardation of the enzymatic decomposition. Also with respect to the synergism between acetylcholine and adrenaline in certain cases (63) and that between succinylcholine and choline (255, 256) such a mechanism has been considered.

In a detailed review on adrenaline-acetylcholine relations in the nervous system, clearly showing their complexity, Burn (63) discussed the possibility that adrenaline (or its oxidation products) might possess anticholinesterase activity, as described for the latter by Waelsch and Wackow (242, 347).

Apart from the fact that Ellis (104) was unable to find evidence for cholinesterase inhibition by adrenochrome, any possible activity of adrenaline certainly cannot be of general importance in this respect, since in denervated skeletal muscle, adrenaline also enhances the effect of tetramethylammonium iodide, which is not decomposed enzymatically. Burn (63) suggested that an effect of adrenaline on the permeability of cell membranes towards acetylcholine might play a part in a possible mechanism. Nevertheless, to cite the author: "More facts are needed".

With regard to the effect of choline on the action of succinylcholine, Osterloh (255, 256) arrived at comparable conclusions. For choline also enhances the neuromuscular blocking effect of decamethonium on the indirectly stimulated gastrocnemius muscle of the decapitated cat.

Similar findings have been reported by Zaimis (365) and by Hutter (161) with respect to the enhancement, by eserine and prostigmine, of the action of decamethonium and choline on mammalian muscle. Löw and Tammelin (226) found a strong synergistic effect of tetraethylpyrophosphate (TEPP) on the neuromuscular blocking action of succinylcholine, which could not be explained satisfactorily by an influence on the rate of hydrolysis. Moreover, with succinylcholine, the spontaneous hydrolysis is very probably far more important than the enzymatic hydrolysis. Osterloh supposes that in the synergism between succinylcholine (or decamethonium) and choline a mutual intensification of neuromuscular blocking effects may be of primary importance (*cf.* the antagonism between *d*-tubocurarine and choline (161)).

Though it is conceivable that under simplified experimental conditions a sparing action with respect to acetylcholine, exerted by anticholinesterases, is

mainly responsible for a synergistic effect *in vitro*, in more complex situations *in vivo* an explanation will not be found by focussing attention exclusively on this site of loss. As suggested by Osterloh, interactions at the site of primary action will have to be considered too (191): ". . . potent and specific inhibition of cholinesterase may exert direct actions on effector cells. An inhibitor combines with an enzyme by virtue of an affinity between certain active groups of the enzyme and the inhibitor molecules. Inasmuch as acetylcholine is able to combine with either the enzyme molecule or some "receptor group" of the effector cells, it is likely that enzyme and cell receptor have certain chemical or physical properties in common. It is therefore reasonable to assume that an enzyme inhibitor might also combine with the receptor groups of the effector cells, leading either to the initiation of a response similar to that evoked by acetylcholine or to cholinergic blockade".

Holaday *et al.* (154), studying ganglionic transmission after inhibition by anticholinesterases, concluded that the effect of high doses of di-*iso*propylfluorophosphonate (DFP) is probably due to an action unrelated to its anticholinesterase effect. In their opinion, possible mechanisms include an interference with the responsiveness of ganglion cells.

Cohen *et al.* (76), who analyzed the structure of neuroreceptors in striated muscle and also the relationship between the pharmacological action of neuromuscular drugs and their inhibitory effects on esterases, could differentiate between depolarizing and competitive neuromuscular agents (*e.g.*, decamethonium and gallamine (Flaxedil®), respectively) and ganglion-blocking compounds (pentamethonium) when comparing their interactions with several types of esterases. Concluding from their results, the authors suggest that the pattern of anionic sites on the endplate receptor is reflected in the structure of cholinesterases.

As this mapping of effector site and enzyme surface advances, it may become possible to understand in more detail the combined attack on a complex site of primary action and its consequences with respect to synergistic effects.

In an important contribution to the solution of this problem Cohen and Posthumus (75) studied the influence of pretreatment with anticholinesterases on the effect of neuromuscular agents (using the isolated frog musculus rectus abdominis). They observed an enhanced response not only towards choline esters, but also towards compounds not attacked by true cholinesterase (*e.g.*, butyrylcholine and non-ester compounds like decamethonium and choline). Evidently cholinesterase inhibition cannot be the sole cause of this enhanced response. The authors, with a plausible argumentation, suggest that, after reaction of the receptor with an anticholinesterase, the subsequent interaction of an effector with specific sites (discussed in detail with regard to their possible types) results in an enhanced effect. If their hypothesis can be substantiated, this type of synergism may, perhaps, rightly be designated a true "sensitization" of a complex effector site.

Since the sensitivity of the motor endplate to acetylcholine is increased after

denervation (89), it might be worthwhile to analyze how far the action of certain anticholinesterases may be considered as a "pharmacological denervation." This possibility was discussed by Fleckenstein *et al.* (106, 107) with regard to the action of cocaine on adrenergic systems. (See next section. See also, however, in this connection, a recent paper by Snell and McIntyre (307), who—by histochemical methods—obtained evidence for a gradual reduction in the cholinesterase concentration at the endplate after denervation.)

VI. SYMPATHOMIMETIC AMINES

In 1910 Fröhlich and Loewi (114) found that cocaine, in combination with adrenaline, has a synergistic effect on the pressor and mydriatic actions of the latter. Since then several investigators, in corroborating this finding (*e.g.*, 20) and also analyzing other compounds for a possible synergism with adrenaline and other sympathomimetic amines, have tried to discover the mechanism of the action.

Apart from cocaine and various other local anaesthetics, compounds like hydroxyhydroquinone (17) and ephedrine (116) have also proved to be active in this respect. While, in the case of hydroxyhydroquinone and other reducing substances, it is plausible to assume that the synergistic effect implies a protection, by preventing the chemical oxidation of adrenaline, a different explanation is needed for the synergistic activity of ephedrine, cocaine, and other compounds. Clark (73), in comparing these cases, thought the simplest explanation to be "the assumption that the cell receptor is a complex structure and that cocaine alters it in some manner so that either the rate of association of adrenaline is increased or its rate of dissociation is decreased".

Gaddum's claim, however, that the action of ephedrine has to be ascribed to an inhibitory effect on amine oxidase—oxidizing adrenaline enzymatically (40)—introduced a new line of investigation. McGregor (228), Tripod (339), and Philpot (264) extended Gaddum's hypothesis by relating the action of cocaine and other local anesthetics to amine oxidase inhibition (for amine oxidase and amine metabolism in general, see Blaschko (39)). Bacq (19) has shown, on the basis of detailed studies in which the ephedrine effect is demonstrated to be a direct action and not an indirect one *via* adrenaline, that Gaddum's arguments, particularly with regard to ephedrine, do not stand criticism.

Though the inhibition of adrenaline oxidation by local anesthetics (264) may contribute to their synergistic effect, this cannot be the sole cause, since no parallelism is found between synergism and degree of enzyme inhibition (21). Moreover, cocaine does not enhance the action of either tyramine or phenylethylamine (both very good substrates for amine oxidase), but completely suppresses both of them (18).

Miyake (244) reported that the inactivation of adrenaline by liver extracts in the presence of hemoglobin is certainly inhibited by cocaine, procaine, dibucaine, stovaine, and tutocaine, but that—on account of a number of observations

with different test objects—the synergistic effect *in vivo* cannot be explained exclusively on the basis of a theory of enzyme inhibition.[1]

In two recent papers by Fleckenstein *et al.* (106, 107), this conclusion was underlined again. They also pointed out the fact that (using the cat nictitating membrane) the actions of corbasil and dihydroxyephedrine, neither of which is attacked by amine oxidase, are enhanced even more than that of adrenaline. In their extensive analysis of the action of sympathomimetic amines on the above mentioned test object, the authors found that, in the chronically denervated membrane, the effect of sympathomimetics of the catechol series (*e.g.*, adrenaline) is enhanced (236, 325) in the same way as in the normal membrane under the influence of cocaine. They conclude that, very probably, the action of cocaine must be considered as a "pharmacological denervation".

An important argument in favour of this view is the fact that the action of another group of sympathomimetics (*e.g.*, tyramine, phenylethylamine) is reduced, both by denervation and by administration of cocaine. This would mean that the synergistic effect brought about by cocaine implies an influence on the responsiveness of the effector site. Fleckenstein *et al.* suggest that this may be connected with an annihilation (by denervation, or by administration of cocaine) of the normal accommodation of the nictitating membrane muscle to noradrenaline.

In that case, consideration should be given to the fact that the synergism would occur in a "damaged" system: the synergistic compound (cocaine) reduces the normal supply of noradrenaline, which is restored or replaced in a certain sense by administration of noradrenaline or of related sympathomimetic amines, which are less effective in the normal situation since they are then more or less superfluous.

It seems probable that, with this investigation, Fleckenstein *et al.* have touched upon the essence of the cocaine action, and this type of analysis should be extended also to local anesthetics in general.

In this connection it is very interesting to note that Griesemer *et al.* (131) bearing in mind the well-known and marked inhibition both *in vitro* and *in vivo* of monoamine oxidase by iproniazid (1-isonicotinoyl-2-isopropylhydrazine) (363, 364), found a pronounced synergistic effect of this compound on the action of tyramine and phenylethylamine on the cat nictitating membrane. Since the action of adrenaline is not influenced significantly, this is just the reverse of the relations found with cocaine, and the other substances discussed above.

This finding does not prove that amine oxidase inhibition is the main cause of the synergism, but there are nevertheless several reasons for assuming that it is an important factor in this case. Monoamine oxidase is much more strongly

[1] In a recent communication (Foster, R., Ing, H. R. and Varagic, V.: Alpha Cocaine. Brit. J. Pharmacol. **10**: 436–441, 1955) it is reported that α-cocaine is inhibitory to amine oxidase to the same extent as cocaine, but that this isomer of cocaine is practically inactive as a synergist when combined with adrenaline. These results are clearly incompatible with the view that the synergism under discussion is based on an inhibition of amine oxidase.

inhibited by iproniazid than by isoniazid (isonicotinoylhydrazine) and with the latter no synergism was found. Adrenaline and noradrenaline are rather poor substrates for the enzyme and ephedrine is not attacked: none of these compounds was influenced synergistically by iproniazid (for an antagonistic effect towards adrenaline, see (132)).

Sjoerdsma *et al.* (302) advanced arguments in favour of the theory that monoamine oxidase plays a major part in the metabolism of 5-hydroxytryptamine (serotonin) and they reported a marked prolongation of the serotonin effects *in vivo* (rats) by means of iproniazid.

Though the synergism exhibited by iproniazid in the above-mentioned cases cannot be ascribed exclusively to amine oxidase inhibition, yet it seems a justifiable conclusion that, with tyramine, phenylethylamine and serotonin, amine oxidase activity is an important limiting factor and that inhibitors of this enzyme are potential synergists.

With regard to the effect of adrenochrome on adrenaline action and the mutual influence of chemical mediators (enhancement of adrenaline action on the iris dilatator by acetylcholine) reference is made to Lecomte and Fischer (200) and to Rüegg and Langemann (288).

VII. ANALGESICS

Apart from some isolated observations on enhancement of the analgesic effect of morphine in combination with amphetamine (123) or adrenaline (158), the mechanisms of which have not been analyzed, the interaction between analgesics and parasympathomimetics has attracted considerable attention.

Slaughter *et al.* (303, 304) found that, in the cat, several actions of morphine are influenced synergistically by physostigmine and neostigmine (Prostigmine®), a finding corroborated later in different test objects (72, 108, 361).

Starting from the point of view that a cholinergic mechanism plays an important part in analgesia (analgesics are known to cause parasympathomimetic symptoms and to be inhibitory to cholinesterase *in vitro* (26, 362)), Slaughter *et al.* considered the synergism to be caused by an extra effect of physostigmine, etc. *via* cholinesterase inhibition.

While corroborating the facts, this explanation was questioned by Komlos *et al.*, since they found that the influence of atropine on these synergisms varied with the test object (186, 195). Therefore, a systematic investigation of the effect of parasympathomimetics on the action of analgesics was undertaken. In mice the analgesic effect of morphine and several synthetic analgesics was enhanced when combined with physostigmine, neostigmine, carbamylcholine (carbachol, Doryl®) or pilocarpine, whereas tetraethyl pyrophosphate (TEPP) was not synergistically active (270). Thus compounds not affecting cholinesterase (carbamylcholine, pilocarpine) are highly active synergists, while a strong inhibitor (TEPP) is inactive in this respect. From these results the authors deduce that a common cholinergic mechanism cannot be of decisive importance to the synergism.

By investigating the effect of analgesics (injected subcutaneously in mice

and dogs, in doses of equal analgesic activity) on the cholinesterase activity of the brain, no parallelism was found between analgesic action and degree of enzyme inhibition (188).

Moreover, it was proved that this inhibition was practically the same for morphine alone or in combination with neostigmine (which caused a pronounced synergistic effect). The fact that choline, a weakly active parasympathomimetic is distinctly active as a synergist in combination with analgesics (194), also makes it probable that some properties of the synergists other than their parasympathomimetic activity are of importance.

Knoll and Komlos (187) considered the possibility that the parasympathomimetics might act by a competitive antagonism with respect to molecules of the analgesics adsorbed to "silent receptors" (124), which would render available more molecules for the primary action. Experiments with peptone, based on the observation by Storm van Leeuwen (318) that, *in vitro*, peptone is able to liberate morphine from protein-morphine complexes, showed that—contrary to expectations—the effect of the latter was reduced in combination with analgesics. This reduction could be counteracted, however, by administration of parasympathomimetics, conceivably by a displacement mechanism.

Similar experiments with human serum (189) proved that the analgesics are bound to it and that the degree of this binding can be reduced by, *e.g.*, addition of choline (dialysis experiments). Suramin (Germanin®), known for its affinity to proteins, possesses the same property as choline in this respect and enhances the analgesic action of morphine. Taking these facts into account, the authors assume that the competition at what we have called sites of loss between analgesics and synergists (without analgesic activity) plays a major part in the synergisms described.

Subsequently Pórszasz *et al.* (271), by carrying out an analysis of the effect of physostigmine and carbamylcholine on the distribution of pethidine in intact animals (mice, rats), tried to locate such a competition more exactly. In combination with carbamylcholine, the pethidine level in the brain was enhanced (130), although that in blood and in the liver was not significantly changed.

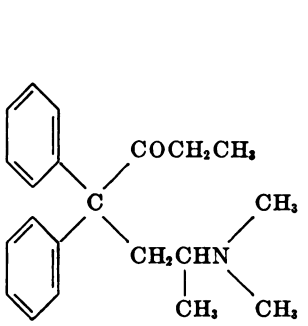
The exact mechanism of the synergism between analgesics and parasympathomimetics has not yet been revealed by these investigations, but important contributions have nevertheless been made to the attainment of that aim.

Mercier *et al.* (238) analyzed the effects of sparteine in combination with various analgesics and established that in mice the action of certain representatives, *e.g.*, morphine, is enhanced, while that of others, *e.g.*, dihydrocodeine, is not influenced at all. A similar differentiation was found with analgesic drugs outside the morphine series: thus benzoxazolone is enhanced in its action, whereas that of antipyrine remains unchanged.

Subsequently, several methonium salts and related compounds were also found to be active as synergists for morphine (240). In all of these cases indications as to the mechanism of action are lacking.

Synergism in combinations of analgesic drugs with various compounds having some structural features in common has been reported. Mercier and Marinacce

(239) found that the antispasmodic diethylaminoethylphenyl-*p*-methoxybenzylacetate (P_4; XXXIV) markedly enhanced the effect of morphine (mice) and, to a lesser extent, that of pethidine.



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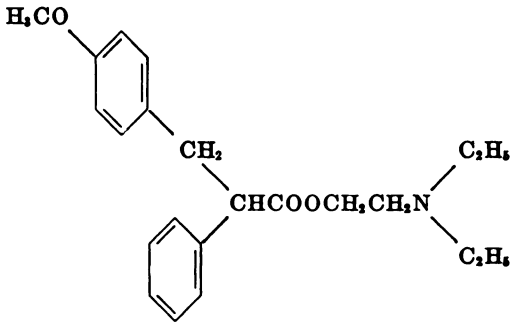
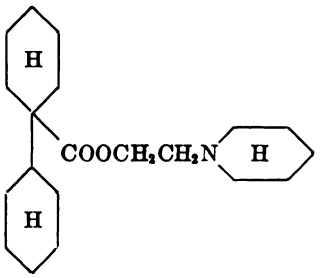
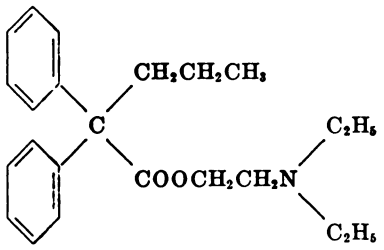


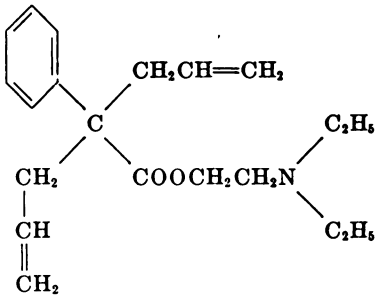
XXXIV



XXXV



XXXVI



XXXVII

Angibeaud *et al.* (9) combined methadone (XXXIII), an analgesic which possesses antispasmodic properties, and morphine, which does not, with the antispasmodic dihexyverine (2-(1-piperidyl)ethyldicyclohexyl-1-carboxylate; XXXV), which lacks analgesic activity. The guiding idea was that methadone suffered a certain loss of analgesic action at sites responsible for the antispasmodic effect. Replacing this latter component of the methadone action by a "pure" antispasmodic might then result in an enhanced analgesic effect. Though the relations are certainly more complex, the results of experiments on mice were not incon-

sistent with the hypothesis, since synergism was found in combination with methadone, but not with morphine.

A large number of papers has appeared on the synergistic action of diethylaminoethyldiphenylpropylacetate (SKF 525A; XXXVI) in combination with a variety of drugs. Cook *et al.* (78, 79) showed that this compound enhanced the analgesic action of morphine, codeine, methadone, meperidine, etc., in rats, without influencing either the LD_{50} (of morphine or meperidine) or the respiratory depressant action (of morphine) in the same animals. This clearly demonstrates the different locations of the morphine molecules in the organism, which can apparently be influenced selectively (see also the "graded" antagonism obtained with morphine antagonists (254, 299)).

The interesting fact that SKF 525A is synergistically active in combination with drugs of very different structures (see also the following sections) prompted Brodie *et al.* (14, 15, 81) to analyze the mechanism of the action and to look for a common factor in the different cases. It was shown that the synergist inhibits the rate of metabolism of, *e.g.*, hexobarbital (83) and pentobarbital and also retards the demethylation of meperidine, aminopyrine and ephedrine (13), thereby prolonging the duration of their actions. It was proved[2] that all the metabolic systems inhibited by the synergist are located in liver microsomes and that they require reduced triphosphopyridine nucleotide (TPNH) and oxygen. The exact location of the inhibitory effect has not as yet been detected. It seems evident, however, that the mechanism of the synergism is an action at sites of loss.

VIII. CENTRAL NERVOUS SYSTEM DEPRESSANTS (HYPNOTICS, NARCOTICS)

Much attention has been paid to the effect of very diverse agents (42, 198, 312, 360) on the intensity and duration of the depression of the central nervous system by barbiturates and allied substances. In many cases a synergism has been observed, but in most instances there is very little knowledge concerning the mechanism by which this is brought about.

For the present, the fact that in different test animals the results are often widely divergent causes difficulties in drawing conclusions of more general value. An analysis of the cause of these variations may ultimately contribute, however, to the elucidation of the mechanism underlying the synergistic effects.

Particularly when the second compound has in itself a depressant action, difficulties in interpretation may easily arise. This is clearly demonstrated by the studies on combinations of barbiturates with alcohol. A number of authors have claimed that synergism occurs (95, 251, 252, 278, 289, 305). In our opinion their conclusions will have to be reevaluated, however, in the light of a more critical analysis by Gruber (133). This author did not find evidence for anything more than an additive effect, when taking into account the differences in character of the depressants (long acting, short acting) and the different methods of administration.

[2] For a review which appeared after the preparation of this paper, see Brodie, B. B.: Pathways of drug metabolism. J. Pharm. Lond. **8**: 1–17, 1956.

In looking for an explanation of the (supposed) synergism, several authors (95, 253, 278) obtained indications that this cannot be based on changes in either the rate of absorption (or elimination) of the drugs, or the distribution of alcohol in the various parts of the central nervous system. On this account, Sandberg (289) considered an increase in the sensitivity of the tissues in the central nervous system to be a plausible cause, but solid arguments to support this contention are so far lacking.

It is known that the antihistamines may cause drowsiness and sedation in patients. Since the evaluation of this property in test animals has sometimes met with difficulties, because stimulation of the central nervous system may interfere, Winter (353) sought to demonstrate the sedative action of antihistamines in animals by analyzing the influence of superimposing an antihistamine upon administration of a drug of known depressant action. It was proved that Tripelennamine Hydrochloride U.S.P. (Pyribenzamine®, 2-[benzyl(2-dimethylaminoethyl)amino]pyridine); Pyrilamine Maleate U.S.P. (Mepyramine Maleate B.P., Neo-Antergan®, 2-[(2-dimethylaminoethyl)(*p*-methoxybenzyl)amino]pyridine); Diphenhydramine Hydrochloride U.S.P. (Benadryl®, 2-(benzohydryloxy)-N,N-dimethylethylamine) and Promethazine Hydrochloride B.P. (Phenergan®, 3277 R.P., N-(β-dimethylamino-α-methylethyl)-phenothiazine) will prolong the sleeping-time produced by hexobarbital in mice, when injected subcutaneously half an hour prior to intraperitoneal injection of the barbiturate.

While Heinrich (143), under comparable conditions, obtained similar results in rats, with combination of hexethal and several antihistamines, Cronheim and Ehrlich (87) found that pretreatment of rats with four tripelennamine analogues did not result in any effect on the action of pentobarbital. This clearly demonstrates the absence of a relation between antihistaminic activity and synergism with barbiturates, a conclusion also arrived at by Ambrus *et al.* (6), who, in mice, corroborated Winter's results, but also found histamine to be synergistically active with hexobarbital (7).

Lightstone and Nelson (211), while confirming the synergistic effect of common antihistamines with pentobarbital in rats, also paid attention to the mode of action. No influence of the antihistamines on the rate of barbiturate detoxication by the liver was found, but in an experiment with Ambodryl (β-(*p*-bromobenzhydryloxy)ethyldimethylamine, the bromoanalogue of Benadryl) and pentobarbital, the barbiturate concentration in the brain was significantly higher than after administration of pentobarbital alone (equalling that of the control at waking). This points to an increased rate of penetration of the barbiturate into the brain under the influence of the antihistamine. In this connection the authors refer to effects of antihistamines on enzyme systems. They are known to inhibit, *e.g.*, pyruvate oxidation in brain tissue (156) and to interfere selectively with glutamate oxidation in brain tissue slices, while not affecting that of glucose (66). It would be most important to analyze how far such activities on the enzyme level may be responsible for the depressant action of the antihistamines and in which way they may influence the distribution of barbiturates.

The combination of barbiturates with analgesics has been investigated to a very limited extent only. Reutner and Gruhzit (284), as well as Cronheim and Ehrlich (86), reported synergism in combinations of barbiturates with methadone in dogs, but data about the mechanism of the effects observed are lacking completely.[3] The same is true for the relatively early observation on the prolongation of barbiturate action in rabbits by sympathicolytic agents (44).

Several reports (67, 118, 126, 127, 172, 357, 358) on an often very strongly enhanced effect of barbiturates in different test animals by means of prior administration of tetraethylthiuram disulfide (TETD) have invited rather extensive discussions concerning its cause. The suggestion by Persky *et al.* (262), that an inhibition of aldehyde dehydrogenase may be involved in barbiturate action, induced Graham *et al.* (126) to consider the inhibitory effect of TETD on this enzyme (185) as the basis of the synergism observed. Giarman *et al.* (118), studying thiopental anesthesia in mice and its prolongation by TETD, ascribed a similar role to the inhibition of xanthine oxidase, inferring that this oxidase has a function in thiopental metabolism. While, so far as we know, this inference has not yet been substantiated, Graham *et al.* (127) expressed some doubt concerning the correctness of their previous opinion, because of the fact that the influence of TETD on barbiturates (*in vivo*) is not affected by ascorbic acid or by reduced glutathione, whereas these compounds reverse the potent inhibitory effect of TETD on the dehydrogenation of alcohol by liver aldehyde dehydrogenase *in vitro* (125).

In trying to analyze the TETD effect, the possibility of a direct action on the brain must also be considered. This was suggested by Winters *et al.* (358) with respect to the synergism between TETD and thiopental in rats. The prolongation by TETD of the depressant effect of barbital, which is not metabolized in the body, may also point to this possibility (356). From the same point of view, Kok and de Jongh (192) studied the effect of TETD on the oxygen consumption of rat brain slices. A saturated solution in medium (0.7 mg %) was without effect, but became depressant after stimulation of the oxygen uptake by addition of 2,4-dinitrophenol. Serum from TETD-treated rats, however, showed no inhibitory properties. The TETD concentration in the brain of such animals being as yet unknown, a definite conclusion concerning a depressant action of TETD on brain respiration *in vivo* cannot be drawn.

With regard to the mechanism of TETD action it must be concluded that, though some leads have been obtained, questions for the moment outnumber convincing answers.

Turning to the effect of compounds which in themselves are devoid of depressant properties, it should be noted that Lamson *et al.* (199) caused sleep to

[3] After completion of this review, a paper by Glassman and Seifter (Glassman, J. M. and Seifter, J.: The effect of analgesic agents on barbiturate responses in mice. J. Pharmacol. **115:** 21–27, 1955) was consulted. This paper describes prolongation of the effects of hexobarbital and pentobarbital in mice by premedication with different analgesics. References to some other papers concerning the enhancement of barbiturate action by morphine are given.

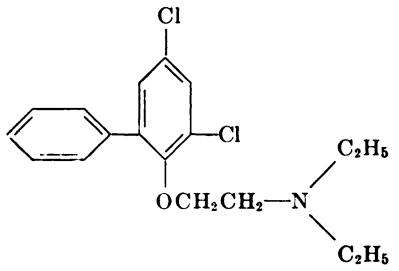
recur in guinea-pigs by intraperitoneal injection of nonhypnotic substances at the moment the animals awoke from barbiturate anesthesia. This effect was reported for glucose and its metabolic products, such as hexose diphosphate, lactate, pyruvate, succinate and fumarate. Malonate was equally active, but sucrose or sodium chloride produced no such effect. The authors interpret these phenomena as a "potentiating effect" on barbiturate anesthesia. Richards *et al.* (285) corroborated these findings and extended this type of experiment by testing a variety of substances unrelated to carbohydrate metabolites. Intraperitoneal injection of a large number of organic and inorganic compounds proved to be effective (guinea-pigs, rabbits). On intracardiac injection, water, blood and polyvinylpyrrolidone had the same effect. No influence on the blood barbiturate level could be detected, but analysis showed that the effect could be brought about at barbiturate levels lower than those found when the animals awoke after the injection of barbiturate alone. Interesting as these effects may be, they very probably belong to a class of synergism different from any so far discussed because of their very unspecific character. No plausible explanation of their mechanism(s) can be offered as yet.

The synergistic effect of SKF 525A (XXXVI) is not restricted to analgesics (see Section VII), since it was also found to be very strong in combination with hexobarbital in mice (80, 338), though not in itself possessing any depressant action. The same result was obtained with other barbiturates and central nervous system depressants (77, 234).[4] The synergist shows some selectivity, since the action of barbital, thiobarbiturates and methylparafynol (3-methyl-1-pentyn-3-ol) is not enhanced.

As with the analgesics, it has been demonstrated (14, 15, 81, 82) that the synergist inhibits the metabolism of barbiturates. An important argument in support of the view that this property of SKF 525A is the main cause of its synergistic effects is found in the absence of synergism with barbital, a barbiturate not metabolized in the organism.

In a recent paper Neubert and Herken (249) reported upon the synergistic

[4] In the meantime it has been shown (Fouts, J. R. and Brodie, B. B.: Inhibition of drug metabolic pathways by the potentiating agent, 2,4-dichloro-6-phenylphenoxyethyldiethylamine. J. Pharmacol. **115:** 68–73, 1955) that the compound indicated in the title:



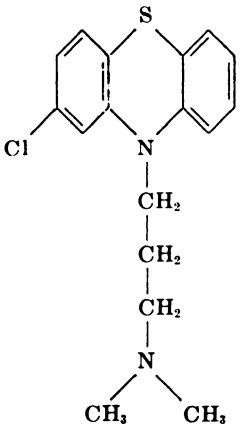
also prolongs the hypnotic activity of hexobarbital in mice by inhibition of its bio-transformation. The compound, though considerably different in structure from SKF 525A, appears to block the same drug enzyme systems.

action of β-diethylaminoethylphenyldiallyl acetate (XXXVII), a compound structurally related to SKF 525A, in combination with hexobarbital. The N-β-diethylaminoethylamide of the parent acid was far less active. This raises the possibility, already considered by Cooper *et al.* (81), that the active agent proper would be the acid, which, *in vivo*, is generally formed more easily from the ester than from the amide. Cooper *et al.* found SKF acid (diphenylpropylacetic acid) and SKF 525A to be equally active on enzyme systems (*in vitro*), the ester being considerably more effective *in vivo*.

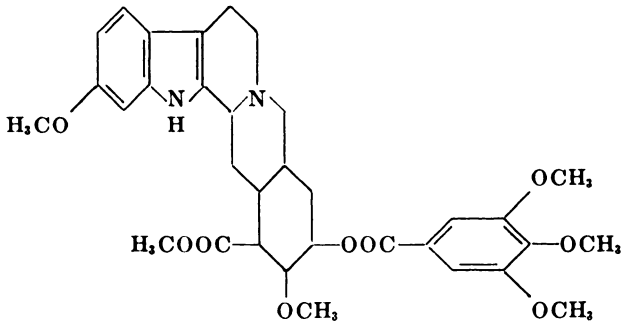
Neubert and Herken (*loc. cit.* 249) found that their synergist was most effective in combination with barbiturates which are metabolized in the organism. This again favours the view expressed above concerning the mechanism of action.

The compound JL1078 (XXXV), the synergistic properties of which in combination with analgesics (9) have already been discussed, proved to be similarly active with a number of barbiturates, though to a lesser extent than SKF 525A (50).

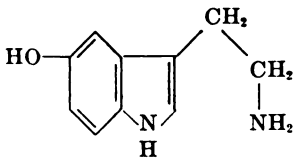
Mirsky and Giarman (243) studied the combinations of thiopental with TETD, SKF 525A and α-tocopherol phosphate (α-TPh), all of which are syner-



XXXVIII



XXXIX



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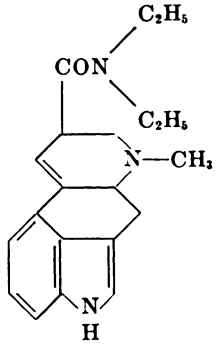


XLI

gistically active. In the first examples the results were sometimes different from those obtained by the authors previously quoted, probably owing to different methods of administration and to differences between the test animals. For α-TPh it was found that the synergist influenced the level of thiopental in the plasma, but not in the fat or the brain. Alpha-TPh being an inhibitor of thiopental oxidation by rat liver slices, it was concluded that the mechanism of the synergistic action is primarily based on the inhibition of barbiturate metabolism.

Though it can be assumed with compounds of the SKF 525A type that the synergism is largely due to an interference with the detoxication of the drugs, the synergism exerted by chlorpromazine (XXXVIII, 85) and by the Rauwolfia alkaloid reserpine (XXXIX) in combination with hypnotics should be explained in a different way, since these compounds do not affect the rate of metabolic transformation of the drugs (48). The concentration of the drugs in the brain is not enhanced under the influence of chlorpromazine or reserpine. The difference from SKF 525A was also shown when animals just awakening from barbiturate anaesthesia were given chlorpromazine or SKF 525A. In the first case sleep recurred immediately, whereas in the second case no effect was observed. Brodie *et al.* concluded that most probably chlorpromazine and reserpine act by increasing the sensitivity of the central nervous system. We do not know the actual meaning of this, however, and a further analysis of this kind of action will be eagerly awaited. Most important in this connection is the finding of Shore *et al.* (300) that 5-hydroxytryptamine (serotonin; XL), as a synergist with hexobarbital, is of the same type as chlorpromazine and reserpine. This synergistic effect can be counteracted by prior administration of lysergic acid diethylamide (LSD; XLI), well-known for the production of mental disturbances. Subsequently it was found that LSD also diminishes the synergism between reserpine and hexobarbital (301). The synergistic action, which serotonin has in common with reserpine and which is antagonized by LSD, suggested to the authors that part of the pharmacological activity of reserpine might be mediated by a serotonin release (266). This hypothesis is supported by the observation that the administration of reserpine to dogs results in an increased excretion of 5-hydroxyindoleacetic acid (a major metabolite of serotonin) in the urine.

(For the synergism between chlorpromazine and *Rauwolfia serpentina* in the treatment of hypertension, see 102).

IX. MISCELLANEOUS COMPOUNDS ACTING ON THE NERVOUS SYSTEM (ANTICONVULSANTS)

The polyvalent nature of the synergistic potency of SKF 525A has already become apparent from the foregoing sections. The compound has proved able to enhance or to prolong the effect of other agents acting on the nervous system.

This was reported, *e.g.*, by Macko *et al.* (230) and by Navis *et al.* (248) for the spinal depressant properties of mephenesin (3-(*o*-toloxy)-1,2-propanediol) and of α-tubocurarine, respectively, and the central stimulant action of strychnine and *d*-amphetamine is likewise enhanced (248).

These findings induced Swinyard *et al.* (331) to study the influence of SKF 525A on anticonvulsant drugs. The effect of seven antiepileptic compounds (three hydantoin derivatives, two barbiturates, 3,5,5-trimethyloxazolidine-2,4-dione and phenylacetylurea) in mice (maximal electroshock seizure test) was compared with that in mice pretreated with SKF 525A. Synergism was observed with all the compounds except phenylacetylurea. Neurotoxicity was also generally enhanced except in the oxazolidinedione.

Since Maas *et al.* (227) had shown that the synergist decreases the rate at which mephenesin is metabolized to *o*-toloxylactic acid, a similar action on the bio-transformation of the anticonvulsants was considered plausible. It has not yet been ascertained, however, to what degree this actually takes place. Swinyard *et al.* obtained several indications that an interference with drug metabolism is not the only factor operating in this synergism. Perhaps the situation is even more complex, since SKF 525A does possess a slight anticonvulsant activity of its own.

Bertrand *et al.* (29) investigated the combination of diphenylhydantoin with chlorpromazine in rats by the same technique as that used by Swinyard *et al.* (331). The protecting action of the anticonvulsants was clearly intensified in the presence of the phenothiazine derivative. As in the other cases where chlorpromazine acts as a synergist, the mechanism of this action is as yet obscure.

X. CHEMOTHERAPEUTIC AGENTS, INCLUDING ANTIBIOTICS AND ANTIMETABOLITES

The combination of other compounds with chemotherapeutic agents has usually been studied not so much with the object of attaining an enhanced action *per se*, as of reducing side effects and overcoming drug resistance. The combination may certainly effect a better result than that obtainable with the single components, but the difference is rather of a qualitative than of a quantitative nature. Further, in the complex (*in vivo*) situation of a diseased organism, where the degree of recovery may be influenced by therapeutic effect on several functions, the attack by a combination of drugs may be preferable because of its polyvalency.

Gibbs (119) alluded to this when distinguishing, apart from the specific synergism relative to a given function (which is the main subject of the present review) "a functional synergism, whereby a multifunctional complex is affected appropriately through the different functions associated" and "a therapeutic synergism, in which the different functions are utilized to focus on a common target".

These latter types of synergism, very important in medicine (263), lend themselves with difficulty to an analysis like that applied in the foregoing sections. For our purpose we shall select only those cases where either relatively less complex situations, or the fact that the investigations were carried out *in vitro*, allow a more quantitative interpretation and/or the opportunity of finding indications concerning the cause of the synergisms observed.

Mixtures of sulphonamides may possess therapeutic advantages because higher doses can be applied without producing crystalluria and also because the

incidence of undesired side effects often appears to be reduced. Proof of a real enhanced activity is lacking as far as we know. Lehr (202, 203) obtained indications of such an effect *in vitro*, with mixtures of sulphathiazole and sulphadiazine on *Streptococcus haemolyticus*, *Staphylococcus aureus* and pneumococci.

Schweinburg and Rutenburg (291) studied fairly extensively the *in vitro* sensitivity of bacteria to mixtures of sulphonamides as compared with the single drugs. Several strains of both gram-positive bacteria, as *Staphylococcus aureus*, and streptococci (hemolytic and non-hemolytic strains) and gram-negative micro-organisms: *Escherichia coli*, *Aerobacter aerogenes*, *Bacillus proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella Friedländeri* and various shigellae, were exposed to sulphadiazine, sulphamerazine, sulphamethazine and sulphathiazole, separately and in binary or ternary mixtures. It was found that in ten out of the seventy strains tested, the activity of the mixture surpassed that of the single drugs. This occurred almost exclusively in the gram-positive group. So far we are completely ignorant about the background of these effects and, in particular, of the difference in reaction between gram-positive and gram-negative micro-organisms, since no analysis of these phenomena has been carried out. This would be of importance, since a real synergism in mixtures of compounds with the same site of action cannot easily be imagined (see II, 1), the more so as in a large number of cases a mixture proved to be less active than one of its components.

Sulphonamides are inactivated *in vivo* by transformation into their N^4-acetyl derivatives and are excreted in the urine in this form, which sometimes causes kidney damage by crystallization. Johnson (170) considered the possibility of reducing the loss of active sulphonamide by an inhibition of the acetylation process. The acetylation of sulphanilamide by pigeon liver extracts was inhibited by a number of amides of aromatic and heterocyclic acids (*e.g.*, 5-bromosalicylamide, salicylic hydrazide, isoniazid). Similar effects have been obtained *in vivo* (rabbits; after an oral dose of sulphanilamide together with *p*-aminosalicylic hydrazide), manifested by a twofold increase in the blood level of free sulphonamide (171). This synergist is thus clearly acting at a site of loss for the active drug. Some of the compounds, most active *in vitro*, failed to show an effect *in vivo*, which may be due to a rapid distribution in the tissues and to a rapid excretion.

With the progress of chemotherapy in tuberculosis (49, 94, 109, 313), during which streptomycin, *p*-aminosalicylic acid, thiosemicarbazones, isoniazid and related drugs were introduced into clinical practice, the use of drug combinations has become routine, the object being to prevent the development of drug resistance (frequently observed with single drugs) as far as possible. The most effective combinations have been selected on an empirical basis, and the question of how resistance is reduced remains completely unanswered. This is not surprising, since the mechanisms of action of the single drugs are still largely unknown. Since these drugs, belonging to different classes of compounds, most probably act along different lines, a combined attack might well give rise to a synergistic effect, but for the present data for discussion are lacking. By focusing attention not exclusively on drug-bacterium but also on host-drug-bacterium relation-

ships, the possible importance of drugs, not tuberculostatic *in vitro* but influencing environmental conditions of the mycobacteria *in vivo*, has been considered. Certain surface-active polyoxyethylene ethers may belong to this group (84, 197), and, with a tuberculostatic drug, a synergism might well occur with respect to the curative effect.

The question whether, in total extracts of cinchona bark (totaquina) or in mixtures of pure alkaloids, a synergism or merely an addition is observed, as compared to the effect of quinine alone, has provoked a lengthy discussion. In our opinion this was ended when Knoppers and Nieuwenhuyse (190), by detailed experiments on bird malaria (canary, *Plasmodium relictum*), arrived at the conclusion that addition does occur. However, a few years later Baranger and Filer (22), also from experiments on bird malaria (*Pl. relictum*, *Pl. gallinaceum*), again concluded that certain mixtures possess greater activity than the sum of the activities of their components. But there is no clear-cut evidence for this view.

According to Greenberg *et al.* (128) a distinct synergism is found when Chlorguanide Hydrochloride U.S.P. (Proguanil Hydrochloride B.P., Paludrine®; 1-(*p*-chlorophenyl)-5-isopropylbiguanide) is acting together with sulphadiazine on avian malaria (chick, *Pl. gallinaceum*). Complete protection was obtained by combining $\frac{1}{4}$ and $\frac{1}{32}$ to $\frac{1}{64}$ of the effective protective doses of chlorguanide and sulphadiazine, respectively. Blood concentrations of the drugs offered no explanation for the synergistic effect. It is probable that the enhanced activity is due to the fact that the components are antagonists of different intermediates in a biosynthetic chain essential to the plasmodium (see the discussion on antimetabolites at the end of this section[5]).

Since primaquine (8-(4′-amino-1′-methylbutylamino)-6-methoxyquinoline), though active against primary and secondary exoerythrocytic forms of *Pl. vivax*, is somewhat deficient in its activity against the asexual blood forms (trophozoites), Alving *et al.* (5) were led to the simultaneous administration of a suppressive drug, such as quinine or chloroquine (4-(4′-diethylamino-1′-methylbutylamino)-7-chloroquinoline) in the treatment of clinical attacks of mosquito-induced vivax malaria.

On comparing the effect of different schedules of drug administration, they obtained convincing evidence of a synergistic effect of both quinine and chloroquine on the curative action of primaquine. In such a complicated situation the search for the mechanism of this effect will certainly be difficult.

In order to simplify this type of analysis, we have tried to find a synergist for quinine among some derivatives of cinchona alkaloids which, in themselves, are devoid of antimalarial activity. For that purpose the 9-chloro-9-desoxy derivatives of quinine, quinidine, cinchonine and cinchonidine were chosen (74, 173, 272) as analogues of a more lipophilic structure. In a series of unpublished experiments on chick malaria (*Pl. gallinaceum*), carried out by de Jongh *et al.*, incompletely protecting doses of quinine were combined with increasing doses of the

[5] See also in this respect a recent communication (Joyner, L. P. and Kendall, S. B.: Synergism in the chemotherapy of *Eimeria tenella*. Nature, Lond. **176**: 975, 1955), describing a strong synergistic effect in the combination of Daraprim (pyrimethamine; 2,4-diamino-5-chlorophenyl-6-ethylpyrimidine) and sulphamezathine.

inactive chlorodesoxy derivatives. The derivatives of quinidine and cinchonine proved to be practically inactive as synergists, whereas those of quinine and cinchonidine enhanced the effect of underdosage of quinine, chlorodesoxycinchonidine being the most active synergist. The following data from one of the experiments with this compound may be illustrative. The percentages of infected erythrocytes in the control, with synergist alone (100 mg/kg) and with quinine alone (10 mg/kg) were 82, 81 and 58, respectively. If the synergist was added to quinine (10 mg/kg) in amounts of 20, 50 or 100 mg/kg the percentage of 58 was reduced to 17, 10 or 5.

It should be noted that the configuration of the synergistically active compounds is the same as that of quinine, while the inactive derivatives belong to a different steric series. This points to the importance of the spatial form of the molecule and is probably indicative of a displacement mechanism, the site of which, possibly the site of loss for quinine, still remains to be determined.

As soon as different types of clinically useful antibiotics had become available, studies on combined antibiotic action, both *in vitro* and *in vivo*, were started. Jawetz *et al.* (134, 166–169, see also 8, 293, 294, 295) analyzed a large number of combinations in different micro-organisms and from their work it is clear that a certain drug pair cannot generally be termed synergistic or antagonistic, since the results vary with the micro-organism tested. Some classification proved possible, however, since within the bactericidal group of penicillin, streptomycin, bacitracin and neomycin, mixtures often resulted in synergism, whereas with the bacteriostatic chloramphenicol, aureomycin and terramycin they gave addition only. When combining an antibiotic of the first group with one of the second, the results depended on the sensitivity of the test bacteria to the drug of the first group. The rate of bactericidal action with synergistic combinations was generally found to be higher than with one of the components, provided that the antibiotics were, in fact, administered simultaneously. Until now, the work in this interesting field has been largely descriptive, and the synergism has in no case found an explanation. Interesting facts await further analysis, *e.g.*, the finding of Barr *et al.* (23) that tyrothricin and carbomycin display a synergism against gram-negative organisms, while apparently being antagonistic when applied to the gram-positive group. Leonardi and Campus (204, 205, 206) observed a sort of synergism *in vitro* between streptomycin and sulphathiazole against *E. coli* in the presence of serum. The antibiotic action appears to be reduced by the serum, and this effect can be counteracted by simultaneous or previous addition of the sulphonamide. The same result is obtained with sodium benzoate and penicillin. According to the authors, this synergism cannot be ascribed to a possible antibacterial action of sulphonamide or benzoate, but is caused most probably by liberation of the antibiotic from an inactive serum-antibiotic complex (193).

While the studies on synergism in connection with chemotherapeutic agents and antibiotics, discussed above, were merely empirical, the search for synergistically active combinations of antimetabolites, especially that by Hitchings *et al.* (103, 152, 153), has been guided by knowledge concerning their modes of action.

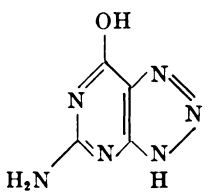
In the biosynthetic chains leading to purines and polynucleotides (nucleic

acids), *p*-aminobenzoic acid, folic acid, citrovorum factor, pyrimidine and purine bases function in sequence. Since antagonists to these intermediates are known (sulphonamides, analogues of folic acid and purines) Hitchings *et al.* used these compounds in a systematic study of multiple biochemical blocking. It could be envisaged that the action of a certain antimetabolite would render the organism more susceptible to antimetabolites acting at subsequent loci in the biosynthetic chain leading to an essential metabolite.

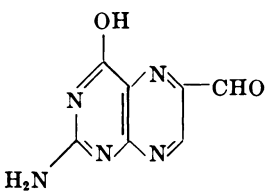
It was found that in growth inhibition of *Lactobacillus casei*, combinations of pyrimethamine (5-*p*-chlorophenyl-2,4-diamino-6-ethylpyrimidine) with its 3′,4′-dichlorophenyl analogue (compounds with identical site of action) resulted in an additive effect only, but that the combination of pyrimethamine and 6-mercaptopurine (acting at different loci) showed a strong synergism. With *Streptococcus faecalis* likewise a strong synergism was observed in all combinations of antifolic acids with antithymines (*e.g.*, 6-azathymine), whereas with antipurines (8-azaguanine) synergism was either weak or absent. With *Lactobacillus casei*, 6-mercaptopurine in combination with 2,6-diaminopurine showed interference, the inhibition being the same as that obtained with the weaker component.

Though interpretation of the interactions observed cannot be straightforward in all cases, in a large number of them synergism is "a regular and predictable event" (103), and where the results are unexpected, they may give leads to better understanding of the connection between metabolites. In the combinations of sulphonamides and diaminopyrimidines synergism has also been observed *in vivo*. Eyles and Coleman (105) found that in a mixture of sulphadiazine and pyrimethamine, acting against experimental toxoplasmosis in the mouse, the amounts of the drugs could be reduced to $\frac{1}{8}$ and $\frac{1}{24}$, respectively, of their median effective doses, when acting alone. Greenberg and Richeson (129) got comparable results when testing compounds for synergism with sulphadiazine as an antimalarial against *Pl. gallinaceum* in the chick. 2,4-Diamino-5-(*p*-chlorophenoxy)-pyrimidine and its 6-methyl derivative proved to be active in this respect. The effective doses of the diaminopyrimidines and of the sulphonamide could be reduced to $\frac{1}{4}$ and $\frac{1}{16}$, respectively, in the mixture.

Synergism within this group of compounds must sometimes be explained in a different way, as has been shown by Shapiro *et al.* (297, 298). The carcinostatic action of 8-azaguanine (XLII) was enhanced by folic acid, which in itself was inactive against the mouse breast carcinoma studied. When it was found (151, 287) that, in mammalian tissues, 8-azaguanine is enzymatically deaminated to 8-azaxanthine (inactive as a carcinostatic agent), Shapiro and Gellhorn (297)



XLII



XLIII

considered the possibility that folic acid or its degradation product (2-amino-4-hydroxy-6-formylpteridine (XLIII)) might inhibit the deamination of azaguanine *in vivo*. Subsequently it was shown (298) that the formylpteridine is inhibitory to the deamination of 8-azaguanine by tumor extracts. Furthermore, it was found that the non-carcinostatic pteridine acts as a synergist with 8-azaguanine against a mammary adenocarcinoma, and it is plausible to assume, together with the authors, that this effect is due to the inhibitory action of the synergist.

XI. VITAMINS AND HORMONES

During somewhat extensive studies on interrelations of vitamins, synergisms have been observed in a relatively large number of cases. This is not surprising because a functional coupling often occurs, *e.g.*, vitamins of the B-complex as coenzymes in multienzyme systems, and because, in principle, the possibilities of co-operation are similar to those discussed for antimetabolites in the foregoing section, but in a positive sense for the biological function.

In several other instances the synergism appeared to be based on stabilization of one vitamin by another, the latter acting, *e.g.*, as an antioxidant. Thus vitamin E in various kinds of experiments proved to protect vitamin A, resulting in an increased storage of vitamin A in the liver of the test animals (16, 246), an increased growth promoting effect of vitamin A (140, 148, 149), or a retarded depletion of its reserves in the liver under certain experimental conditions (91). It will be clear that this type of synergist need not necessarily possess vitamin character.

We shall refrain from discussing the papers separately, as the material has been reviewed by Moore (247) and by Lecoq *et al.* (201). However, interesting as the subject may be, only limited attention has been paid to possibilities of synergistic effects between the structurally related D vitamins (324). Some data about the E vitamins (α-, β-, and γ-tocopherol) have been reported by Hickman and Harris (147).

For various steroid hormones, acting in sequence with respect to a certain function, synergism of the type indicated above for vitamins, has been noticed (196, p. 462). The interaction between androgens and oestrogens may also imply a synergistic action, as has been shown by Ito *et al.* (165), when taking the weight of seminal vesicles, anterior prostate or intramedial lobe (hamster) as a measure.

In view of the close structural relationship between certain androgens and oestrogens, the former being the more lipophilic, it might be possible that in some actions of oestrogens, addition of androgens would result in a synergistic effect on the basis of competition at sites of loss, but so far as we know an analysis from this point of view has not as yet been carried out. It has been reported that (unsaturated) fatty acid fractions may enhance the effect of androgens (97, 164); their mode of action remains to be investigated.

The interaction of corticoid hormones and sex hormones has been studied in a number of tests. Taubenhaus (334) found that, with respect to the inhibitory action on granulation tissue, cortisone and either oestrone or testosterone act synergistically.

XII. MITOTIC POISONS

In the elaborate investigations by Lettré *et al.* on the antimitotic action of various compounds in fibroblast cultures (from mesenchymal tissue of chick embryo), colchicine and its derivatives have been studied most comprehensively, together with other agents. In a number of these combinations the antimitotic effect of colchicine was considerably enhanced.

The synergists belong to very different classes of compounds and may be either weakly active or inactive as mitotic poisons. Narcotine (a weak mitotic poison) and antimitotically inactive, structurally related members of the aporphine group (*e.g.*, bulbocapnine) enhance the effect of threshold doses of colchicine. Phlorhizin, a phosphatase inhibitor, is even able to activate a dose of colchicine which has in itself no effect. Considering not only that analogies exist between muscle contraction and contraction of cell elements (occurring during mitosis), but also that adenosine triphosphate (ATP) counteracts the colchicine effect, Lettré favours the view that these synergists (many of them muscle poisons) act either by a direct effect on contractile systems in the cell or by affecting processes leading to formation of ATP.

Several steroid hormones (such as oestrone, testosterone, progesterone, cortisone) and some structural analogues of corticoid hormones are likewise active as synergists. Indications are that their synergistic activity is not directly dependent on the structural features decisive for the hormonal action.

Local anaesthetics such as xylocaine and procaine were proved to enhance the effect of a threshold dosage of colchicine, the former being by far the more active. In these cases no connection with the mechanism of contraction is known and the way in which hormones and local anaesthetics act here is so far completely obscure.

However, different as these synergists may be, there is some analogy in their molecular patterns and the localization of certain groups therein. It would seem worthwhile, therefore, to investigate the influence of the synergists on colchicine in simplified model systems, which might give information as to possible displacement mechanisms.

The relevant papers do not need separate reference, as they have been summarized in a recent review by Lettré (207).

XIII. SYNERGISM BY REVERSIBLE INHIBITION OF EXCRETION MECHANISMS

Those of the synergisms so far discussed which could be explained—at least in part—on the basis of a competition at sites of loss, have been either of type A or of type B (Figure 1).

The finest illustration of type C, in which an excretion mechanism represents the site of loss, is to be found in the impressive series of investigations by Beyer *et al.*, on the selective inhibition of renal tubular excretion.

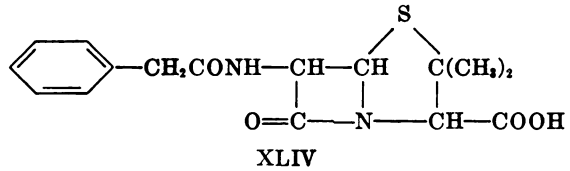
When penicillin was introduced in therapy, consideration was soon given to the question of reducing the somewhat high rate of excretion (which takes place mainly by way of the renal tubules) of the antibiotic, in order to maintain the

desired blood level for a longer time after an injection of a given amount of the drug.

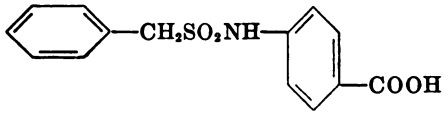
Rammelkamp and Bradley (277) achieved this aim by administration of diodrast (diethanolamine salt of 3,5-di-iodo-4-pyridone-N-acetic acid), while Beyer *et al.* (38) obtained the same results with the aid of *p*-aminohippurate. Both compounds are excreted by the same tubular mechanism and at a comparable rate and therefore, when present with penicillin, they compete with the drug for the excretion mechanism and thus retard its elimination (33).

Para-aminohippurate has been applied successfully in a few cases in clinical practice (12, 216, 217), but the very large amounts required hampered a more general introduction.

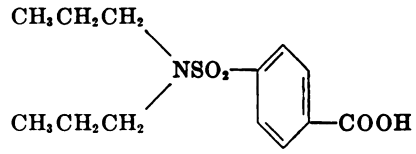
For this reason Beyer *et al.* sought to inhibit the penicillin-excreting transport mechanism reversibly and selectively, by means of a compound refractory to excretion by this mechanism. By way of lucid deductions, starting from a plausible concept on the enzymatic nature of metabolic processes, the requirements for such a selective inhibitor were set forth, and they could be met in 4′-carboxyphenylmethanesulfonanilide (carinamide, XLV) (30, 34, 35). Together with penicillin this compound almost completely suppresses the tubular excretion of the antibiotic, thus limiting excretion to glomerular filtration, which represents about one fifth of the normal rate (88, 157).



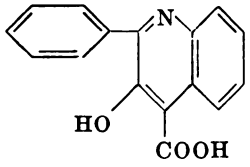
XLIV



XLV



XLVI



XLVII

Though there is a strong temptation to refer more fully to the brilliant work of Beyer *et al.* in this field (for a review see 31), comments must be limited to the results of direct importance to the present discussion.

Of a series of N-alkylsulfamylbenzoic acids, *p*-(di-*n*-propylsulfamyl)-benzoic acid (probenecid, Benemid®, XLVI) (37) proved to be still more effective as a

synergist for penicillin than carinamide (345). (With the aid of the whole series, Beyer (32) illustrated most instructively which factors are basic to the development of useful inhibitors of renal transport mechanisms.)

When comparing the structures of penicillin (G, XLIV) and that of the most effective synergists, it is suggestive that a certain structural analogy, though admittedly a remote one, may be of importance in this respect. In this connection it is interesting that 2-phenyl-3-hydroxycinchoninic acid (XLVII) has about the same activity as carinamide in sustaining higher blood levels of penicillin (36, 366).

If a competition for some specific enzymatic component of an excretion mechanism really is the underlying principle of the synergisms observed, structural analogy may well be of importance. It might be worthwhile to investigate whether further generally useful, selective synergists of this type may be obtained by starting from the structure of compounds, the excretion of which it is required to restrict, provided that sufficient information is available about the excretion process itself.

XIV. STABILIZATION OF AN ACTIVE COMPOUND TO BIO-TRANSFORMATION BY STRUCTURAL MODIFICATIONS ("AUTOSYNERGISM")

In a number of cases we have seen that synergistic action consists in an inhibitory effect on the metabolism of the active compound. The synergist, as it were, effects an external stabilization of the compound. It is conceivable that such a protection might also be obtained by structural modifications of the drug, causing little or no impairment of the primary action, but rendering the substance less liable to chemical or enzymatic degradation (internal stabilization). In comparison with the original active compound, the modified drug would then possess a "built-in" synergist and the effect might be described as "autosynergism".

Though in this picture the relations are certainly over-simplified, since it would often be very difficult to distinguish between an effect of structural modifications on the activity itself as distinct from an effect on the stability, possibilities such as these are nevertheless worth bearing in mind.

Investigations along these lines have been carried out, especially with local anaesthetics, having regard to the high and prolonged anaesthetic activity of xylocaine (2,6-dimethyl-N-diethylaminoacetanilide). In this series of diethylaminoacetanilides, which are attacked *in vivo* at the —NH—CO— bond (235, 335), the above-mentioned effect may also be operative. This appears from the fact that, starting from diethylaminoacetanilide, the stepwise introduction of ortho methyl substituents increases both the degree and the duration of the anaesthetic action and decreases the rate of hydrolysis (292).

Dvoretzky and Richter (99), in attempting to prolong the relatively short duration of benzocaine action, achieved their aim with the ethyl and *n*-propyl esters of 2,6-dimethyl-4-aminobenzoic acid.

Childress *et al.* (71) reported β-diethylaminoethyl-2,6-dichloro-4-aminobenzoate to possess an intradermal local anaesthetic activity three and one half times higher than that of the unsubstituted procaine.

Rabjohn *et al.* (276) found that, of a series of β-diethylaminoethyl esters of benzoic acid sterically hindered by alkyl substituents in the nucleus, β-diethylaminoethyl-2,3,5,6-tetramethylbenzoate in particular would act over a much longer period than procaine. (For a systematic study, with a large number of compounds, on substitutions increasing the stability of esters and amides in human serum see (208) and (122).)

Since we are dealing mainly in this section with local anaesthetics, a few data on synergism with these drugs in mixtures may be added. Higuchi and Lachman (150) showed that the rate of hydrolysis of benzocaine in aqueous solution is markedly decreased in the presence of caffeine, with which the anaesthetic forms a complex. Analysis proved that the ester, in the form of the complex, is not hydrolyzed to a measurable extent. Though its value *in vivo* still has to be determined, synergistic effects based on protection in complexes might also occur in other cases.

Serembe (296) pointed out the incompatibility of procaine and chloramphenicol: the toxicity of the anaesthetic in humans is strongly enhanced by the antibiotic, so that convulsions may result. There are indications that the metabolic transformation of procaine is inhibited, since, in the presence of chloramphenicol the blood level is raised and more unchanged procaine is found in the urine. The concentration in the brain is also increased.

In view of older work of Stender and Amsler (314), who found that analgesics will enhance the effect of local anaesthetics (183, 349), Wiedling (351) investigated the influence of methadone on xylocaine action. When applied prior to injection of the anaesthetic, the action of the latter was prolonged; intravenous injection of methadone at the moment when xylocaine action ended re-established local anaesthesia. The mechanism of this synergism has not yet been investigated, but it must be remembered that analgesics may display some local anaesthetic effect of their own.

Purposeful modification of the structure of a compound, in order to arrive at a greater stability under *in vivo* conditions, has been mainly limited to the group of local anaesthetics.

In some respects the work on acetylcholine analogues has had the same tendency, *e.g.*, the clinically used acetyl-β-methylcholine and carbamylcholine are hydrolyzed enzymatically at a much lower rate than acetylcholine. Attention was directed, however, predominantly towards a study of substrate specificity of cholinesterase (3, 4, 121, 290).

It has been reported that the biological activities of cortisone and hydrocortisone are strongly enhanced by introduction of either a halogen atom (especially fluorine) into the 9 α-position (112, 113, 117, 210, 330) or a double bond into the 1,2-position (51, 111, 145). Particularly in the case of the 9 α-fluoro compounds one is inclined to ask whether the enhanced activity may be brought about, at least in part, by a lesser degree of biotransformation, the more so since these substances are active on oral administration. Although a few objections may be raised to such an interpretation, since the sodium-retaining activity seems to be increased more than the glucocorticoid activity, a comparative analysis of the metabolism of the various corticoids is nevertheless desirable.

XV. EPILOGUE

When surveying the extensive material discussed in the foregoing sections, it must be admitted that a great number of questions with respect to synergism cannot be answered satisfactorily at the moment, either because the relations are too complex to be unravelled by present methods of analysis or because a purposeful analysis has not yet been carried out.

On the other hand, a relatively large proportion of the cases could 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 may be identified as loci of unspecific adsorption (storage at "silent receptors"), as enzyme surfaces functioning in the metabolism or in the detoxication of the agent, or as excretion mechanisms. In our opinion, this type of synergist constitutes a rather neglected counterpart (or even, a special form) of the amply investigated antimetabolites. In both cases the action is based on an (physico-chemically speaking) identical competition with the active compound, resulting in different effects because of different sites of action. While the analogues, termed antimetabolites, act mainly at the sites of primary action (thus causing an antagonism), the competition with the synergists takes place at sites where the primary active compound is lost or wasted, causing an over-all synergistic effect with respect to the measured end-effect.

In antimetabolites a structural analogy with the metabolites is generally compulsory, since the interaction with the site of primary action is specific. Metabolite analogues, however, are not necessarily antimetabolites in this sense, as structural variations may result in a compound, the affinity of which to the site of primary action is less than that to other "receptors", onto which part of the active compound may also be adsorbed. It is in this group of analogues that synergists may be found. Though structural analogy may thus be of importance, it is not a *conditio sine qua non*, since, *e.g.*, an enzyme, transforming the drug may also be inhibited by compounds other than substrate analogues.

A systematic exploration of the possibilities of finding synergists to active compounds can therefore be made along two different lines. First, they may be sought among structural analogues not antagonizing the primary action. Such investigations would, however, be largely "trial and error" (just as with the antimetabolites), though indications are that analogues with an enhanced lipophilic character may offer a better chance. In this connection it would be worthwhile, when testing series of related compounds for pharmacological activity, not to select exclusively the most active members. Before rejecting less active or inactive compounds, it would be desirable to analyze, with mixtures of underdosages of a highly active compound and supplementary quantities of a weakly active or inactive analogue, how far the same effects can be attained as with a larger amount of the highly active substance alone.

A second approach may be found by studying the mechanisms by which the active compound is metabolized (detoxified) in, or excreted from, the system. Inhibitors for these mechanisms, which may or may not be structural analogues of the agent, are potential synergists.

Synergism may also occur when the components of a mixture are acting "in series" towards the same end-effect, *e.g.*, when antimetabolites are competing with different intermediates in a biosynthetic chain (X, see also II, 2). It appears that synergisms of pharmacologically active compounds with the same type of action also belong to this class.

Though synergistic effects in general cannot be explained solely by "a competition at sites of loss", we hold the view that this mechanism is at the root of a large number of these effects and that this concept may prove a useful tool in future investigations.

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