Antispirochetal Interference between Antibiotics and Arsenoxide

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IT HAS BEEN REPORTED RECENTLY that BAL (dimercaptopropanol) inhibits the antispirochetal effect of penicillin, bacitracin, and chloromycetin as it does that of the metal-containing (As, Au) compounds, whereas streptomycin, aureomycin, terramycin, and subtilin are not inhibited by the dithiol. This BAL reversal of antiprotozoan action is a specific one, inasmuch as the antibacterial action is conserved and seems to be related to the chemoreceptor mechanism (*1*). Exploring further the mechanism of antispirochetal action, we have studied the effect of combined treatment with known antiprotozoan agents.

Therapeutic experiments were carried out on mice infected (20 hours before) with an African strain of *Borrelia duttoni*,[1] against which the effective (subcutaneous) doses[2] for each drug investigated had been accurately determined (*2*). Characteristic details of the combination experiments appear in Tables 1–3. It was found that the antispirochetal activity of arsenoxide was additively or synergistically enhanced by combined treatment with the antibiotics streptomycin, penicillin, or bacitracin: the same result was obtained with arsenoxide and myochrysin. In contrast, simultaneous treatment with arsenoxide and the antibiotics terramycin, aureomycin, or chloromycetin both decreased the immediate action of the antibiotic and the delayed one of arsenoxide. This phenomenon of interference with arsenoxide activity appeared with effective, as well as ineffective, doses of the antibiotics. For example, 1.5 mg/kg terramycin—a dose that does not inhibit the rapid increase of the spirochetes—interfered noticeably with the effect of 5 mg/kg arsenoxide (which, given by itself, clears the blood stream of parasites within 24 hours). On the other hand, the interference effect disappeared if the dose of either arsenoxide or the antibiotic was increased sufficiently above the therapeutic range. Effective doses (5–10 mg/kg) of arsenoxide, for example, inhibited the immediate effect (3–5 hours) of 4 mg/kg terramycin, but not that of 8 mg/kg. Conversely, 2–4 mg/kg terramycin or aureomycin inhibited the delayed action (22–45 hours) of 5 mg/kg arsenoxide, but not that of 10–20 mg/kg. It should be mentioned that, as a result of interference with the early action of the antibiotic, the *immediate* antispirochetal effect of the latter combinations was lower than that corresponding to antibiotic treatment alone.

TABLE 1

THERAPEUTIC INTERFERENCE BETWEEN TERRAMYCIN AND ARSENOXIDE IN THE *Borrelia duttoni* INFECTION OF MICE
(Expt No. 110)

Treatment	No. spirochetes in 25 dark fields (Hours after treatment)				
	0	3	5	22	44
None	37	120	200	500	1000
	50	125	200	450	900
	25	120	225	550	750
Arsenoxide, 5 mg/kg	50	75	25	0	0
	40	40	10	0	0
	25	65	12	0	0
Aureomycin, 4 mg/kg	35	8	1	0	0
	50	4	0	0	0
	40	6	0	0	0
	25	0	0	0	0
Arsenoxide, 5 mg/kg + aureomycin, 4 mg/kg	40	50	85	4	3
	40	40	75	80	1
	50	40	75	10	0
Arsenoxide, 10 mg/kg + aureomycin, 4 mg/kg	40	40	50	0	0
	40	25	40	0	0
	35	20	12	0	0
	25	20	10	0	0
	35	22	20	0	0
Arsenoxide, 20 mg/kg + aureomycin, 4 mg/kg	40	12	4	0	0
	40	1	2	0	0
	40	15	2	0	0
	35	25	1	0	0
	50	20	35	0	0

Chloromycetin, aureomycin, and terramycin, characterized by a similar wide antibiotic spectrum, interfere with the antispirochetal action of arsenoxide. The similarity of these antibiotics as antibacterial, antiprotozoan, and antirickettsial agents, and as producers of cross-resistant strains (*3–5*), finds analogy in their common ability to interfere with the antispirochetal activity of arsenoxide. From the majority of the re-

[1] Strain received in 1949 from the Pasteur Institute, Paris.

[2] The minimal *clearing* and *reducing* doses—i.e., the doses giving total disappearance, respectively 80–95% reduction of the circulating spirochetes, within 3–5 hours—have been determined for the subcutaneous method of administration. The reduction of the spirochetemic curve appears with greater delay, but it is more persistent in the case of arsenoxide and streptomycin than in that of the other antibiotics. Arsenoxide doses of 2.5–5.0 mg/kg give 80–95% reduction in less than 22 (and more than 6) hours; to obtain clearing within 5–6 hours, arsenoxide doses of 20 mg/kg are required. The drop in the spirochete count appears more promptly, although with a greater tendency to rise again, with the other antibiotics. The subcutaneous reducing doses were the following: terramycin, aureomycin, 2–4 mg/kg; bacitracin, 7 mg/kg; subtilin, 8 mg/kg; penicillin, 8–10 mg/kg; chloromycetin, 35 mg/kg; streptomycin, 75 mg/kg. The clearing doses were 50–100% higher.

TABLE 2

THERAPEUTIC INTERFERENCE BETWEEN TERRAMYCIN AND ARSENOXIDE IN THE *Borrelia duttoni* INFECTION OF MICE

Treatment	No. spirochetes in 25 dark fields (Hours after treatment)				
	0	3	5	22	44
Expt No. 108					
Terramycin, 4 mg/kg	25	3	3	20	380
	35	10	7	50	500
	35	18	10	35	500
Arsenoxide, 5 mg/kg	35	35	20	0	0
	50	50	22	0	0
	35	75	15	0	0
Terramycin, 4 mg/kg + arsenoxide, 5 mg/kg	35	25	20	18	1
	35	35	18	6	0
	35	35	70	20	25
Expt No. 131					
Terramycin, 1.5 mg/kg	25	75	60		8
	25	75	125		12
Terramycin, 3 mg/kg	50	2		0	3
	25	4		0	0
Arsenoxide, 5 mg/kg	25	80		0	0
	35	80		0	0
	50	60		1	0
Terramycin, 1.5 mg/kg + arsenoxide, 5 mg/kg	25	60		15	0
	60	75		3	25
	35	50		5	5

ports (*5–10*) it would seem that they also share the ability to interfere with the antibacterial action of penicillin and streptomycin.

Of the three antibiotics interfering with arsenoxide, only chloromycetin is inhibited, as are penicillin, bacitricin, and arsenoxide itself, by BAL in regard to its antispirochetal effectiveness. On the basis of the assumption that BAL-reversibility is related to the action on chemoreceptors (*1*), it can be concluded that the antibiotic interference with arsenoxide is not bound to this action.

In all our experiments the interference phenomenon appeared as a decrease of activity of both drugs used for combined treatment (antibiotic and arsenoxide). The phenomenon is mostly manifest at *minimal effective dose* ranges (or even below) and resembles a decrease of dosage in all its manifestations (reduction of the parasite count, relapses).

To interpret these findings, we arrived at the following working hypothesis, which seems to agree with the known facts and the present observations. *Therapeutic interference* is the result of a reciprocal competition between effective drugs in an *elective* process of fixation-penetration on or in the microorganism. This competition—which presumes a similar elective mechanism of fixation-penetration—reduces the concentration of the competing drugs below the levels required to affect the receptors to the point of measurable therapeutic response. The competition in the process of fixation-penetration can take place between drugs acting on identical or on different receptors—i.e., through the same or through a different type of biochemical lesion.

1) The strongest supporting evidence for this thesis lies in Jancsó's experiments: pretreatment with pararosaniline protects trypanosomes against lethal photosensitization to acriflavine, as a result of decreased fixation of the latter drug (*11*). Earlier, Hirschfelder and Wright (*12*) attributed to "a simple surface reaction" the antagonism between acriflavine and triphenylmethane dyes on the inhibition of CO_2 production by yeast cells. This effect was obtained by staining the cells with ineffective methyl violet or brilliant green doses and exposing them to acriflavine, or vice versa, after exposing them first to ineffective acriflavine concentrations and then to the other dye. In our opinion, other evidence can be seen in the "separated interference" experiments of Schnitzer (*13*): trypanosomes treated with pararosaniline in one host, after passage to another (mouse), were not affected by therapeutic acriflavine doses.

2) The existence of an optimal dose for interfering effect established in 1927 (*14*) for pararosaniline/acriflavine and pararosaniline/arsphenamine, since confirmed in a number of other cases, shows that the competition can be overcome by an excess of one of the therapeutic agents. In the examples presented above, the immediate effect of the antibiotic, as well as the delayed action of the arsenical, was re-established by

TABLE 3

THERAPEUTIC INTERFERENCE BETWEEN CHLOROMYCETIN AND ARSENOXIDE IN THE *Borrelia duttoni* INFECTION OF MICE (Expts 107 and 108)

Treatment	No. spirochetes in 25 dark fields (Hours after treatment)				
	0	3	5	22	44
Chloromycetin, 15 mg/kg	50	35	75	350	+++
	50	50	40	40	+++
	25	40	40	500	+++
Chloromycetin, 60 mg/kg	40	0	0	0	0
	25	0	0	0	0
	25	1	0	0	0
Arsenoxide, 5 mg/kg	50	50	22	0	0
	35	50	20	0	0
	35	60	20	0	0
Arsenoxide, 10 mg/kg	35	25	10	0	0
	50	35	6	0	0
	35	35	6	0	0
Chloromycetin, 15 mg/kg + arsenoxide, 5 mg/kg	25	35	20	2	15
	35	25	22	20	20
	25	25	25	12	25
Chloromycetin, 60 mg/kg + arsenoxide, 5 mg/kg	25	5	5	2	35
	35	12	8	4	50
	25	10	6	3	35
Chloromycetin, 60 mg/kg + arsenoxide, 10 mg/kg	35	5	3	2	2
	35	6	8	4	0*
	25	10	5	1	1

* Relapsed within 24 hr.

increasing the dosage of one or the other drug. These observations prove that the receptors of the cell did not lose their sensitivity to the therapeutic action of the interfering drugs; provided the drug arrives in sufficient quantity a therapeutic effect will take place.

3) Fractions of the minimal dose of terramycin, aureomycin, and chloromycetin are sufficient to interfere with the therapeutic effectiveness of arsenoxide. This is another fact confirming the view that interference is independent of a cellular lesion (and of the receptors). Therapeutically ineffective doses of one drug are sufficient to compete with the cellular fixation mechanism of the other drug to an extent that reduces its uptake below effective levels.

4) The possibility that chemoreceptors influence this phenomenon is minimized or excluded by other facts which demonstrate that the interfering effect is independent of therapeutic action. The fundamental observation that led to interference studies was made with an inactive drug. Morgenroth and Rosenthal (*15*) reported in 1911 that the trypanocidal activity of antimonyltartrate was inhibited by hexatantalate, an ineffective compound that could, nevertheless, induce drug resistance to antimonials. The inhibiting effect of the inactive hexatantalate was obtained later for certain arsenicals (e.g., arsphenamine), but not for others (arsenoxide) (*16*). Interference between two *active* drugs was observed first by Browning and Gulbransen in 1922 (*17*) with pararosaniline/acriflavine and a trypanosome strain that was resistant to pararosaniline itself. Pararosaniline-resistant strains showed the same interference phenomenon with pararosaniline/arsphenamine and pararosaniline/arsecatine (*16, 18*) as did normal strains. Thus the sensitivity of the microorganism to the therapeutic effect of the drug is not a determining factor in the interference phenomenon. In fact, Hasskó (*19*) quantitatively demonstrated that certain therapeutically ineffective triphenylmethane dyes decrease the fixation of acriflavine in the trypanosomes.

In relation to these observations we investigated whether penicillin, aureomycin, terramycin, and chloromycetin—which are inactive in the *Trypanosoma equiperdum* infection of mice—have any influence on the trypanocidal activity of arsenoxide. No measurable effect was found. We also established that chloromycetin, aureomycin, and terramycin do not influence the host toxicity of arsenoxide.

5) The characteristic time/effect curves of the interfering drugs in our study permitted the demonstration of the *reciprocal* nature of the interference between active drugs. This reciprocal effect is most suggestive of a competitive mechanism. It is difficult, in general, to attribute therapeutic effect to one or the other of the interfering drugs. We are, however, under the impression that the reciprocity of the interference is much more frequent than has been demonstrable so far; and, consequently, we consider that the positions of the "interfering" and the "interfered" drugs may be only relative, depending on the particular conditions of the experiment.

6) The possibility that the competitive process takes place at the level of the receptors (i.e., that it should be biochemical in nature) rather than in the fixation process is considered unlikely—a view substantiated not only by the facts outlined but by logic. (*a*) If two active drugs are acting on the same sensitive receptors (i.e., by identical biochemical lesion), it is not conceivable that the displacement of one active drug by another should result in a lower therapeutic effect, except in the case of extreme quantitative differences of activity, which in the examples studied do not occur. (*b*) If the two drugs are acting on different receptors, the effect of the lesions determined by one drug could not influence those attributable to the other in any way except synergistically. An interesting example is the interference described for arsenoxide and aureomycin (or terramycin), in view of the fact that, in accordance with their dithiol reactivity, these drugs are assumed to have different receptors.

7) We assume that the underlying cause for competitive fixation is an analogous absorption-fixation pattern of the interfering drugs. Hypothetically this might depend on a similar mechanism of cellular uptake (e.g., analogous binding forces), or on the fixation of the two drugs by the same specialized zones of the cellular surface. It would be difficult to imagine the number of possibilities which a polyphase structure such as that of the protoplasm might possess as far as mechanisms of fixations are concerned, considering that even the surface of homogeneous materials presents differentiated patches of adsorptive power in relation to various substances (*20*). Quastel and Wooldridge (*21*), studying the effect of various chemicals on resting *Bacillus coli,* a quarter of a century ago, expressed the view that the cell surface is composed of active centers "made up of a number of groupings each of which plays its part in determining the access of a substrate to the centre."

Without the concept of a differentiated and elective cellular absorption (fixation-penetration), it would be extremely difficult to explain why interference does not occur each time two active drugs, each of which is no doubt becoming bound to the microorganism, are used.

Possibly it is in relation to an analogous mechanism of uptake that the three antibiotics which interfere with arsenoxide (and possess similar spectra) are the same antibiotics which exert—like arsenoxide itself—a high *in vitro* action against the spirochetes (*2*).

Therapeutic interference (which is the name given to the phenomenon by Browning and Gulbransen) can take place between an antibiotic and a totally different type of compound (arsenoxide), as well as between two antibiotics (*6–10*). Also, in bacterial infection, we can cite a case of interference between an antibiotic and a metallic compound: gold-sodium-thiomalate interferes with the therapeutic activity of subtilin in the hemolytic streptococcus infection of mice, as noted by one of us in collaboration with B. S. Schwartz in 1949 (*22*).

These findings seem to indicate that antibiotics pos-

sess the same aptitude as other drugs to enter into antagonistic pairs. No doubt the "antibiotic antagonism" is only a particular example in the broader field of the therapeutic interference, and it could be examined in this light. It is claimed (*23*) that the interference of chloromycetin with the antibacterial action of penicillin is related to the bactericidal function of the latter antibiotic, since interference appeared only in this early phase of action; further, that the bactericidal action of penicillin is not exerted on the microorganism in a state of bacteriostasis determined by the interfering antibiotics (*9*). In view of the fact that in all cases of interference there is an optimal time element—as established first in 1911 (*15*)—which may be related to the time period required for fixation, it remains questionable whether the conditions described as the cause of the interference between two antibiotics are not rather incidental.

We realize, of course, that the assumption of a differentiated process of fixation does not simplify the question of the mechanism of antiprotozoan action; in fact, for its understanding, new questions have to be answered (the mode and factors responsible for it, grouping of drugs from standpoint of fixation, etc.). No single mechanism of action is conceivable, however, that could account for the complex and highly specific manifestations of antiprotozoan action, such as the selective action of chemically similar drugs, or the different dispersion of antimicrobial spectra. These could be better understood as being linked to a number of superimposed mechanisms which may vary to different extents from one to another drug/microorganism system.

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News and Notes

Centennial Convention, A.Ph.A.

MORE than 1500 pharmacists from all parts of the world converged on Philadelphia Aug. 17–22 for the 1952 convention of the American Pharmaceutical Association, which was observing its 100th anniversary. The association was founded in 1852 by 24 progressive pharmacists; today, a hundred years later, it lists a membership of over 25,000, from all branches of pharmacy.

The convention officially opened Aug. 17 with an address by Clarence E. Pickett, honorary secretary of the American Friends Service Committee. A symphony concert by 45 musicians, recruited largely from the Philadelphia Orchestra, was a part of the opening exercises. The concert was under the direction of Norman Black. Hugh C. Muldoon, dean of the College of Pharmacy, Duquesne University, Pittsburgh, and chairman of the Committee on the Centennial Celebration, presided at this program, and Don E. Francke, president of the A.Ph.A., gave a short address of welcome before Dr. Pickett spoke.

In his address Dr. Pickett urged Americans to have increased interest in the peoples of other nations, and to accept the humanitarian responsibilities forced upon America as the leading world power. He further stated that if people at large would apply the same integrity to their lives and their understanding of world conditions as the pharmacist and the scientist apply in their everyday work, the world would be far better for all.

The business sessions of the convention began on Aug. 18 with the first meeting of the House of Delegates and the first general session. The House which, as the association's governing body, is made up of delegates from all branches of pharmacy, including teaching, manufacturing, wholesaling, retailing, law enforcement, and research, heard committee reports, named committees, and considered organizational plans. At the first general session, welcoming addresses by the local committee were followed by a tribute to the past presidents of the association by Robert L. Swain, editor of *Drug Topics*, and a past president of the A.Ph.A., who spoke on "The A.Ph.A.