THE BACTERIOSTATIC ACTION OF BASIC DI- AND TRIPHENYLMETHANE DERIVATIVES

PART I. THE INHIBITION OF BACTERIOSTATIC ACTION BY INACTIVE COMPOUNDS OF SIMILAR STRUCTURE

BY E. FISCHER, M. GENSELOVICH and P. RONA

From The Research Department of Szabó Hnos., Kessler & Cía., Buenos Aires, Argentina

Received November 26, 1951

DURING our earlier experimental work on the bacteriostatic activity of certain di- and triphenylmethane dyes[1,2,3,4] we have observed some facts suggesting that, among methane carbon atom derivatives of these dyes, only those with a "potential quinoid structure" were able to inhibit the growth of *Staphylococci* and *Streptococci*. We have noted[1,2] that the leucobases (R_3CH), the leucoamines ($R_3C{\cdot}NH_2$) and the leucocyanides ($R_3C{\cdot}CN$) of basic triphenylmethane dyes were practically inactive, while the corresponding dye cation ($R_2C = R$), carbinol bases ($R_3C{\cdot}OH$), leucosulphonic (bisulphite) and leucosulphinic (hydrosulphite) derivatives ($R_3C{\cdot}SO_3Na$, $R_3C{\cdot}SO_2Na$) exerted a strong bacteriostatic effect of equal degree. It is a well known fact that carbinol bases possess a tautomeric quinoid ionised form whose cation appears in their dye salts. Bisulphite and hydrosulphite derivatives, for their part, are unstable compounds, transformed more or less rapidly, in contact with air, into coloured dye salts (vat dyes).

Still more conclusive have been the results of experiments with diphenylmethane derivatives.[3,4] The bacteriostatic activity of these is far weaker than that of the triphenylmethane homologues, but the stability of their methane carbon atom derivatives allows a more clear cut separation between "essentially non-quinoid" and "potentially quinoid" structures. The non-quinoid compounds R_2CH_2 (bis[dimethylaminophenyl]methane) and $R_2CH{\cdot}NH_2$ (leuco-auramine) were practically negative, whereas both the compounds R_2CHOH (the "potentially quinoid" Michler's hydrol) and $R{\cdot}C(NH_2){=}R$ (the quinoid auramine dye) showed bacteriostatic effects of the same degree. The sulphonic derivative ($R_2CH{\cdot}SO_3H$), which is, contrasting with its unstable triphenylmethane homologues, a stable non-quinoid compound, did not exert, correspondingly, any noticeable bacteriostatic activity. The non-quinoid Michler's ketone ($R_2C = O$) was also inactive.

The most important common feature of active di- and triphenylmethane derivatives, which distinguish them from inactive compounds of similar structure, is constituted in our opinion by their "potentially quinoid" character. An actually quinoid structure, however, cannot be looked upon as a requisite for the bacteriostatic action, since quinoid dye cations and actually non-quinoid carbinol bases have a quantitatively equal effect. Especially noteworthy is, from this point of view, the similarity of the bacteriostatic action of Michler's hydrol, non-quinoid at the *p*H of the bacteriological medium (7·0) and that of auramine, quinoid cation at the

same *p*H. Marini-Bettòlo,[7] confirming the comparable antimicrobic efficacy of triphenylmethane dyes and the corresponding carbinol bases, considers their higher state of oxidation responsible for this phenomenon. We should emphasise that the circumstance that both dyes, cation and carbinol basis, are oxidised compounds, does not account for their similar bacteriostatic efficiency, as Michler's ketone, a still more highly oxidised derivative, is inactive. It would be necessary to admit that a degree of oxidation represented by both the quinoid dye cation and the carbinol basis is needed for the efficiency. We mention in this connection, that Ingraham[5] tried to explain the antimicrobic activity of triphenylmethane dyes by postulating a balancing effect on the oxidation-reduction potential (*cf.* our discussion of Ingraham's work[6]).

Our experimental results, on the inhibition of the bacteriostatic effect of triphenylmethane dyes by their leucobases,[3,4] are also connected with this problem. The demonstration of such an inhibition acquires special importance in view of the well known theory of Fildes and Woods (competition with essential metabolites), applied with great success to explain the antimicrobic effects of sulphanilamides and other substances. Northey,[8] referring to our work, remarks that "there are cases of antagonism between similarly constituted chemotherapeutic drugs where it is difficult to picture the antagonist as being essential to the metabolism of the parasite."

It seemed advisable, therefore, for us to reinvestigate the antagonism between active and inactive derivaties in a more quantitative manner. As shown in a previous paper,[2] experiments with triphenylmethane leucobases are technically difficult because their very low solubility in water does not allow quantitative measurements.

In the hope of encountering more favourable conditions among diphenylmethane dyes, we have investigated the leucobases of both the bis-4:4′-(dimethylaminophenyl)methane and bis-4:4′-(aminophenyl)-methane series. The very slightly water-soluble leucobases of the former series, i.e., tetramethyl-*pp*′-diaminodiphenylmethane and leucoauramine, did not offer any advantage over leucomalachite green and leucomethyl violet. We had more success with the corresponding member of the second series, *pp*′-diaminodiphenylmethane, a fairly water-soluble compound, which had no bacteriostatic effect on *Staphylococcus aureus* in concentrations as high as 1 : 5000 (0·02 per cent.), while a 0·0025 to 0·005 per cent. concentration prevented the growth inhibition of a 0·005 per cent. concentration of Michler's hydrol. To counteract the effect of a 0·0025 per cent. concentration of the latter, a 0·0025 to 0·00125 per cent. concentration of diaminodiphenylmethane was required (see Table I). We deduce from these results, that one molecule of the reduced substance is sufficient to suppress the action of one or two molecules of the oxidised compound. The antagonistic action of diaminodiphenylmethane is not limited only to diphenylmethane derivatives, for 3000 molecules of it inhibited also the action of one molecule of methyl violet, a triphenylmethane dye of high bacteriostatic efficiency (1 : 8,000,000) (see Table II).

The antagonism between the reduced and oxidised forms may, perhaps,

TABLE I

GROWTH OF *Staphylococcus aureus* IN BROTH

Michler's hydrol alone		Michler's hydrol and					
		DIAMINODIPHENYLMETHANE			DIAMINOBENZOPHENONE		
Dilution of Michler's hydrol		1:20,000	1:40,000	1:80,000	1:20,000	1:40,000	1:80,000
1:20,000	−	+	±	−	+	−	−
1:40,000	−	+	+	±	+	+	±
1:80,000	+	+	+	+	+	+	+
0	+	+	+	+	+	+	+

\+ Normal growth in 24 hours
− No growth in 24 hours
± Growth in some experiments and no growth in others

TABLE II

GROWTH OF *Staphylococcus aureus* IN BROTH

Methyl violet alone		Methyl violet and	
		DIAMINODIPHENYLMETHANE	DIAMINOBENZOPHENONE
Dilution of methyl violet		1:5,000	1:5,000
1:2,000,000	−	−	−
1:4,000,000	−	−	−
1:8,000,000	−	+	+
1:16,000,000	+	+	+
0	+	+	+

suggest that these exert opposite influences on the oxidation-reduction potential. Nevertheless, such a possibility has been excluded by subsequent experiments realised with *pp'*-diaminobenzophenone, a non-methylated homologue of Michler's ketone. This substance is a highly oxidised non-quinoid derivative of the diaminodiphenylmethane series that has been reported as slightly active by Auhagen[9] and by Kuhn, Möller and Beinert[10] against *Streptobacterium plantarum*. This activity corresponded to the sulphanylamide type, having been opposed by a 1:2000 concentration of *p*-aminobenzoic acid. Jensen and Schmith[11] did not observe, however, any bacteriostatic effect of this substance on *Pneumococcus*. In our own experiments with *Staphylococcus aureus*, diaminobenzophenone did not exert a growth-inhibiting action in concentrations as high as 1:5000 (0·02 per cent.), behaving, in this respect, exactly like diaminodiphenylmethane. Applied simultaneously with Michler's hydrol and with methyl violet, diaminobenzophenone showed, furthermore, an antagonistic effect of the same magnitude as diaminodiphenylmethane, one molecule inhibiting the effect of 1 or 2 molecules of the hydrol, and 3000 molecules inhibiting the effect of one molecule of methyl violet (Tables I-II).

These results confirm our working hypothesis, according to which the bacteriostatic effect of basic di- and triphenylmethane derivatives is linked in some way to their "potentially quinoid structure," being the essentially non-quinoid substances of the same series—independently of their lower or higher state of oxidation—not only non-bacteriostatic, but even inhibitory for the bacteriostatic effects of active "potentially quinoid"

derivatives. It is difficult to consider this kind of antagonism as an example of competition with essential metabolites and it does not seem to have anything to do with opposing effects of reduced and oxidised substances on oxidation-reduction potential. We think it more likely that the non-toxic derivatives block the way for the penetration of the chemically related toxic agents to the susceptible parts of the cell. The toxicity of di- and triphenylmethane derivatives for bacterial cells is probably connected with their specific chemical configuration and not with the degree of their basicity or state of oxidation.

REFERENCES

1. Fischer, Hoffmann and Prado, *Science*, 1944, **100**, 576.
2. Fischer, Hoffmann, Prado and Boné, *J. Bact.*, 1944, **48**, 439.
3. Fischer, Garcés and López, *Science*, 1945, **102**, 507.
4. Fischer, Garcés and López, *J. Bact.*, 1946, **51**, 1.
5. Ingraham, *J. Bact.*, 1933, **26**, 573.
6. Fischer and Muñòz, *J. Bact.*, 1947, **53**, 381.
7. Marini-Bettòlo, *Ist. botan. univ. lab. crittogam.* (*Pavia*), 1947, **3**, 265.
8. Northey, *The Sulfonamides and Allied Compounds*, Reinhold, 1948, 1st ed., 492.
9. Auhagen, *Z. physiol. Chem.*, 1942, **274**, 48.
10. Kuhn, Möller and Beinert, *Ber. dtsch. chem. Ges.*, 1942, **75B**, 711.
11. Jensen and Schmith, *Z. Immunitätsf.*, 1942, **102**, 261.