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# LXXVI. BACTERICIDAL ACTION OF SOME ORGANIC COMPOUNDS OF MERCURY.

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Though many organic compounds of mercury have been prepared, especially during the last few years, and much laboratory and clinical work has been done in ascertaining the possibilities of their application as medicaments, there is as yet a scarcity of systematic information regarding the bactericidal action of such substances, although such knowledge must form the basis on which the use of these products in medicine depends.

The most important work of this kind done so far is that of Schoeller and Schrauth. They showed [1910] that, beginning with a simple compound such as hydroxymercuribenzoic acid HO—Hg—$C_6H_4$.COOH, there was little change in bactericidal activity when HO— was replaced by bromine or chlorine, a marked diminution on replacement by iodine or cyanogen, still greater reduction on replacement by sulphur, and that activity almost vanished when HO— was replaced by a second benzoyl radical as in mercuridibenzoic acid.

In a second paper [1912] they showed that by the insertion of other substituents in hydroxymercuribenzoic acid the bactericidal action could be markedly influenced. Thus it was increased by halogen or alkyl groups or by a second hydroxymercuri residue and diminished by hydroxyl, sulphonic acid, or amino groups. These observations relate for the most part to new substituents in the *ortho*-position to the carboxyl group, but some evidence was obtained that the orientation of substituents also affected the intensity of bactericidal action, *e.g.* in $C_6H_3$(COONa)(Hg.OH)($CH_3O$) the three substituents in positions 1:2:4 were less efficient than in positions 1:2:6. Two species were used in these experiments, viz. *B. anthracis* and *Staphylococcus pyogenes aureus*. There was little evidence of any specificity in action, but it must be remembered in this connection that all the compounds were not tried on each species.

In a third paper [1916] the same authors dealt with the influence of the same substituents in the absence of the carboxyl group, the latter being replaced by hydroxyl. Here bactericidal action was intensified to start with, the carboxyl group being a weakening factor, but the influence of the substituents already referred to remained the same so far as it was tried, with the

addition of the nitro-group to the augmenting series. The three mercurated isomeric cresols arranged themselves in these trials in the following order of diminishing activity: *meta*, *ortho*, *para*.

Reference may also be made to Miss de Witt's work [1921] which relates mainly to the possibility of using organic compounds of mercury in tuberculosis, and covers a large number of substances ranging from mercuric chloride to complex products including chloromercuri derivatives of dyes such as methylene blue, trypan blue, methylene green, etc. The results are expressed in terms of mercury required to inhibit the growth of human tubercle bacilli on melted nutrient agar; and from the present point of view they are chiefly of interest in showing the variation in the amount of mercury (g. per litre) required for this purpose, depending on the organic or inorganic residue with which it is associated, the least effective being mercuric chloride (0·093) and the most active, acetoxymercuriallyl alcohol, $HO.CH_2CH : CH.HgO.Ac$ (0·012), chloromercurimethylene blue (0·014) and chloromercurifluorescein (0·016).

There are numerous other papers on the subject but the foregoing account is sufficient to show the general position.

It occurred to us that valuable information might be obtained by starting with a comparatively simple substance, mercurating it and a suitable selection of its derivatives and determining the bactericidal action of the resulting mercury compounds, keeping throughout to strictly comparable conditions, and using for comparison certain standard mercury compounds, which are already in more or less general use in medicine.

Of the numerous possible starting points for such an investigation we selected benzaldehyde mainly because, when we began this work, no mercurated derivatives of aromatic aldehydes had been made. The substances so far prepared have been described and their constitution discussed elsewhere [Henry and Sharp, 1922, 1924], and in the present paper only their bactericidal action need be discussed. For the elucidation of certain points it proved desirable to make a number of other organic mercury compounds, for example, those of the alkyl phenols, from which we hoped to obtain information as to the effect of replacing the aldehyde group by alkyl groups. Further, for certain clinical trials which became necessary in the course of this work, derivatives soluble in oils had to be made, and this was done by converting the hydroxymercuri compounds into salts of higher fatty acids, such as oleic, myristic, chaulmoogric, etc. These salts, which are described briefly later on, are readily soluble in almond, olive and other oils and such solutions are suitable for chemotherapeutic experiments on animals where aqueous solvents are inadvisable or useless. The bactericidal tests were all made by the methods described below, using *Bacillus typhosus*, Rawlings.

*B. typhosus* was selected as the test organism in preference to *B. coli* for the reasons put forward by Anderson and McClintock [1912] viz., (1) the greater reliability of the strains in the former organism, (2) the proof that the growth in the medium is actually due to *B. typhosus* and not to accidental

contamination is easily made by using specific agglutination with a high titre serum.

*Aqueous solutions.* Most of these mercury compounds are insoluble in water but form sparingly soluble sodium or potassium salts and these were used for the tests carried out in water. The solutions were diluted to a suitable series of known concentrations with sterile distilled water and quantities of 5 cc. mixed with 0·2 cc. of a 24 hours broth culture of *B. typhosus*, Rawlings, filtered through paper. This was allowed to interact for 5 minutes at 20° and a 3 mm. platinum loopful taken into 10 cc. of sterile broth and incubated at 37° for 48 hours. The tubes showing growth in the highest concentration were tested with typhoid serum and a control serum for specific agglutination.

*Serum solutions.* It is now well known that the bactericidal action of many substances is reduced when they are used in presence of serum and to ascertain how this medium affected mercury compounds some trials were made with normal horse serum, which had been passed through a Berkefeld candle and heated at 58° for 2 hours. The serum in this case was used as a diluent in place of sterile distilled water as in the previous test.

*Oil solutions.* For the reason already stated (p. 514) it was desirable to see how the use of oil as a solvent for oil-soluble mercury compounds affected their use as bactericidal agents and these tests were carried out as follows. The reagents were diluted in sterile olive oil. 5 cc. quantities were tubed and to these varying dilutions, 0·02 cc. of the filtered broth culture was added; the tubes were then closed with sterile rubber corks and the contents vigorously shaken. After 5 minutes' interaction in a water-bath at 20° cultures were taken as before.

## Results of Experiments.

The substances are indicated by typical formulae as far as possible, *e.g.* in Table I, substance No. 1 is 3 : 5-dihydroxydimercuri-2-hydroxybenzaldehyde, which is represented thus $C_6H_2[(CHO)(Hg.OH)_2(OH) = 1 : 3 : 5 : 2]$.

From the results given (Table I) it is clear that the introduction of a mercury residue into the molecule of an organic compound enhances the bactericidal action of the latter: thus, whilst a 0·5 % solution of *m*-hydroxybenzaldehyde only kills *B. typhosus* after 4 hours' action, solutions of all the mercury derivatives of *m*-hydroxybenzaldehyde are much more active bactericidal agents, the most potent towards *B. typhosus* being hydroxymercuri-4-nitro-3-hydroxybenzaldehyde (No. 7, Table I) which kills in 5 minutes at a concentration of 0·004 %, whilst the least active are the dihydroxydimercurinitro-3-hydroxybenzaldehydes (Nos. 4 and 8, Table I) which are toxic in 5 minutes at a concentration of 0·06 %. It is noteworthy in this connection that so far from enhancing the bactericidal action, as stated by Schoeller and Schrauth, the introduction of a second mercury residue appears to reduce it, all the dimercuri derivatives of nitro-*m*-hydroxybenzaldehyde (Nos. 4, 6, 8, in Table I) being less active than the monomercuri derivatives (Nos. 3, 5, 7, 9, in Table I).

Table I. *Salts of mercurated hydroxybenzaldehydes, alkylphenols and certain related substances in water.*

| No. | Substance used | Toxicity to *B. typhosus* % kills | Toxicity to *B. typhosus* % fails | Mercury in toxic concentration g. % |
|---|---|---|---|---|
| | *Mercurated hydroxybenzaldehydes.* | | | |
| 1 | $C_6H_2[(CHO)(HgOH)_2(OH)=1:3:5:2]$ | 0·0202 | 0·013 | 0·016 |
| 2 | $C_6H_2[(CHO)(HgOH)_2(OH)=1:3:5:4]$ | — | 0·03 | * |
| 3 | $C_6H_3[(CHO)(HgOH)(OH)=1:2:3]$ | 0·0085 | 0·0056 | 0·005 |
| 4 | $C_6H[(CHO)(HgOH)_2(OH)(NO_2)=1:2:4:3:6]$ | 0·06 | 0·05 | 0·04 |
| 5 | $C_6H_2[(CHO)(HgOH)(OH)(NO_2)=1:2 \text{ or } 4:3:6]$ | 0·01 | 0·009 | 0·005 |
| 6 | $C_6H[(CHO)(HgOH)_2(OH)(NO_2)=1:2:6:3:4]$ | 0·02 | 0·01 | 0·013 |
| 7 | $C_6H_2[(CHO)(HgOH)(OH)(NO_2)=1:2 \text{ or } 6:3:4]$ | 0·0038 | 0·0029 | 0·002 |
| 8 | $C_6H[(CHO)(HgOH)_2(OH)(NO_2)=1:4:6:3:2]$ | 0·06 | 0·05 | 0·04 |
| 9 | $C_6H_2[(CHO)(HgOH)(OH)(NO_2)=1:4 \text{ or } 6:3:2]$ | 0·0095 | 0·0086 | 0·005 |
| 10 | $C_6H_2[(CHO)(HgOH)(OH)(NO_2)=1:5:4:3]$ | 0·117 | 0·095 | 0·06 |
| | *Mercurated alkylphenols.* | | | |
| 11 | $C_6H_2[(CH_3)(C_3H_7)(HgOH)(OH)=1:4:?:2]$ | 0·038 | 0·017 | 0·021 |
| 12 | $C_6H[(CH_3)(C_3H_7)(HgOH)_2(OH)=1:4:2:6:3]$ | 0·033 | 0·016 | 0·023 |
| 13 | $C_6H_3[(C_4H_9)(HgOH)(OH)=1:3:4]$ | 0·043 | 0·032 | 0·024 |
| 14 | $C_6H_2[(C_4H_9)(HgOH)_2(OH)=1:3:5:4]$ | — | 0·012 | * |
| 15 | $C_6H_3[(C_5H_{11})(HgOH)(OH)=1:3:4]$ | 0·035 | 0·028 | 0·018 |
| 16 | $C_6H_2[(C_5H_{11})(HgOH)_2(OH)=1:3:5:4]$ | — | 0·022 | * |
| | *Miscellaneous mercury compounds.* | | | |
| 17 | Hydroxymercuri-*o*-nitrophenol† | 0·0021 | 0·001 | 0·0011 |
| 18 | Hydroxymercuri-*m*-nitrophenol | 0·005 | 0·004 | 0·0028 |
| 19 | Dihydroxydimercuri-*m*-nitrophenol | 0·01 | 0·009 | 0·007 |
| 20 | Hydroxymercuriacetylsalicylic acid† | 0·6 | 0·4 | 0·26 |
| 21 | Hydroxymercuridibromofluorescein† | 0·3 | 0·2 | 0·069 |

* Strongest concentration possible.

† Commercial preparations issued and used as sodium salts.

In order to confirm this result mono- and di-mercuri derivatives of *m*-nitrophenol were prepared, when it was found that the dimercuri derivative (No. 19, Table I) is only half as active as the monomercuri compound (No. 18, Table I).

On arranging these results as in Table II, to show the percentage of metallic mercury in the concentration toxic to *B. typhosus*, it becomes clear that the bactericidal equivalent, thus expressed in terms of metallic mercury, depends in a marked degree on the nature of the organic residue to which it is attached.

When these effects are considered in detail it is seen that the two groups —CHO and —OH taken together are most effective in the *meta*-position to each other and least effective in the *para*-position. The entrance of a nitro-group when CHO : OH = 1 : 3, enhances the effect in position 4 (No. 7) but exerts no influence in positions 2 or 6 (Nos. 5 and 9) though it should be remembered that these compounds are not strictly comparable since the

position of the mercuri-group is not the same in all three. These results differ from those of Miss de Witt [1921] with mercurated nitrophenols in which the combination HgOH : $NO_2$ : OH was much more active than HgOH : OH and was at its best with HgOH : $NO_2$ : OH = 4 : 2 : 1.

Table II.

| No. in Table I | Substance | Mercury in toxic concentration g. % |
|---|---|---|
| 17 | Hydroxymercuri-*o*-nitrophenol* ... ... ... ... | 0·0011 |
| 7 | Hydroxymercuri-4-nitro-3-hydroxybenzaldehyde ... | 0·002 |
| 18 | Hydroxymercuri-*m*-nitrophenol ... ... ... ... | 0·0028 |
| 3 | 2-Hydroxymercuri-3-hydroxybenzaldehyde ... ... | 0·005 |
| 9 | Hydroxymercuri-2-nitro-3-hydroxybenzaldehyde ... | 0·005 |
| 5 | Hydroxymercuri-6-nitro-3-hydroxybenzaldehyde ... | 0·005 |
| 19 | Dihydroxydimercuri-*m*-nitrophenol ... ... ... | 0·007 |
| 6 | 2 : 6-Dihydroxydimercuri-4-nitro-3-hydroxybenzaldehyde | 0·013 |
| 1 | 3 : 5-Dihydroxydimercuri-2-hydroxybenzaldehyde ... | 0·016 |
| 15 | 2-Hydroxymercuri-*p*-iso-amylphenol ... ... ... | 0·018 |
| 11 | Hydroxymercuricarvacrol ... ... ... ... | 0·021 |
| 12 | 2 : 6-Dihydroxydimercurithymol* ... ... ... ... | 0·023 |
| 13 | 2-Hydroxymercuri-*p*-*tert*-butylphenol ... ... ... | 0·024 |
| 8 | 4 : 6-Dihydroxydimercuri-2-nitro-3-hydroxybenzaldehyde | 0·04 |
| 4 | 2 : 4-Dihydroxydimercuri-6-nitro-3-hydroxybenzaldehyde | 0·04 |
| 10 | 5-Hydroxymercuri-3-nitro-4-hydroxybenzaldehyde ... | 0·06 |
| 21 | Hydroxymercuridibromofluorescein* ... ... ... | 0·069 |
| 20 | Hydroxymercuriacetylsalicylic acid* ... ... ... | 0·26 |

* Commercial preparations.

Table III. *Comparative tests in serum, broth and water.*

| | Medium | | | | | | |
|---|---|---|---|---|---|---|---|
| | Water | | Broth | | Serum | | |
| Substance | Kills % | Fails % | Kills % | Fails % | Kills % | Fails % | Mercury in toxic concentration g. % |
| Mercury succinimide | 0·001 | 0·0001 | 0·001 | 0·0001 | 0·1 | 0·01 | Water 0·0005<br>Broth 0·0005<br>Serum 0·05 |
| 2-Hydroxymercuri-3-hydroxybenzaldehyde 20° (time 5 minutes) | 0·0085 | 0·005 | 0·05 | 0·01 | 0·05 | 0·01 | Water 0·005<br>Broth 0·03<br>Serum 0·03 |
| Time 24 hours; 37° | 0·0005 | 0·0001 | 0·001 | 0·0005 | 0·01 | 0·005 | Water 0·0003<br>Broth 0·0006<br>Serum 0·006 |
| Time 48 hours; 37° | 0·0005 | 0·0001 | 0·001 | 0·0005 | 0·01 | 0·005 | Water 0·0003<br>Broth 0·0006<br>Serum 0·006 |

In the case of the alkyl phenols the increase of bactericidal activity due to the introduction of mercury residues is much less marked; thus whilst carvacrol, thymol, *p*-*tert*-butylphenol and *p*-*iso*-amylphenol are all toxic to *B. typhosus*, under these conditions, at a concentration of about 0·03–0·04 %, the activity of the mercury derivatives (Nos. 11–16, Table I) is of about the same order, but owing to the sparing solubility of the salts of these derivatives in water

the results are perhaps not entirely trustworthy. For the same reason no conclusions can be drawn regarding the effect of replacing the aldehyde group (as in No. 2) by alkyl groups (as in Nos. 14 and 16).

It is clear from the results in Table III that there is a considerable reduction in the bactericidal action of both organic and inorganic compounds of mercury when they are used in serum in place of water: broth however has little or no effect on the activity of mercury succinimide though it reduces the activity of the hydroxymercurihydroxybenzaldehyde from about one-sixth to one-half of that shown in water.

*Experiments in oil.* As it is desirable for clinical work in some cases to use mercury compounds in oily media, a number of these compounds have been converted into oil-soluble derivatives by converting the —Hg.OH residue into a radical containing the residue of one of the higher fatty acids. Thus if 2-acetoxymercuri-3-hydroxybenzaldehyde is heated at 100° with excess of myristic acid for 1 hour it dissolves and on extracting the cooled product with hot light petroleum (B.P. 40–60°) to remove excess of myristic acid there is left undissolved 2-myristoxymercuri-3-hydroxybenzaldehyde

$$C_6H_3(CHO)(OH)(HgO.CO.C_{13}H_{27})$$

as a colourless indistinctly crystalline powder, melting at 130° and decomposing at 200°. Found Hg = 36·44, calculated Hg = 36·56 %. This product is readily soluble in oils, especially on warming, and in like manner similar derivatives with higher fatty acids can be obtained from the various mercury compounds mentioned in Table I.

A number of such compounds have been made from 2-acetoxymercuri-3-hydroxybenzaldehyde and their bactericidal action on *B. typhosus* has been determined in olive oil. The characters of the principal compounds of this type made are as follows:

2-Chaulmoogroxymercuri-3-hydroxybenzaldehyde $C_6H_3CHO.OH.HgO.CO.C_{17}H_{31}$ M.P. 117°
2-Lauroxymercuri-3-hydroxybenzaldehyde $C_6H_3CHO.OH.HgO.CO.C_{11}H_{23}$ M.P. 131–3°
2-Oleoxymercuri-3-hydroxybenzaldehyde $C_6H_3CHO.OH.HgO.CO.C_{17}H_{33}$ M.P. 110–115°
2-Capryloxymercuri-3-hydroxybenzaldehyde $C_6H_3CHO.OH.HgO.CO.C_7H_{15}$ M.P. 130–1°

The results of the bactericidal tests in oil are as follows:

Table IV. *Higher fatty acid derivatives of 2-hydroxymercuri-3-hydroxybenzaldehyde* (No. 3, Table I).

| Acid used and formula of compound | Toxicity to *B. typhosus* | | Mercury in toxic concentration |
|---|---|---|---|
| | % kills | % fails | g. % |
| 1. Caprylic acid $C_6H_3(CHO)(OH)(HgO.CO.C_7H_{15})$ | 0·025 | 0·01 | 0·011 |
| 2. Lauric acid $C_6H_3(CHO)(OH)(HgO.CO.C_{11}H_{23})$ | 0·025 | 0·01 | 0·0096 |
| 3. Myristic acid $C_6H_3(CHO)(OH)(HgO.CO.C_{13}H_{27})$ | 0·025 | 0·01 | 0·0091 |
| 4. Oleic acid $C_6H_3(CHO)(OH)(HgO.CO.C_{17}H_{33})$ | 0·025 | 0·01 | 0·0083 |
| 5. Chaulmoogric acid $C_6H_3(CHO)(OH)(HgO.CO.C_{17}H_{31})$ | 0·0076 | 0·0038 | 0·0026 |

The results of these experiments in oil indicate that the activity of 2-hydroxymercuri-3-hydroxybenzaldehyde used in the form of salts of the higher

fatty acids is reduced by about half in this medium, except in the case of the chaulmoogric acid salt where the activity is doubled, due no doubt to the influence of the chaulmoogric acid, which itself exhibits considerable bactericidal action.

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