A contribution to the chemotherapy of bacterial infections

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IT IS CURRENTLY THE GENERAL OPINION that only protozoal infections can be attacked by chemotherapeutic means. For protozoal infections, a number of effective drugs are available; for example, germanin for trypanosome infections, neostibodan for kala azar, plasmochin and atebrin for malaria, and salvarsan and its derivatives for spirochetes, especially syphilis.

For coccal infections, there have been no reasonably effective chemotherapeutants known. Protozoa and spirochetes represent relatively advanced groups of living organisms, and the more highly developed an infectious agent is, the more loci it offers for attack by chemotherapeutants. An advance in the chemotherapy of pneumococcus infections has been made by Morgenroth, but optochin is used mostly for direct application on the infection focus, as is vuzin—another derivative of hydroquinine—in streptococcal infections. In systemic infections we have found no clear-cut effect of these preparations in our experimental animals. Also, the silver compounds recommended for therapy of septic infections have proved to be inadequate in practice. Indeed, critical observation often indicates that they cause a detrimental effect on the course of the illness.

A prerequisite for the systematic search for chemotherapeutically effective substances is always a suitable model system. With streptococci it is possible to produce reproducibly in mice a fatal infection. We have used for our studies a hemolytic strain of streptococcus which came from a fatal human infection.

The first chemical compounds which we found to be effective in streptococcus infections were a series of compounds of gold. These compounds produced a significant effect in animals and also showed an unquestionably favorable influence on streptococcus infections in humans. These gold compounds however had an important disadvantage. They could not be used in doses high enough to produce a certain chemotherapeutic effect and could not be used over a long period of time. For in long-term treatment, there was the danger of gold toxicity developing. Skin rashes and kidney damage appeared, which disappeared when the drug was stopped, but returned when the therapy was begun again.

Success with gold compounds in

the treatment of streptococcus infections as well as syphilis has also been reported by Feldt, who recommends as the most effective agent a gold thioglucose preparation.

Because of the disadvantages mentioned, we turned our attention to other chemical classes which were pure organic compounds without any metallic groups and which showed in mouse experiments an indication of activity. We were aware of a series of azo and acridine compounds which had shown a relatively good effect during *in vitro* disinfection experiments against streptococci. This occasional excellent *in vitro* activity almost completely disappeared when these substances were injected into the animal body.

Azo compounds have often excited therapeutic interest. Among the acidic azo compounds, trypan blue has been found effective against trypanosomes as well as against leprosy. Of the neutral azo compounds, diacetylaminoazotoluene has been used as an agent for the promotion of healing after accidents. The oldest of the bactericidal basic azo compounds is 2, 4-diaminoazobenzene, whose hydrochloride has been known for a long time under the name chrysoidin and has been used as a bacterial stain. Eisenberg showed in 1913 that chrysoidin inhibited the growth of Gram-negative bacteria in a dilution of 1/1000 and Gram-positive in a dilution of 1/10,000. Eisenberg himself had entertained the thought that this occasionally highly active dye might have a chemotherapeutic effect in infections. He emphasized at the same time what a long way it is from the most excellent *in vitro* results to a decisive *in vivo* result. This demonstrable *in vitro* bactericidal activity of such basic azo compounds was mentioned again by Lockemann and Ulrich in 1934. They tested preparations which contained as their important ingredient 2,4-diaminoazobenzene. A further development in the basic azo compounds over chrysoidin was the use of the pyridine component in phenylazo-2,6-diaminopyridine and 2′-butoxypyridyl-5,5′-azo-2,6-diaminopyridine. These and other related compounds were used in practice as urinary antiseptics and were also recommended by the manufacturer for gonorrhoea. We could not demonstrate any therapeutic effect of these compounds in animals infected with streptococci and staphylococci.

In the course of our studies, we hit upon a group of very nontoxic azo compounds which indeed had no significant disinfection action *in vitro* against streptococci but showed a clear effect in mouse experiments. In this group was Prontosil, which Mietzsch and Klarer had synthesized in 1932. With Prontosil we can show the best chemotherapeutic effect in streptococcus infections in animals that we have ever seen. Prontosil is the hydrochloride salt of 4′-sulfonamid-2,4-diaminoazobenzene.* This is a red crystalline powder with a melting point of 247–251°, which is soluble to 0.25% in cold water but is more readily soluble in warm water.

The nontoxicity of this preparation can be seen from the toxicological data. The animals tolerated orally the following doses: mice, 500 mg./kg.; rabbits, 500 mg./kg.; cats, 200 mg./kg. Higher doses were vomited up.

Subcutaneously, a 20g. mouse can be given 1–2 cc. of a 0.25% solution. As a suspension, a 4% solution of the dye can be injected under the skin of mice in 1–2 cc. without any skin necrosis developing and without any generalized toxic symptoms occurring. . . .

Weese and Hecht have shown that Prontosil is pharmacologically an ex-

* [See page 199 for this structure.]

tremely indifferent compound. Even with rapid injections of 10 mg./kg. intravenously there was no change in blood pressure or heart function in cats and rabbits. The smooth muscle of the uterus, large intestine and small intestine were not affected either *in situ* or isolated. The physiological function of these organs was not influenced by Prontosil. In subcutaneous injections up to 1 g./kg., no toxicity in animals was observed, while intravenous injections showed no production of thrombosis as the result of damage of the blood vessel walls.

Prontosil shows in mice a selective chemotherapeutic effect against streptococci.

In our studies we have used a highly pathogenic *Streptococcus hemolyticus* from a human case of streptococcus sepsis. We cultured the streptococci in egg broth. Dilutions of 1/1000, 1/5000, 1/10,000 and 1/100,000 of a 24-hour culture were used. In general 0.3 cc. of a 1/10,000 dilution was sufficient to kill mice within 24 hours when injected intraperitoneally. For the experiments we usually infected the animals with 10 times the lethal dose, so that all of the infected, untreated controls died with certainty within 24 hours, or at latest 48 hours, from the sepsis which developed in them. In the sick animals hemolytic streptococci could be demonstrated in the heart blood and in almost all organs, even 1–2 hours after infection.

For subcutaneous and oral treatment of the infected mice we used the dye dissolved in water up to 0.5% or suspended in water up to 1–4%. For a treatment with a single dose, 1/10 to 1/50 of the highest tolerated dose was sufficient to show a clear effect. If the infected mice were treated with this dose for 3–5 days, then they generally showed a complete cure from the otherwise fatal infection. From time to time we could show a clear effect at a dose 1/100–1/500 of the tolerated dose, especially when the infection was not quite so acute and the controls did not die until later than 24–48 hours.

The table on page 198 shows an experiment in which the animals were infected with a 1/1000 dilution of a 24 hour culture, although the infection was somewhat slower than usual. . . .

The results of our animal experiments have been confirmed in the clinic. Prontosil will be tested in the clinic under the name "Streptozon."

Whether Prontosil acts directly or indirectly against the pathogen in the body cannot be decided as yet.* It is remarkable that *in vitro* it shows no noticeable effect against streptococci or staphylococci. It exerts a true chemotherapeutic effect only in the living animal. In pneumococcus and other infections Prontosil shows no noteworthy effect, so that it seems to be specific for streptococci, while acting somewhat on staphylococci. These observations argue against a nonspecific, general, and indirect effect of Prontosil on the organism, such as a nonspecific activation of the reticuloendothelial system.

Therefore, in the future the physician will have to determine also with bacterial infections immediately the specific character of the infection. From the chemotherapy of the protozoal infections it has been known for a long time that our most effective chemotherapeutic preparations affect only certain protozoa, while others, which cannot be distinguished morphologically from those which are affected, show no sensitivity to the agent.

In order to determine the specific character of a bacterial infection as soon as possible, a close cooperation

* [See comment, page 199.]

Streptococcus experiment of Dec. 20, 1932.

Infected with 1/1000 dilution of egg broth culture, 0.3 cc. intraperitoneally.

Treated 1½ hour after the infection, orally.

Animal			*Date*						
No.	*Weight*	*Dose*	*Dec. 21*	*Dec. 22*	*Dec. 23*	*Dec. 24*	*Dec. 25*	*Dec. 26*	*Dec. 27*
201	14 g.	0	+	(+)	(+)	—			
202	14 g.	0	+	—					
203	14 g.	0	+	—					
204	17 g.	0	+	—					
205	19 g.	0	+	—					
206	14 g.	0	+	+	—				
303	18 g.	0.2 cc. 0.01%	+	+	+	+	+	+	+
304	19 g.	0.2 cc. 0.01%	+	+	+	+	+	+	+
305	18 g.	1.0 cc. 0.01%	+	+	+	+	+	+	+
306	14 g.	1.0 cc. 0.01%	+	+	+	+	+	+	+
307	16 g.	0.2 cc. 0.1%	+	+	+	+	+	+	+
308	15 g.	0.2 cc. 0.1%	+	+	+	+	+	+	+
309	17 g.	1.0 cc. 0.1%	+	+	+	+	+	+	+
310	17 g.	1.0 cc. 0.1%	+	+	+	+	+	+	+
311	14 g.	0.2 cc. 1.0%	+	+	+	+	+	+	+
312	17 g.	0.2 cc. 1.0%	+	+	+	+	+	+	+
313	18 g.	1.0 cc. 1.0%	+	+	+	+	+	+	+
314	14 g.	1.0 cc. 1.0%	+	+	+	+	+	+	+

Key: + alive and well, (+) sick, — dead

between the clinician or physician on one side and the bacteriologist on the other side will be necessary. But even this ideal cooperation will not always achieve the desired result, since many times it is not possible to demonstrate the pathogen in the blood, especially in the early stages. . . .

Since this problem will not be quick to be solved, it should be given our attention immediately.

Summary. Prontosil shows an effect on animals infected with streptococci such as has not been observed before. It is possible to protect infected mice which would die within 24 hours by giving them subcutaneous or oral doses of Prontosil. Also chronic infections with streptococci in rabbits show a favorable result. Prontosil seems to show the best effect on streptococcal sepsis in the mouse but has some effect in staphyloccoccus infections. In pneumoccoccus infections as well as in other infections, it has not been possible to show any effect in experimental animals.

Comment

Domagk's discovery of Prontosil is a direct fulfillment of the dreams of Ehrlich when he first formulated his concepts of chemotherapy (see page 176). The methods used were based directly on Ehrlich's, and the success of the work results directly from the use of these methods. The basic idea was to test the action of chemical compounds directly in the infected animal, without regard to whether they had any effect in the test tube. In addition, a large number of compounds should be tested, representing as many of the possible modifications of certain basic structures as could be used.

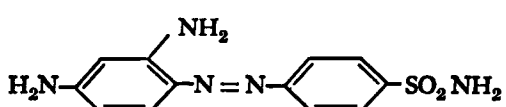
Prontosil is not active against bacteria

in vitro, as Domagk noted. It is quite active in infected animals. This difference is due, as we know now, to the fact that Prontosil is broken down in the animal into the active agent, sulfanilamide, as shown in the formulas below.

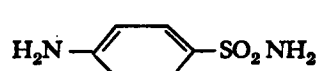
With the discovery of this fact, it was possible to embark on a program of synthesis around the sulfanilamide structure. This program has led to the large number of sulfa drugs known today.

The sulfa drugs were the first chemical substances that had any real effectiveness against bacterial infections. In particular, their action against streptococcal infections is noteworthy, since streptococcal infections are among the most frequent and most acute that occur in man. When it was possible to conquer these infections with chemical compounds, a major breakthrough in medicine had occurred. In addition, this success was probably instrumental in encouraging a group at Oxford University to attempt to make practical use of Fleming's discovery of penicillin (see page 185). Therefore, although the sulfa drugs have been replaced in many medical uses by antibiotics, their historical importance is quite great. In addition, they still have wide usage for certain infectious diseases, especially urinary tract infections, where they are often the drugs of choice.

The mode of action of sulfanilamide and the other sulfa drugs against bacteria has been understood since the work of Woods, whose paper is presented next.



Prontosil



Sulfanilamide