Staff Meeting Bulletin
Hospitals of the . . .
University of Minnesota

Sulfanilimide
(Prontosil)

Volume VIII Thursday, April 29, 1937 Number 26

STAFF MEETING BULLETIN
HOSPITALS OF THE . . .
UNIVERSITY OF MINNESOTA

Volume VIII | Thursday, April 29, 1937 | Number 26

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Published for the General Staff Meeting each week during the school year, October to May, inclusive.

Financed by the Citizens Aid Society

William A. O'Brien, M.D.

I. LAST WEEK

Date: April 22, 1937

Place: Nurses' Hall
Recreation Room

Time: 12:15 to 1:15 P.M.

Program: Movie: India on Parade

Abstract: Tuberculosis of the Skin

Present: 107

Discussion: C. Laymon
H. E. Michelson
R. V. Ellis
O. H. Wangensteen
K. W. Stenstrom
W. T. Peyton
E. Klaveness

II. MOVIE

Title: Romantic Mexico

Released by: RKO --

III. ABSTRACT

PRONTOSIL AND RELATED COMPOUNDS (SULFANILIMIDE)

R. E. Mattison

History

Early in 1935 a startling chemotherapeutic success was announced by Domagk in Germany. Hemolytic Streptococci of human origin were injected into the peritoneum of 26 mice. An hour and one-half later 12 of them received by stomach-tube a single dose of a dark red dye, the hydrochloride of 4 sulphamido 2-4 diaminoazobenzol which had been synthesized by Mietsch & Klarer. All of the treated mice survived. Of the 14 other animals which served as untreated controls, 13 were dead of their streptococcal infection within 3 days and the last succumbed on the fourth day.

Domagk also described a similar compound which was prepared by Mietsch and Klarer; which he at first called Streptozon S but later changed the name to Prontosil S. This is disodium salt of 4 sulphamidophenyl, 2 azo, 7 acetylamino, 1 hydroxynaphthalene, 3 : 6 disulphonic acid.

About the same time in France, Levaditi and Vaisman used a similar compound synthesized by Girard. This substance was similar in composition to Prontosil and called Rubiazol. They obtained somewhat similar results except that the treated mice did not as a rule survive indefinitely after a single dose of the drug, but lived a few days longer than the controls.

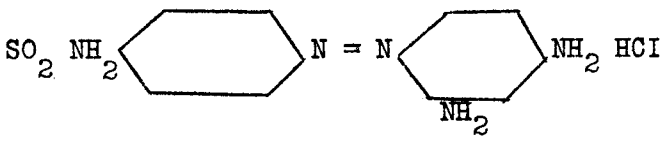
Somewhat later T. Trefouel , Mme. Trefouel, F. Nitti, and D. Bovet experimented with several substances derived from prontosil and found that many of them had similar anti-streptococcic powers and described considerable experimental work on the activity of the hydrochloride of p-aminophenyl sulfonamide, showing that the diazo group was not necessary.

Since the descriptions of these various chemotherapeutical agents English and American workers have to some extent confirmed the experimental work and several German, French, English and American writers have published their clinical results.

Chemistry

Prontosil - Streptozon

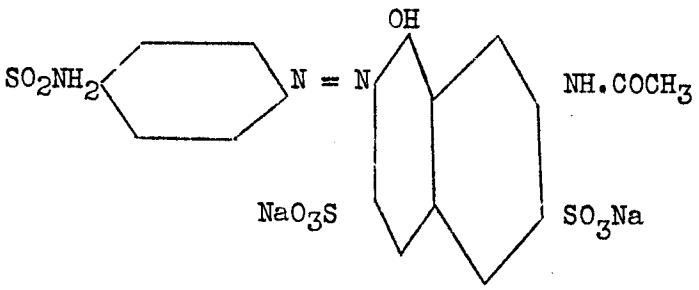
The first substance described by Domagk was called prontosil. This is an azo-dye with sulfonamide group, the formula being the hydrochloride of 2:4 diamino azo benzene 4 sulphonamide.



It has also been called sulphonamide chrysoidin. It is a red crystalline powder with a melting point of 247 to 251° and is soluble in water up to .25%.

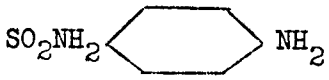
Prontosil S - Streptozon S - Rubiozol.

This is the disodium salt of 4 sulphonamide phenul-azo-1 oxy- 7 acelylamino naphtholene-3 : 6 disulphonic acid.



This substance is also a red-dye but is more soluble in water than Prontosil (up to 4%).

Sulfanilimide - Para amino benzene Sulfonamide (Prontylin Proprietary Compound sold by Winthrop)



Sulfanilimide is the official name accepted by the Council on Pharmacy and Chemistry of the American Medical Association. This is a wuite crystalline powder soluble up to 0.8%.

Experimental Work:

Domagk-

Twenty-six mice were inoculated intraperitoneally with a human streptoccus hemolyticus culture which was virulent for mice. The amount was 3cc. of 1:1000 dilution or 10 x M.L.D. Twelve of these mice were given .02 to 10 mg. of Prontosil by mouth 1½ hour later. All of the treated mice were alive on the eighth day whereas of the untreated mice, 11 died on the second and 2 on the third day and one on the fourth day.

He also showed that 500 mg./kg orally or 125 to 250 mg./kg were tolerated as non-tosic doses to mouse and 500 mg./kg and 25 mg. intravenously were tolerated non-toxic dose to rabbit.

There was practically no effect on the streptococci in vitro.

Horlein-

Showed that prontosil S was just as effective as prontosil.

Rabbits and mice tolerate 500 mg./kg. body weight by mouth and subcutaneously.

Intravenously mice tolerate 1 cc. of the 0.25% solution per 20 gm. body weight and rabbits up to at least 10 cc. of the 0.25% solution. The substances could be given to rabbits by mouth for long periods, e.g., 0.1 to 0.2 gm./kg. body weight for 14 days without harming the general condition of the animal without causing any alteration in the composition of the blood or pathological change in the urine. When given in large quantities the dye penetrates into all the tissues although accumulation of dye in any particular organ or cells could not be demonstrated.

No change in the blood pressure or heart function could be demonstrated after the rapid intravenous injection of 10 mg/kg. body weight. It was noted that there was no response of smooth muscle of bowel or uterus to similar injection. There was no local change when injected subcutaneous and thrombosis as a result of injury to vessel wall was never noted after injection (intravenous).

Prontosil S.

Mice can be given 1 cc. of 4% solution intravenously 2 cc. subcutaneously and 1 cc. by mouth without harm.

Rabbits will tolerage 100 to 200 mg./kg body weight intravenously.

Levaditi and Vaisman

Their experiments were with rubiazol which had same formula as prontosil S. When administered per os to mice in a single dose of 0.005 gm. this compound caused the animals to survive 6 days

longer than control animals. All of the animals were infected intraperitoneally with 0.3 cc. of a bullion culture of a strain of hemolytic streptococcus which killed the animals in 24 to 30 hours. After a subcutaneous injection of the same amount of the drug the survival was often 10 to 17 days. If care is taken to repeat the injection the animals lived for 8 to 20 days or even indefinitely. This curative activity was manifest if the injection of the drug was made eight hours after the intraperitoneal innoculation.

When rubiazel was given as a preventive it was found to be active if the animals were infected 48 hours later. Rubiazol does not exert any bactericidal action in vitro with regard to the streptococcus and it does not seem to prevent the growth of this organism.

Nitti & Bovet

These men undertook experiments on eight strains of human hemolytic streptococci which were virulent for mice.

Mice were inoculated intraperitoneally with 24 hour culture in bouillon and ascitic fluid. Both treated and non-treated mice which died within 8 days following inoculation were autopsied and a search was made for the streptococcus in the blood of the heart by direct examinations and cultures.

Eight strains of hemolytic streptococci obtained from grave or fatal human infections were used. The virulence of these strains varied between ½ cc. and 1/20 cc. of a 24 hour culture. When injected into the peritoneum prontosil was given by mouth or subcutaneously either in a single dose or repeated daily. Practically no effect could be found. The animals treated and the control generally died at the same time and they all presented a streptococcic septicemia which was verified by cultures of blood from the heart. It should be noted that the animals treated never resisted inoculation of a single fatal dose of streptococci. The human streptococci used in these experiments were not very virulent for mice.

The authors repeated these experiments using a human hemolytic streptococcus which was pathogenic for mice in a dose between 1/1000 and 1/150,000 cc. of a 24 hour culture. Prontosil was sometimes found to have a definite action against this very virulent strain. The best results were obtained by daily ingestions of 0.00125 gm. and in some fortunate cases a part of the animals treated survived one or several fatal doses of streptococci. The subcutaneous route furnished the most inconstant results and in most of the cases a single injection of 0.0025 gm. only caused the treated animals to live a few days longer than the control animals. It should be noted that the treated animals which died presented large numbers of streptococci in their blood.

Hence it was found that prontosil, in some cases, might protect mice against an intraperitoneal inoculation of a very virulent streptococcus but even in these cases the action was inconstant and it is probable that the good results reported by Domagk were due to the fact that he used a streptococcus which was particularly sensitive to prontosil.

Levaditi & Vaisman

Prontosil when injected as a .05 gm. aqueous or oil suspension subcutaneously in mice protected 60 to 100% of these mice if they were injected 5 to 10 days later with virulent streptococcus hemolyticus. The average was 80% protected. A second inoculation was given to the surviving animals on the 23rd day and 30 to 100% (generally 100%) survived. When inoculated for the third time on the 50th day following the prophylactic injection of prontosil all of the remaining mice died from the injection.

J. Trefouel, Mme. Trefouel, Nitti & Bovet

First experimented with para-aminophenylsulphonamide.

Mice were inoculated with a strain of hemolytic streptococcus isolated from a fatal puerperal septicaemia. The inoculation was made into the peritoneum with 1/20,000 cc. of a 24 hour culture in

bouillon and ascitic fluid. Under these conditions the five control animals died in less than 48 hours and the streptococcus was found in the blood. Six mice were inoculated under the same conditions and were given two successive doses at an interval of one day 1.25 and 2.5 mg./20 gm. body weight para-aminobenzenesulphonamide orally. Three of the animals died between the fourth and sixth day and the other three lived more than ten days. These results were almost identical with those obtained with prontosil under the same conditions.

Similar experiments were carried out with rabbits.

Colebrook and Kenny

Trials were first made with strains of streptococci freshly isolated from human puerperal infections.

Although occasionally the treated animals survived a little longer than the untreated controls, there were practically no survivals, and the experiments were regarded as negative, failing to confirm Domagk's claim. Six different strains were employed in such tests.

However, the next series of experiments were carried out with a highly virulent passage strain obtained from a puerperal fever case. With this strain, striking curative results were obtained in mice, although the animals only survived indefinitely if a series of 6 or 7 doses were given over a period of several days. It is of interest to note that one mouse survived after treatment although hemolytic strepticocci were already in the circulating blood in considerable numbers before the drug was administered.

Prophylactic experiments on mice

Mice were injected with 50 mg. of prontosil in aqueous suspension (10%), and four days later were given a dose of streptococcal culture into the peritoneum. The mice showed no toxic effect of the drug at any time. Nine out of 12 animals which did not receive the drug were dead within 3 days. Only 2 of the 12 treated mice succumbed to streptococcal infection within that period and two died somewhat later.

Animal experiments with para amino benzyl sulfonamide:

Curative effects were marked, fifteen mice being injected with virulent hemolytic streptococcus culture. Six of these were treated with varying amounts of p-aminobenzylsulfonamide, at 9 hours and 24 hours, and 2, 3, 4, and 5 days. The nine control animals were inoculated with varying amounts of virulent streptococcus culture. One control animal survived and one treated animal died on thirty-second day of streptococcus infection. The remaining treated animals were killed on the thirty-fifth day and showed no infection (cultures sterile).

No prophylactic effect was obtained when 40 to 50 mg. of the drug was given in aqueous suspension subcutaneously four days before the inoculation of virulent culture. This they stated was probably due to absorption of the drug from the subcutaneous depot more rapidly than in the case of prontosil.

Experimental work with related substances

Geissedet, Despois, Galliot and Mayer showed that two substances whose formula appear below had the same action as prontosil in experiments with mice.

$$C_6H_5.CH_2.NH.C_6H_4.SO_2NH_2$$

and

$$NH_2.SO_2.C_6H_4.NH.CH_2.SO_3.Na$$

This showed that the azo linkage is not necessary but that the sulfonamide $SO_2{:}NH_2$ probably is.

Forneou, J. & Mme. Trefouel, Nitti & Bovet

Prepared 130 substances related to prontosil and paraminophenylsulfonamide. They found that the ortho and meta com-

pounds were practically inactive. Also when the SO_2NH_2 group was replaced, the compounds had no activity at all. When the H of the SO_2NH_2 group was replaced with an alkyl group the therapeutic activity was decreased to a varying degree but always decreased. Replacement of the NH_2 group or blocking it reduced the activity but did not completely destroy it.

They also found that the addition of other radicles, to the other benzene ring of prontosil does not increase its activity and that by adding a third radicle ortho or meta to the SO_2NH_2 group decreased the therapeutic activity.

Buttle, Gray & Stephenson

These workers confirmed the work of Trefouel, Nitti and Bovet that paraminobenzylsulfonamide had a curative action on infected mice the same as prontosil. They found that for a comparison between different drugs it is necessary to use mice infected at the same time as streptococci grown under apparently identical conditions vary somewhat in virulence. They showed that the protection shown with paraminobenzylsulphonamide was better than that obtained with prontosil.

Twelve mice in each group were injected with 50,000 organisms.

Group I - control - no mice survived.

Group II - 2 mg. dose of each drug given orally and repeated eight times.

In the case of prontosil two mice survived, in the case of p.-aminobenzylsulfonamide six mice survived.

Group III - 10 mg. oral doses repeated eight times. Three mice survived when treated with prontosil and nine mice survived when treated with p-aminobenzylsulfonamide.

Group IV - Twelve mice treated with 50 mg. oral doses of paraminobenzylsulfonamide repeated eight times resulted in survival of all the mice.

Long and Bliss

Therapeutic effects of prontosil S and paraminobenzylsulfonamide were compared in mice inoculated with hemolytic streptococci. Sixty-four mice in control group all died within the first forty-eight hours.

Eighty-four mice treated with prontosil S being given 125 to 285 mg. over period of 5-14 days. 13 or 15.4% died in first 48 hours, 6 or 7.1% died in next 8 to 74 days, or total mortality of 62.9%.

Eighteen mice treated with 90 to 117 mgm. of paraminobenzylsulfonamide over period of seven days. There were no deaths in first 48 hours; 1 mouse died in next 5 days, 5.5% and 5 or 27.7% died in next 8 to 64 days, leaving a mortality of 33.2%.

Experiments with animals with organisms other than hemolytic streptococci

Domagk reports "improvement" in the condition of rabbits with chronic staphylococcus infections.

However experiments with staphylococci have so far failed to show any protection. The staphylococcal strains available will only kill mice with a very large dose and it has been shown that there is no protection with a large dose of relatively avirulent streptococci

Buttle, Gray and Stephenson have experimented with mice infected with meningococci with favorable results. The organisms were injected intraperitoneally in a suspension of 5% mucin. The degree of protection with one strain of meningococci (Type II) is of the same order as that with streptococci. Protection against another strain of lower virulence was also obtained.

H. Proom

P.-aminophenylsulphonamide protects mice from 10,000 to 1,000,000 M.L.D. of meningococci when injected intraperitoneal. By comparison, this drug

protects mice from 10,000 M.L.D. of hemolytic streptococci. A possible explanation of the difference is advanced namely the meningococcus cannot establish a focus of infection in mice whereas the hemolytic streptococcus can.

Long and Bliss have conducted in vitro experiments on other organisms and have found that a 1/10,000 concentration of P.-aminobenzylsulfonamide in serum broth markedly inhibited the growth of alpha-hemolytic streptococci, gamma streptococci, pneumococcus types I and II, several varieties of Neisseriae from the throat, micrococcus tetragenus, haemophilus influenzae, and haemophilus haemolyticus. The growth of staphylococcus aureus, typhoid bacilli, paratyphoid A & B, enteriditis, Flexner and Shiga and several other gram negative bacilli was not affected by this concentration of the chemical.

Horlein

States that prontosil as well as Prontosil S had a particularly marked effect on Type III pneumococcus, the pneumococcus mucosus. Chemotherapeutically, therefore, group III pneumococci are to be classed as streptococci rather than pneumococci.

The more recent literature has more evidence of the action paraminobenzylsulfonamide and its derivatives against the Type III pneumococcus and the results in general show less action of these drugs against this organism than against streptococcal and meningococcal infections.

Method of Action

The method of action has not been proven as yet but considerable experimental work has been done along these lines.

Domagk first showed that prontosil and prontosil S had no effect on the streptococcus in vitro.

The effect of prontosil on smears taken from the peritoneal cavity of infected animals is quite marked. In the control animals large numbers of cocci, together with degenerating cells damaged white blood corpuscles as their fragments are found from 24 to 48 hours after infection. On the other hand in animals successfully treated with prontosil no cocci and no damaged or disintegrated cells are found, but only a few leucocytes, monocytes, or lymphocytes, in good condition.

Provided treatment with sufficient dosage was begun early enough, none of the pathological tissue changes found in the streptococcal infection of mice were present in those animals which were successfully treated with prontosil. In other cases, the further spread of tissue damage which was already present was prevented.

Its meagre action on pneumococcal and other infections and its specific action in streptococcal infections tends to disprove the theory of a non-specific general and indirect action of prontosil on the body, of a nature of a non-specific activation of the reticulo-endothelial system.

Levaditi & Vaisman

The findings seemed to prove that rubiazol acted on the organism and increased defensive reactions, particularly phagocytosis. In certain cases there was a simple arrest in the multiplication of the streptococcus without a fatal destruction of this organism. Permanent sterilization was obtained by repeating the injections.

In some cases an animal which was apparently cured died suddenly of a generalized streptococcic infection on the twentieth day. In other cases there was a progressive sterilization of the animal. As the derivative used did not seem to have any appreciable bactericidal properties in vitro, it was necessarily concluded that it acted through the intermediary of the organism and that it did not have a direct antiseptic action. The mechanism of this action is not known. Experiments showing that the blood of a mouse impregnated with this drug did not con-

fer a refractory state to other mice. On the other hand, the liver did not transform rubiazol into a bactericidal derivative.

Trefouel, Mme. Trefouel, Nitti & Bovet

In their experiments with compounds related to prontosil, they thought that in vivo prontosil and prontosil S were reduced to p-aminobenzylsulphonamide which was the drug that caused the action of these cpds. To support this theory they showed that the results obtained are almost identical in the case of the two drugs and that p-aminobenzylsulphonamide had a marked bacterio static action on streptococci in vitro.

Colebrook & Kenny

The chief positive facts which emerge are that multiplication of the streptococci in the peritoneal cavity is prevented and that this happens quickly--within a few hours. At present it appears very unlikely that the cocci are actually destroyed either by the drug itself or by some compound formed from it in the animal body.

These workers found that serum from animals and patients treated with prontosil S had a marked bacteria static effect on the growth of the organism. It was noted that this growth retarding influence of the patients sera does not come into operation until after the first four hours of incubation.

The leucocyte count of patients and of animals under treatment has shown somewhat valuable results. Some showed a temporary rise with a fall back to the original level and others showed a fall after injection. A leucocytosis however was maintained. The possibility that the cocci in the treated animals might cease to elaborate certain toxic products which may be directly responsible for death is under consideration but these workers at present know of nothing to support it.

They mention that the possibility that the drug activates the reticulo endothelial system or some other tissue which may play a part in checking invasion by the organism has to be considered.

Long & Bliss

These workers in America confirmed the experimental evidence that prontosil S has no bacteria static action in vitro, whereas paraminobenzylsulphanamide has.

They also found that the serum from rabbits previously injected intravenously with prontosil S showed no bacteriostatic action. However, if sodium formaldehyde sulfoxalate (a reducing agent) was also given to the rabbit at the same time as prontosil S, the serum isolated from the rabbit thereafter gave a bacterio-static effect.

They concluded that reduction of the prontosil then had to take place before any therapeutic action was begun.

Colebrook & Kenny

Somewhat later these workers confirmed the work of Long & Bliss that 1:10,000 dilution of paraminobenzylsulphonamide inhibits the growth of hemolytic streptococci. Prontosil S showed no inhibition of growth in vitro.

These workers also found that human or monkey blood or serum obtained after the administration of prontosil or sulphonamide will inhibit the growth of hemolytic streptococci. This action is more marked in these species than it is in the case of rabbits, guinea pigs or mice. They also have evidence that prontosil is reduced to sulphonamide in the animal body.

Long & Bliss

These observers further showed that prontosil S reduced by cysteine hydrochloride had the bacterio static effect of paraminobenzylsulphonamide in vitro.

Further observations on the mode of action of these compounds lead them to believe that they act by inhibiting the growth of betahemolytic streptococci and that they injure these organisms in

such a way as to permit them to be phagocytosed by the white blood cells. These changes they observed occurred about 6-8 hours after the injection of the chemotherapeutic agent into the animal body. This lag in action suggests the possibility of a change in the chemical substance after the introduction into the animal organism.

Clinical Observation with the Use of Prontosil and Its Derivatives

The first reports referring to the use of the drug in human infections, e.g. erysipelas, puerperal fever and others, appeared in Germany. These clinical reports are unanimously favorable, but their evidential value must be regarded as small since, in most cases, the recovery of patients is unhesitatingly ascribed to the treatment, and too little allowance is made for the tendency to spontaneous cure of these infections. The bacteriological and clinical data supplied are nearly always very scanty, e.g. we are not told whether the cases were all infected by hemolytic streptococci, whether the organisms were present in the blood stream before treatment was commenced, nor in how many of the cases there was present any clinical condition such as generalized peritonitis which habitually connotes a very high mortality. The papers do serve however to indicate that the drug is well tolerated by the human subject and what dosage has given apparently good results.

A brief summary of these papers along with a few from the French Literature therefore should be mentioned.

Otto Scheurer

This writer used prontosil in various diseases, such as erysipelas, angina streptococcus pneumonia, acute and chronic polyarthritis, septic endocarditis and scarlet fever. The mode of administration was .25 gm. oral tablets of prontosil and 20 cc. ampule of .25% prontosil or the two variously combined.

The most striking results were with erysipelas. The temperature dropped from 105° after the first injection (intravenous) or after the third tablet by mouth and would become normal in 2-3 days. The local process became stationary and regressed after defervescence. General condition rapidly improved.

Suppurative anginas were treated by mouth, one tablet t.i.d. Temperature dropped to normal in 4 to 48 hours, while swelling, redness, coatedness of tonsils disappeared.

In pneumonias with bacterio-logic findings of streptococci, the entire picture, fever pulmonary findings alike changed with the onset of prontosil therapy.

Several cases of endocarditis lenta were refractory to most energetic treatment with prontosil.

Scarlet fever. The temperature dropped after the second injection (intravenous). Angina regressed and exanthema faded.

Results in polyarthritis were not uniform. The effect in fresh acute polyarthritis was sometimes very good.

On the basis of his own experiments, in several cases the writer advises two daily injections of 20 cc. of 0.25% solution or 5 cc. of the 2.5% solution and concomitantly 2 tablets t.i.d. Prontosil S injections discontinued after improvement and oral tablets continued several days.

Lother Ley

Treated 79 cases for puerperal fever, 42 prophylactically and 37 therapeutically 20 cc. of .25% solution was used (intravenous), and 5 cc. of 2.5% (prontosil S) solution was used (intramuscularly). Oral and rectal administrations were by tablets.

Rectal administration was by drips. The tablets being dissolved in 5% grape sugar solution or physiologic saline. This was especially used for prophylaxis. In severe cases paraoral and oral administrations were combined.

There were only 4 failures in 79 cases.

W. Kramer

This author treated a total of 23 cases of erysipelas with prontosil. A total failure of the remedy could not be observed in any case. In most patients the defervescence occurred on the second or third day.

This result was compared with 23 unselected cases which were treated without prontosil. The temperatures did not drop until the eleventh day. The characteristic redness soon faded following the administration of prontosil. The edema regressed, the improvement of the subjective condition was still more impressive. Within a short time (at most thirty minutes) after administration of prontosil, the annoying feeling of tension and burning of diseased regions of the skin subsided. Patients appetites increased and they were interested in surroundings.

The author prefers the intravenous administration. The average total dose which his patients received was 40 cc. of 0.25% solution of prontosil.

Meyer-Heine & Huguenin

These writers report 150 cases of erysipelas treated with sulphamido chrysoidin (rubiazol). With few exceptions the results were rapid and constant as regards temperature and local phenomena. Several cases were less convincing because the erysipelas was either mild, recurrent, or subsiding. The following doses were adopted for adults. For the first three or four days - 2 gm. (8 tablets) two at a time, sometime after meals. For the next three days as a rule decreasing doses (6 tablets), then for 3 or 4 days more four tablets. Even after clinical recovery, three tablets may be given with advantage for about 8 days.

The intravenous method, the writers thought at that time, was too experimental.

a. Erysipelas of face. 51 cases. No fatalities. Temperature dropped promptly--lesion regressed. Edema disappeared after third day. The mortality in 1934 was 5% (26 in 508 cases).

b. Erysipelas of the extremities. Treatment was ineffective in 1 of 8 cases treated. In the others the fever disappeared, the local lesions were somewhat slower to regress as in the case of erysipelas of the face.

c. Erysipelas in infants. Recovery of 8 cases. Previous mortality 80%. The temperature was somewhat slower in dropping fifth day and the lesions regressed in similar slow manner.

H. Schranz

This author treated 60 cases of sepsis with prontosil. All cases were studied bacteriologically. Clinically in all cases, high fever and chills were present. Three of the cases died. One of these had gas bacillus infection following fracture. Second case had meningitis following skull injury. The third case died of embolism. At first only 20 cc. doses of 0.25% solution of prontosil were used later 40 and even 60 cc. doses were administered. A dose of this size the author states can be maintained 5-6 days without ill effect.

The author reports good results in osteomyelitis and furunculosis due to staphylococci.

The author also used prontosil prophylactically following labor, Caesarean section and curettage. He also advocates that in severe throat infections a gargle should be combined with 20 cc. of prontosil repeated three times daily.

T. Schreus

Preparations of prontosil were first used by this author in an 18 month old child in moribund state from pyemia with staphylococcus blood culture and numerous staphylococcus abscesses. Two days after administration temperature dropped and gradually became normal, abscesses were drained and child recovered.

This author also treated erysipelas and not a single resistant case was met. Two to six gm. of prontosil was given by mouth and on second day temperature

returned to normal and the progress of the erysipelas was arrested.

E. Anselm

Treated two cases of streptococcal sepsis following an abortion. The diagnosis was confirmed bacteriologically.

Both cases were given 20 cc. prontosil 0.25% solution intravenously daily. The temperature returned to normal on the sixth day in both cases. It cannot be stated with certainty whether the prontosil had any influence on the regression of adnexal changes.

This author also treated pure and mixed infections of staphylococci with apparent good results and concluded the drug should be used on all cases of puerperal infection.

On the basis of his results the author recommends the use of 1-2 daily injections of 20 cc. of 0.25% solution of prontosil I.V. depending on severity of the infection.

G. Scherber

He treated over 40 cases of erysipelas with prontosil and in all of them satisfactory results were obtained. The average duration of the fever in these cases was four days for males and 3½ days for females. The effect was apparently dependent on the size of the dose as well as on the early institution of treatment and on its daily repetition.

The author advises daily intravenous injections of 40 cc. given together with the proper local treatment to shorten the duration of the disease.

Average case treated locally lasts eight days. When local treatment is combined with prontosil therapy the average case lasts four days in men and 3.5 days in women.

He advises blood transfusions in conjunction with prontosil in very severe cases.

Colebrook & Kenny

These authors report their clinical results in conjunction with experimental work.

Thirty-eight cases of puerperal fever were treated in the isolation block of Queen Charlotte's Hospital. At first only the more severe cases (10 in number) were treated by prontosil, but finally there was included every case (28) infected by hemolytic streptococci unless there was any contraindication, i.e., nephritis.

No other "specific" treatment has been employed at the same time, except in the case of two patients, a single small dose of anti-tixic serum (because they presented an intense scarlatiniform rash).

Dosage. At first 20 cc. of the 2.5% solution of prontosil S was injected either subcutaneously or intramuscularly each day and six tablets per diem by mouth. Each tablet containing 0.3 gm. of prontosil. Later the amount given by injection in several cases has been increased to 40 cc. per day (occasionally 60 or even 90 cc.), and this amount has been diminished with the clinical improvement of the patient.

Only the first 20 cc. was given intravenously, the rest intramuscularly. Since the drug is absorbed very rapidly, it is doubtful whether any advantage is secured by intravenous administration.

45% of the cases permitted no conclusion as to the effect of the treatment. All of them would in all probability have recovered without treatment and perhaps as quickly.

42% of the cases left one with the impression that the drug had in all probability hastened or determined recovery from the infection. All these were cases which undoubtedly presented signs and symptoms of septic infection so severe as to give ground for anxiety; and cases which in the light of previous

experience over several years surprised the writers by the prompt clinical improvement and remission of fever following the first few doses of prontosil.

The clinical course of five cases did not run so smoothly. In two of them who recovered one had a suspicion that the treatment might be exerting an unfavorable effect and in one of these improvement did set in as soon as the drug was discontinued.

Mortality rates of treated and untreated cases -- there were three deaths in 38 cases treated, or mortality of 8%.

In the 38 cases admitted immediately prior to the use of prontosil, there were 10 deaths or a 26.3% mortality. In the 38 cases immediately preceding these there were 9 deaths or 23.7% mortality. From 1931 to 1934 the cases were divided into group of 38. The mortality varied between 18 to 28.8%. The average being 22%.

Somewhat later 26 additional cases were added to this list with no deaths.

The totals to date: December, 1936:

Year	No. of Cases	Deaths	Deaths from Peritonitis
	Prontosil Used		
1936	64	3 = 4.7%	1
	Prontosil was not Used		
1935	90	22 = 24.4%	15
1934	120	20 = 16.6%	16
1933	97	20 = 20.6%	14
1932	90	19 = 21. %	16
1931	98	31 = 31.6%	23

Long & Bliss

Type of Infection	No. of Patients	Recovered	Died
Erysipelas and Cellulitis	19	19	0
Septicemia	3	2	1
Puerperal Sepsis	2	2	0
Traumatic Orbital Cellulitis	3	3	0
Osteomyelitis	2	2	0
Cystitis	3	3	0
Peritonitis	1	0	1
Tonsillitis	14	14	0
Peritonsillar Infections	5	5	0
Scarlet Fever	8	8	0
Ludwigs Angina	2	0	2
Otitis Media	9	9	0

The clinical results obtained in the treatment of human beings with infections produced by the beta hemolytic streptococci have been striking. While this series of patients who have received treatment is not large enough for accurate statistical analysis based on mortality figures, in practically every instance marked clinical improvement in the patients' conditions occurred promptly after the administration of paraminobenzenesulphonamide or its derivatives.

Their observations lead them to believe that about 48 hours is required before maximum therapeutic effects can be obtained with paraminobenzenesulphonamide or its derivatives. Because of this time factor, they advocate immediate treatment if an infection is considered to be due to hemolytic streptococci. Striking results, therefore, should not be expected when patients with far advanced infections or those who are moribund are treated.

Rules for estimating the amount of drug needed in the treatment of acute infections

When prontosil S 2.5% solution is used the total amount required during the first 24 hours of treatment is calculated on the basis of 1 cc. for each pound (0.5 kg) of body weight up to 120 lbs. (54.4 kg). For patients over this weight the first day's dose is 120 cc. The total daily amount is divided into six parts and a dose is given every four hours by subcutaneous injection. Prontosil S 2.5% solution is rapidly absorbed and should not be administered by the intravenous or intrathecal route.

Sulfanilimide (Prontylin) tablets are to be used only for oral administration. The total dose for the first 24 hours is calculated on the basis of 3 tablets (1 gm) for each 20 lbs. (9.1 kg.) of body weight up to 100 lbs. (45.4 kg.). In the treatment of acute infections they believe that 5 gm. of this substance represents the maximum daily dose in adults which may be used with safety. This total amount is divided into 4 doses given at intervals of six hours.

In cases of severe infection these authors have given paraminobenzenesulphonamide by hyperdermoclysis. The chemical is soluble up to 0.8% in hot physiological solution. In the preparation of this substance for clinical use, they dissolve 0.8 gm. of the substance in 100 cc. of physiological saline which has been brought to the boiling point and then cooled to 90°C. The solution is then cooled to 37°C and given by hyperdermoclysis. 0.8 gm. in 100 cc. saline for patients weighing up to 40 pounds. 1.6 gm. in 200 cc. saline for patients weighing 40 to 80 pounds. 2.4 gm. in 300 cc. saline for patients weighing 80 to 120 pounds. 3.2 gm. in 400 cc. saline for patients weighing over 120 pounds.

An 0.8% solution of paraminobenzylsulfonamide in physiologic solution of sodium chloride may also be used intrathecally in the treatment of streptococcic meningitis. The general practice in administering paraminobenzylsulfonamide intrathecally should closely follow that recommended for the use of antimeningococcus serum in meningococcus meningitis, namely, that rather complete spinal drainage by lumbar puncture should be instituted and then from 15 to 25 cc. of a warm freshly prepared 0.8% solution of paraminobenzylsulfonamide should be permitted to flow in by gravity. The solution should never be injected under pressure.

In severe infection either form of parenteral administration of either drug should be carried out and in addition prontylic tablets in one half the estimated amount should be given orally about four hours after parenteral therapy has been instituted.

In moderately severe infections, the patient is given the estimated amount of either of the two drugs mentioned. They are never used together.

Mild or relatively chronic streptococcal infections can be controlled by sulfanilamid tablets alone, and the total dose may be reduced.

Maximum treatment should be continued until definite improvement is noted.

In case of septicemia two blood cultures should be sterile before dosage is decreased. Clinical appearance of visible lesion is some indication as to when amount of therapeutic can be decreased.

Three to five gms. of paraminobenzenesulfonamide may be given over period of several days without ill effect.

Schwentker, Gelman & Long

These men report 10 cases of meningococcic meningitis treated with sulfanilimide with one fatality. These patients were given 10 to 30 cc. of 0.8% sulfanilimide in physiological saline for each 40 pounds of body weight subcutaneously. Both forms of treatment were repeated every twelve hours for the first two days and once daily thereafter until definite improvement was evident. The subcutaneous injections were continued longer than the spinal injections in a number of cases.

They conclude that the response to treatment with sulfanilimide in all patients seemed quite comparable to that caused by the specific antiserum.

Toxic Manifestations and Excretion of Prontosil and Related Compounds

Domagk

Non-toxic tolerated doses of prontosil.
Mouse--500 mg./kg oral and 125 to 250 mg./kg subcutaneous.
Rabbit--500 mg./kg oral and 25 mg./kg. subcutaneous.

Horlein

Mice tolerate 2 gm./kg. orally and Intravenously, and 4 gm./kg subcutaneously.

Buttle, Gray, and Stephenson

These men showed effects of large doses of prontosil S and paraminobenzenesulphonamide. Average weight of mouse is 19-22 gms.

Prontosil-S 12 mgm. innocuous to mouse .25 mg. kills four out of 6 and 50 mg. five out of six, 100 mg. kills six out of six.

Paraminobenzenesulfonamide (base on HCL) 50 mg. innocuous, 100 mg. kills two out of six, and 200 mg. kills six out of six.

Long & Bliss

A normal human given 100 cc. of paraoral prontosil-S showed fever 8-12 hours, mental and physical lassitude, and headaches. This work has been repeated.

Patients given up to 5 gms. of paraminobenzylsulfonamide show no toxicity.

Normals show malaise and lassitude. 9 mg. of paraminobenzenesulfonamide were given to mice producing a peculiar vestibular dysfunction and spastic paralysis for three hours followed by a return to normal. This would be equivalent to 25 or 30 gms. for an adult human.

Prontosil and prontosil-S are excreted in part at least by kidneys, giving the urine a yellow-red color of the dye.

According to some of the most recent work prontosil and prontosil-S are changed in vivo to paraminobenzylsulfonamide which is excreted through kidneys partly unchanged and partly as the acetylated derivative. Dogs are unable to acetylate this compound. (Marshall)

When patients are treated exclusively with paraminobenzenesulphonamide, no discoloration of urine or skin is noted.

Colebrook and Kenny report that 75% of the patients show epithelial cells in urine, 40% show red blood cells, and about 25% show increased albumin or positive albuminuria. Other workershave failed to confirm this observation.

Nausea, vomiting, and occasionally diarrhea follows the administration of prontosil-S parorally and administration by mouth and may require discontinuance of the drug orally. However, these responses following paroral administration are only transient in character.

No effect on smooth muscles either in

blood vessels, bowel or uterus has been noted.

The rise in temperature has been mentioned before and during the course of treatment it requires keen observation to detect which is due to medicament and which is due to underlying illness.

Dyspnoea and cyanosis has been reported. They may be due to acidosis or sulphemoglobinemia (3 cases reported by Colebrook and Kenny and three cases by Long & Bliss. This rapidly disappears on discontinuance of the drug.

Clinical acidosis has been reported in two cases by Long & Bliss. One case showed CO_2 combining power of 36.2 Volumes %. This patient subsequently returned when in apparent good health and voluntarily took 6.9 gm. of prontylin in 60 hours. At that time the CO_2 combining power dropped from 59.8 Volumes% to 37.2 Volumes %. The second case showed a CO_2 combining power of 27.7 Volumes %.

Fifteen consecutive patients treated with prontylin were studied as to acidosis. All patients showed a fall in CO_2 combining power varying from 1.9 to 27.3 Volume %. The average being 14.1 Volumes %. The degree of fall was found to show a moderate correlation with the dose of prontylin in grams per kilogram given the previous 24 hours.

Jaundice has been reported in one patient while under treatment and recovery promptly occurred when paraminobenzenesulfonamide was discontinued. This patient has subsequently been given large doses of this drug without recurrence of jaundice.

Sulphemoglobinemia has been reported and there has been mention that saline cathartic administered at the same time may play a role in the production of this condition. Sulfhemoglobinemia has been reported in three cases by Long & Bliss. None of these patients had received saline cathartics.

Methemoglobinemia and a morbilliform rash have been reported in the literature read but no description of these conditions could be found in following bibliography.

Summary

1. Three substances, namely prontosil, prontosil-S, and sulfanilimide, have been introduced as more or less specific chemotherapeutic agents in the field of medicine. These substances show marked curative effects against the hemolytic streptococcus, experimentally. The mode of action is unknown.

2. Two of these substances, namely prontosil-S known as prontosil 2.5% solution and sulfanilamid (prontylin), are available in this country for clinical use.

3. Clinically, these substances seem to be indicated in hemolytic streptococcal infections, meningococcic infections, and possibly in infections due to Type III pneumococcus.

4. Considerable clinical work, both accurate and inaccurate, has been done and is being done to determine the exact status of this drug in the field of therapeutics. However, much more work with accurate bacteriological studies are necessary before definite conclusions can be formed along these lines.

Bibliography

1. Anselm, E.
Deutsche Med. Wchnschr. 61:264, (Feb. 14,) 1935.

2. Buttle, Gray, Stephenson
Lancet, 230: 1286, 1936.

3. Colebrook & Kenny
Lancet. 230: 1279, (June 6) 1936.

4. Colebrook & Kenny
Lancet. 231: 1319, (Dec.5) 1936.

5. Colebrook, Buttle & O'Meara
Lancet. 231: 1323, (Dec.5) 1936.

6. Domagk
Ein Beitrag zur Chemotherapie der bakteriellen Infektionen.
Deutsch Med. Wachenschr. 61: 250, (Feb.) 1935.

7. Fourneau E., J. & Mme. J. Trefouel, Nitti & Bovet
Compt. Dend. Soc. de Biol. 122:258, 1936.

8. Geissedet, Despois, Galliot & Mayer
Comp. Red. Soc. de Biol. 121: 1082, 1936.

9. Horlein, S.
Proc. Ray. Saociety Medicine, 29:213, (Feb.) 1936.

10. Kramer, W.
Munchener Medizin Wchnschr. 83: 608, (Apr. 10) 1936.

11. Levaditi & Vaisman
Comp. rend Societe de Biol. 119:946, 1935.

12. Levaditi & Vaisman
Comp. rend. Societe de Biol. 120:1077, 1935.

13. Levaditi & Vaisman
Compt. rend. Acad. des Sciences, 200: 1694, 1935.

14. Levaditi & Vaisman
Comp. rend. Societe de Biol. 121:803, 1936.

15. Ley, Lothar
Munchener Medizin Wochenschrift, 83: 1092 (July 3) 1936.

16. Long & Bliss
J.A.M.A. 108: 32, (Jan. 2) 1937.

17. Long & Bliss
Bull. Johns Hopkins Hosp. 149, 1937.

18. Long & Bliss
Arch. Surg. 34: 351, (Feb.) 1937

19. Marshall, Cutting & Emerson
Science, 85:202, (Feb. 19) 1937.

20. Meyer-Heine & Hygenin
Presse Medicole. 44:454 (Mar. 18) 1936.

21. Nitti & Bovet
Compt. rend. Soc. de Biol. 119:1271, 1935.

22. Proom
Lancet. 232:16, (Jan.2) 1937

23. Scherber, G.
Wien Med. Wchnschr.
85: 284 (Mar. 9) 1935
346 (Mar. 23) 1935
376 (May 30) 1935

24. Scheurer, O.
Medizuische Klinik. 32:739, (May 29) 1936.

25. Scheus, T.
Deutsche Med. Wchnschr. 61: 255, 1935.

26. Schronz, H.
Munchener Med. Wchnschr. 82: 419 (Mar. 14) 1935.

27. Schwentker, Gelman & Long
J.A.M.A. 108: 1407 (Apr. 24) 1937.

28. Southworth, H.
Proc. of Soc. Exp: Bwl. & Med. 36: 58, (Feb.) 1937.

29. Trefouel, J. & Mme. J., Nitti & Bovet
Comp. rend. Soc. de Biol. 120: 756, 1935.

30. Vermelin & Hartman
Bulletin de la Societe d Obs. & Gyn. 25: 158, (Feb.) 1936.

Note: Para-amino-benzene-sulfonamide was apparently first synthesized by Gelmo in 1908. J. pr. Chem. 77:369, (April 3), 1908.

IV. CASE REPORTS

1. , aged 27, male.
Hospital No.
Admitted 3-31-37;
Discharged 4-14-37.

Sore throat

3-10-37 - Developed a severe sore throat.

Arthritis

3-24-37 - Pain in knees and ankles, associated with swelling of ankles. Subsided in 2 days.

Erysipelas

3-30-37 - Developed a red spot under his left eye, which gradually spread over his face and had become swollen and quite painful.

Desquamation- following scarlet fever?

3-31-37 - Admitted. Physical examination: temperature 104, pulse 96, respirations 24; blood pressure 116/60. Appeared quite ill. Face was quite swollen, especially around the left eye, with marked erythema over upper part of face and left eye; this area was quite tender. Marked injection of pharynx. Lymph glands palpable in cervical region. Slight amount of pitting edema over lower ends of tibiae. Generalized desuqmation of skin.

Laboratory

Urine - very occasional white blood cells. Blood - hemoglobin 71%, white blood cells 22,000, 19% lymphocytes, 77% polymorphonuclears, and 4% monocytes; Wassermann, negative. Blood culture - sterile at the end of 137 hours.

Clinical Diagnosis

erysipelas of the face, probably following scarlet fever (the latter being diagnosed by the history).

Prontylin - Recovery

Treatment

Prontylin by mouth grains V, plus 4 injections of 5 cc. of prontosil 2.5% solution intramuscularly, given every 3 hours following admission. This treatment was supported with general supportive treatment, plus local treatment of Burrow's packs. Patient's temperature was 104° on admission, became normal 43 hours after treatment was instituted, and remained so during his hospital stay. The urine during the treatment showed only an occasional white blood cell. The white blood count 6 days after admission was 11,000, with 60% polymorphonuclears, 38% lymphocytes, 1% monocyte, and 1% basophil.

Summary

Twenty-seven year old, white male developed erysipelas following scarlet fever; treated with prontosil 2.5% solution intramuscularly and prontylin tablets by mouth, associated with local treatment of Burrow's packs. Patient apparently responded very well to treatment, and showed no toxic effects of the treatment.

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2. ____, aged 25, female.
Hospital No.
Admitted, 4-13-37;
Discharged, 4-24-37

Sore Throat

4-12-37 - Developed severe sore throat, characterized by rapid onset. With this, there was chilly sensation, anorexia, general malaise, headache, aching through the joints and back. Symptoms progressively became worse.

4-13-37 - Admitted to hospital. Now has occasional unproductive cough; no pain in the chest. Physical Examination, negative except for examination of throat. Tonsils, atrophic; diffuse pharyngitis; no membrane; no cervical adenopathy. Laboratory: Urine, negative Blood - white blood cells 13,600, 73% pmn's, 23% lymphocytes. Icteric index, 5. Culture of throat on blood agar shows a heavy growth of hemolytic streptococci. Blood culture - sterile after 111 hrs. incubation. Temperature on admission, 99.4°.

Prontolyn

4-14-37 - Temperature 104.8°. Throat diffusely injected. Chest, clear. Chilliness persists. Complaining of increase in soreness of throat. Later in day, became mentally confused. Prontylin grains X given every 3 hours.

Improvement

4-15-37 - Temperature 98.6°. Definitely improved.

Vomiting - Gallbladder Pain

4-16-37 - Temperature normal. Patient hungry; in evening ate supper but vomited. Had attacks of pain in gallbladder area which apparently was a recurrence of the previous gallbladder attacks for which the patient had been hospitalized one year before.

4-17-37 - Throat condition apparently cleared up. Temperature, normal. Gall-

bladder pain still present.

4-22-37 - Gallbladder attack cleared up. Up and around.

4-24-37 - Discharged.

Summary

Case is that of a young girl admitted with a typical severe acute sore throat. Temperature rose to 105°. Was delirious. Placed on prontylin grains x, every 3 hours, with immediate response. Patient was kept in the hospital for an intercurrent gallbladder attack, during which period of time the temperature remained normal. The throat condition cleared very appreciably within 24 hours after administration of prontylin.