A New Class of Antifilarial Compounds[1]

Arnold D. Welch, Lawrence Peters, Ernest Bueding, Arthur Valk, Jr., and Aeme Higashi

Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio

THE OCCURRENCE OF FILARIASIS IN members of the Armed Forces stationed in South Pacific areas led to organization by the National Research Council, in 1944, of coordinated investigations of the chemotherapy of this disease. The potency and the margin of safety of organometallic compounds, now used in the treatment of filariasis, may be improved by continued research; however, it was decided to concentrate the effort of this laboratory on compounds containing no heavy metal, in the hope of disclosing a new approach to the chemotherapy of this disease.

Unfortunately for chemotherapeutic research, the human form of the parasite, transmitted by various mosquito vectors, has not been found in other animal species, and it was necessary to restrict the study of new compounds to animals infested with closely related, but not identical, filarial parasites. For this purpose the most practical approach to the problem was afforded by the use of the wild cotton rat, which frequently harbors *Litomosoides carinii*, a filarial worm that resides in the pleural cavity rather than in the lymphatic system (*1*).

Although the details of the techniques employed in "screening" compounds *in vivo* for possible antifilarial activity underwent many modifications as the studies progressed, all procedures were based on the principle that each compound should be given in an amount approaching that maximally tolerated by the animal host. Further, it was considered that the frequency of dosage should be such as to favor the maintenance of a concentration of drug in the tissue fluids bathing the parasite. It was believed that these precautions might increase the possibility of detecting minimal activity in a compound, as a lead for further study.

In general, compounds were administered intraperitoneally at maximally tolerated doses every 8 hours for a total of 18 doses. Forty hours after the final injection, autopsy was performed, and the adult filariae were removed aseptically from the pleural cavities and placed in a sterile nutrient medium for observation; worms removed from an untreated rat were observed simultaneously. Characteristically, unaffected worms remained motile for at least two days, when observed at room temperature. When motility was absent and did not appear within about 8 hours after the worms were removed from the rats, the filariae were considered dead, and other criteria of death supported the validity of this simple measurement.

The effect of drug treatment on microfilariae, either *in vitro* or *in vivo*, was not studied, since it was known that the susceptibility of the embryos may differ from that of the parent worms, and it was the latter only toward which therapy was directed. Also, it was felt that "screening" of unrelated chemical substances for filaricidal activity *in vitro* would give information more likely to be misleading than helpful. Later, when a lead was obtained from studies *in vivo*, and more had been learned concerning the metabolism of these filariae, studies *in vitro* became of great importance.

Among the many compounds studied, none was found to possess appreciable activity until a member of the group of compounds known as the cyanine dyes was tested. This compound, (1-amyl-2,5-dimethyl-3-pyrrole) (1,6-dimethyl-2-quinoline) dimethinecyanine chloride (Chemotherapy Center #348), was completely curative in the maximally tolerated doses used. On further study it became evident that a very high degree of activity was present, since the intraperitoneal injection of 0.1 mg./kg. at 8-hour intervals for 18 doses regularly killed all filarial worms in all the treated animals. Fortunately for the chemotherapeutic program, the high activity of #348 was found to be shared by many others of a large group of compounds synthesized by L. G. S. Brooker and his associates, of the Eastman Kodak Company and supplied to the Committee on Medical Research of the National Research Council through Parke, Davis & Company.[2]

The cyanines were also strikingly active *in vitro*, not only in causing a rapid disappearance of motility but also in inhibiting the consumption of oxygen by adult filariae.

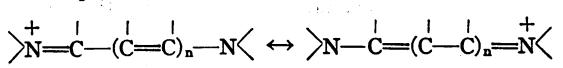
[1] The work described in this paper was done, in part, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Western Reserve University (August 1, 1944–October 31, 1945); in part, under a contract between the Office of the Surgeon General, U. S. Army (November 1, 1945–December 31, 1946); and in part, with the aid of a grant from the U. S. Public Health Service (since January 1, 1947).

[2] From the beginning of the study of this group of compounds, we have enjoyed the finest cooperation from Dr. Brooker and from Parke, Davis Laboratories. Further, this investigation has been facilitated in innumerable ways by Lucille Farquhar, technical aide of the National Research Council, who coordinated studies in this and related fields. Other members of this series of compounds have been studied by R. N. Bieter, H. N. Wright, and their associates at the University of Minnesota.

The oxidative metabolism of the worms was inhibited by concentrations of #348 ranging from 1:25,000,000 to 1:6,000,000, and the decrease in oxygen consumption was associated with a compensatory increase in aerobic glycolysis and a decrease in glycogen synthesis. Under anaerobic conditions no effect on glycolysis was observed. Only with concentrations 1,000–2,000 times higher was the oxygen consumption of mammalian tissue slices or homogenates affected by these compounds, and such concentrations caused severe injury at the site of subcutaneous or intramuscular injection.

For the following reasons it is postulated that these drugs exert their chemotherapeutic effect through inhibiting one or more enzyme systems concerned with oxidative metabolism: (1) Worms removed from rats treated with subcurative doses of active members of the cyanine series showed markedly depressed respiratory activity and an increase in aerobic glycolysis; (2) of the large series studied, every compound active *in vivo* inhibited filarial respiration *in vitro*.

As the study of the cyanines progressed, it became evident that activity in inhibiting filarial respiration *in vitro* could be retained despite rather radical variations in structure. Almost all cyanines (and many styryl) dyes show the effect to some extent, although the activity is by no means always of a high order. The grouping characteristic of the cyanine (and styryl) dyes, whether highly or weakly active, is the amidinium-ion system in which a positively charged quaternary nitrogen is linked

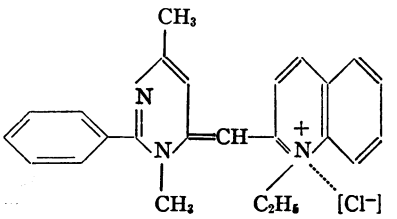


to a tertiary nitrogen by a conjugated chain consisting of an uneven number of members. One or both of the nitrogens may be part of a heterocyclic ring, but activity is not restricted to any particular ring. Any modification which destroys the possibility of amidinium-ion resonance in the compounds causes a disappearance of high activity both *in vitro* and *in vivo*.

Variations in the manner of administration of active members of the series indicated that the drugs were effective when given intravenously, as well as intraperitoneally, but only occasional cures followed massive oral dosage. Cures followed subcutaneous administration of the drugs only when doses equal or close to those maximally tolerated by this route were used. Furthermore, subcutaneous or intramuscular injection caused damage to the tissues which, with certain members of the series, was very severe. The effectiveness of intraperitoneal administration once daily was slightly greater than that following administration every 8 hours; thus, with #348, a total of 1.8 mg./kg. was required when administration was at 8-hour intervals for 6 days, while a total of 1 mg./kg. was curative when the drug was given once daily for 5 days. Actually, cures could be obtained when animals were given a single dose of 1.35 mg./kg.

Since the intravenous route of administration of the drugs appeared to be the only feasible one for man, study of the activity of various members of the series was made in cotton rats, using this mode of administration. Attention was also given to the comparative damaging effects on tissues, in view of the possibility of extravasation.

One member of the series was clearly outstanding; this compound, Chemotherapy Center #863, is probably of the following structure, a name for which is 1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride, although an isomeric structure is possible.



In infested cotton rats, cures were almost invariably produced when #863 was given intravenously in doses of 1.0 mg./kg., repeated 3–6 times at intervals of 1, 3, or even 7 days. This indicates clearly, as did the earlier intraperitoneal studies with #348, that curative results can be correlated rather closely with the total amount of drug administered over a wide range of dosage intervals. This situation resembles that observed with certain organometallic drugs, e.g. as used in the treatment of syphilis. Toxic effects, according to the dosage schedules described, were seen only with individual doses of 10 mg. or more/kg. Accordingly, this route of administration appeared to offer a considerable margin of safety.

Extensive studies of the chronic toxicity of #863 in dogs and in monkeys have been based on the findings in cotton rats. These studies disclosed a mild and reversible renal damage as the only manifestation of chronic toxicity.[3] During intravenous administration there is a transient hypotensive effect, with compensatory tachycardia; this effect is of consequence only when large doses are rapidly administered.

A method for the extraction of #863 from tissues and its spectrophotometric determination at 494 mμ has been devised. The compound rapidly disappeared from the blood of dogs after intravenous administration, but urinary excretion rarely exceeded 5 per cent. Recovery experiments indicated that the drug is metabolically altered. The material is remarkably concentrated in the kidney; when dried from the frozen state, the drug was visible microscopically, predominantly in the convoluted tubules.[4] This finding may account for the observed renal toxicity.

[3] We are indebted to W. B. Wartman for his cooperation in the pathological examination of the tissues of animals subjected to treatment with #863.

[4] These observations were made by Normand L. Hoerr and Arnold Lazarow, to whom our thanks are due.

Studies of the possible effectiveness of #863 in the treatment of human patients infested with the filariae of *Wuchereria bancrofti* have been initiated through the facilities afforded us by the School of Tropical Medicine, San Juan, Puerto Rico.[5] The drug was administered to 27 patients, using various intravenous dosage regimes, without manifestations of systemic toxicity other than transient mild hypotension and tachycardia of no clinical significance. Since the drug usually does not cause an immediate disappearance of microfilariae, in either cotton rats or man, studies of the peripheral blood may be required for many months in order to determine whether sterilization or death of the parent worms has been accomplished.

[5] We are greatly indebted to the director, P. Morales-Otero, for many courtesies; to José Oliver-González, who procured the patients and gave us constant help; and to F. Hernández-Morales, D. Santiago-Stevenson, and Ramón Suárez, Jr., for their clinical aid and excellent cooperation.

Reference

1. CULBERTSON, J. T., and ROSE, H. M. *J. Pharm. exp. Therap.*, 1944, **81**, 189.

Nomenclature of Parenteral Proteases

John H. Ferguson

Department of Physiology, University of North Carolina, Chapel Hill

RECENT INTEREST IN CERTAIN PROteases, which participate in blood coagulation (*5*, *21*) and indirectly related proteolytic phenomena, e.g. fibrinolysis (*3*, *16*, *19*), has run into a confusion of nomenclature which should be settled by general agreement. The story of the *parenteral* proteases and fibrinolytic phenomena was fully reviewed in the monumental work of Oppenheimer (*18*), whose classificatory principles have been widely accepted, with some more modern modifications.

PROTEASE, in the broadest sense, refers to all enzymes associated with protein hydrolysis. PROTEINASE is a general term appropriately used when the substrate is the whole protein molecule. Owing to multiplicity of substrates, enzymes acting under this head must be subclassified, empirically, under such generic terms as (1) *peptases*, (2) *cathepsins* (which has largely replaced the older term, ereptases), and (3) *tryptases*, for the three most common types of animal origin; and (4) *papainases*, for an important group of plant proteinases. It is distinctly intended that these terms group together enzymes on the basis of certain similarities, in the case of the first three, to the well-known alimentary prototypes, pepsin, erepsin, and trypsin(s), *disregarding* differences in origin and certain other differences, e.g. in mode of activation, inhibition, substrate specificity, stability, purity, etc. Even pH optima are currently minimized, as chiefly a matter of protein stability and purity. The recent identification of certain proteinases as crystalline proteins (*17*) and the newest means of testing on pure synthetic polypeptide substrates (*1*) are steps toward characterization of a few of these enzymes by their chemical specificity, in terms, for example, of certain linkages in the substrate molecule. The crystalline enzymes thus studied, however, show multiple points of attack on the protein (polypeptide) molecule. Hence, this approach is more successful as a basis for identification of the *individual* enzymes than as a means of classification (cf. *1*).

The Nobel-laureate work of the Rockefeller investigators (*17*), significantly extending the pioneering efforts of Willstätter and others, clearly outlines the general principle that each proteolytic enzyme really constitutes a *complex system*. This is especially well exemplified in the case of pancreatic trypsin: An inactive precursor or zymogen (TRYPSINOGEN, crystalline) is converted into active enzyme (TRYPSIN, crystalline), particularly through the mediation of an "activator," e.g. ENTEROKINASE (*17*), MOLD KINASE (*11*), etc.[1] A crystalline polypeptide (TRYPSIN INHIBITOR, *pancreatic*) inactivates fully-formed trypsin by formation of a crystalline inactive TRYPSIN + INHIBITOR compound (*17*). A recently crystallized protein TRYPSIN INHIBITOR from *soybean* (*12*) probably acts in the same way. Since there is multiplicity both of inhibitors and of the proteases they inhibit (e.g. chymotrypsin and plasma-tryptase, also), the nomenclature, ANTITRYPSIN, should be used with these reservations. The possibility of KINASE inhibitors, directed against the "activators" rather than against the protease proper, is not fully explored in the work on the pancreatic enzymes.

The "thromboplastic-enzyme" theory of blood coagulation (*5*) draws attention to similarities between the experimental actions of pancreatic trypsin and natural TRYPTASE enzymes demonstrable in, but not yet isolated from, plasma (serum), blood corpuscles (including platelets), and tissue source materials. Very recent work (*6*) is completely confirmatory of this idea and suggests adoption of the following nomenclature, in close analogy with the pancreatic tryptase system:

I. TRYPTASE: active protease, prefixed by name of

[1] Crystalline trypsin assists its own formation from crystalline trypsinogen in an autocatalytic manner, and certain factors, e.g. CALCIUM, are important accessories because they prevent loss of enzyme through side reactions of an inactivating nature (*14*).

A New Class of Antifilarial Compounds

Arnold D. Welch, Lawrence Peters, Ernest Bueding, Arthur Valk Jr. and Aeme Higashi

Science **105** (2732), 486-488.
DOI: 10.1126/science.105.2732.486