Tests of 2,4-Diaminopyrimidines On Toxoplasmosis

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Tests of several compounds of the 2,4-diaminopyrimidine group have shown two members of the group, particularly 2,4-diamino-5-(4′-chlorophenyl)-6-ethyl pyrimidine (DCEP), to be active against toxoplasmosis.

DCEP is an effective antimalarial drug, as shown by Falco and associates (*1*), with a proguanil equivalent of 40 against *Plasmodium gallinaceum* and 200 against *Plasmodium berghei.*

To test their antitoxoplasmic activity, DCEP and related pyrimidines were screened by our laboratory for their effect against *Toxoplasma gondii* in the mouse. We are reporting the results of these screening tests and more extensive tests with DCEP.

Methods

Young mice (weight about 20 gm.) of the NIH general purpose strain and a strain of *T. gondii* isolated by this laboratory from the Norway rat were used in the tests. With an intraperitoneal inoculum of 20,000 organisms, prepared by diluting peritoneal exudate with physiological saline, mice invariably died, usually in 7±1 days. Six mice so infected were used in the screening of each new drug. Untreated controls and clean controls were kept for each group of tests.

Drugs were administered in pulverized diet starting just after inoculation and continuing for 14 days. Dosages are stated as milligrams percent in diet and were at the maximum tolerated dose (MTD) if it was known. Conversion to milligrams per kilogram was made by using an average daily food intake figure of 4 gm. Drug activity was measured by the degree of prolongation of life of treated mice over the controls. Significance was measured by comparing the mean duration of life of the untreated and treated groups by means of the T-test (*6*).

The more extensive tests with DCEP required the determination of the dose-effect relationship against similarly induced infections. Nine or more mice per dosage level were used. The Litchfield and Wilcoxon (*4*) method of calculating the median effective dose (MED) was utilized. Since cure was infrequent, the MED was defined as the dose which permitted half the mice to survive 10 days or longer. Ten days was the period used because the test showed greater sensitivity at that time than at 14 days, thus facilitating comparison with less active drugs and with drugs of limited supply, which necessitated a shorter treatment regimen.

Results

The results of the screening tests are given in table 1; results of one test with sulfadiazine

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Table 1. Summary of screening tests with 2,4-diaminopyrimidines

Drug	Dosage (mg. percent in diet)	Mean days to death (controls)	Mean days to death (experiment)	Delay of death due to drug [1]	Proguanil equivalent [2] *P. berghei*
5-(4′-chlorophenyl)-6-ethyl (DCEP)	8	7.3	10.8	+3.5(S)	200
	12	7.3	10.5	+3.2(S)	----------
5-(4′-chlorophenyl)-6-*n*-amyl	100	6.8	9.6	+2.8(S)	8
5-(4′-chlorophenoxy)-6-methyl	250	6.8	7.4	+.6	.7
5-(4′-nitrobenzyl)-6-methyl	50	6.8	6.8	0	1.5
Sulfadiazine	500	7.3	12.2	+4.9(S)	----------

[1] In calculating this mean all animals still living on the 14th day are considered as having lived just this long; letter (S) indicates significant difference between test and control.
[2] From Falco and associates (*1*).

are included for comparison. DCEP and 2,4-diamino-5-(4′-chlorophenyl)-6-n-amyl pyrimidine were significantly active. DCEP was considered sufficiently effective to warrant further investigation, since several animals not only lived longer than the controls, but also survived indefinitely.

The dose-effect curve for DCEP is shown in the chart. The MED was calculated as 9 mg. percent in the diet (95-percent confidence limits, 15 and 5). This is the equivalent of about 18 mg./kg. per day (calculated on the basis of 4 gm. of food consumed per day). According to data in this laboratory, sulfadiazine has, in comparison, a MED of 40 mg. percent in the diet. Gram for gram, DCEP is more effective, but its therapeutic efficiency is much lower, as 9 mg. percent is close to the MTD, which was found to be 32 mg. percent; whereas, the MTD of sulfadiazine is at least 12 to 24 times the MED.

DCEP appeared to cure some mice; a number of animals survived until killed or challenged 42 days after the day of infection. Table 2 presents these data and data on survival until the end of the 14-day treatment period. The number of mice which survived for 14 days was only slightly smaller than the number surviving for 10 days. Of the 23 mice which survived 14 days, 9 were still living at 42 days. With one exception, all of the mice which died between the fourteenth and the forty-second day died within a week of the end of the treatment period. This was somewhat different from groups given sulfadiazine; post-treatment deaths in these mice occurred frequently two or more weeks after the end of treatment (*5*).

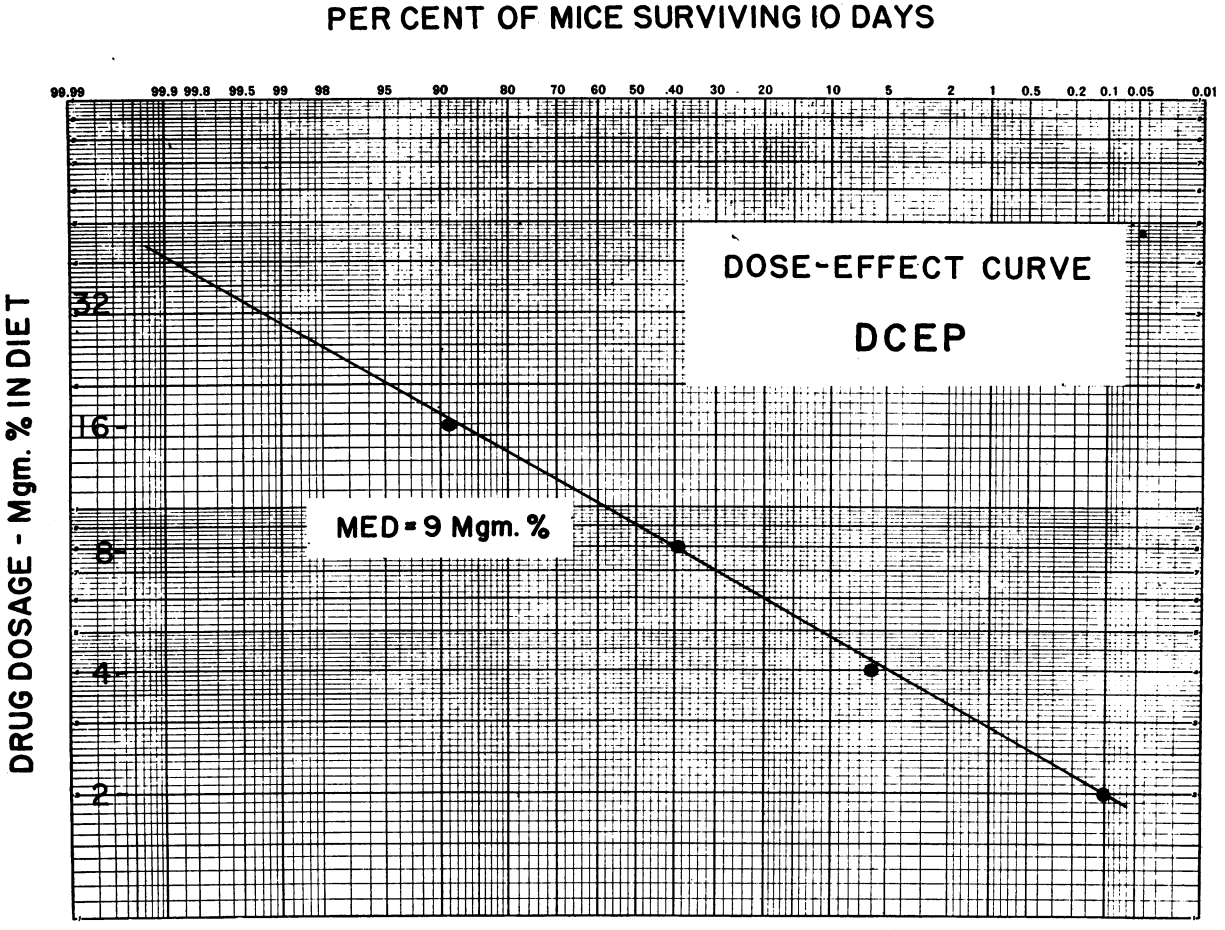
In order to determine if the mice surviving 42 days were cured (free of organisms), four of the nine mice were killed, and suspensions of brain and liver tissue from each were inoculated intraperitoneally (i. p.) into two clean mice. One of the mice inoculated from one survivor died from toxoplasmosis in 6 days; none of the other mice developed the disease during a 42-day observation period.

To determine if surviving animals were immune to reinfection, five of the survivors were challenged with an i. p. inoculation of 20,000 *Toxoplasma* organisms on the forty-second day. Four of the mice died in the usual 7 ± 1 days, but one survived until the tenth day. The last finding may possibly have significance in indicating some degree of immunity.

None of the animals killed on the forty-second day showed any organisms in smears made from brain, liver, spleen, peritoneal fluid, and lung.

Table 2. Survival and possible cure after treatment with DCEP

Dosage (mg. percent in diet)	Number of animals	Survived 10 days	Survived 14 days (end of treatment)	Survived 42 days (cured?)
32 (toxic)	9	4	3	2
16	15	10	9	4
12	6	2	2	2
8	24	11	8	1
4	18	1	1	0
2	9	0	0	0
1	9	0	0	0



Dose-effect curve for 2,4-diamino 5-(4'-chlorophenyl)-6-ethyl pyrimidine.

Discussion and Conclusions

These findings add another chemical group to those known to be active against toxoplasmosis. They also illustrate an instance of parallelism between antimalarial and antitoxoplasmic activity. Experiments are now in progress to determine if sulfadiazine and 2,4-diaminopyrimidines act synergistically as in malaria therapy (*2*) and if pteroylglutamic acid (PGA) antagonizes their effect (*3*).

The investigation so far indicates, but does not prove conclusively, that DCEP is a curative drug in some instances. More animals must be subjected to subinoculation tests, and other animals must be observed for longer periods of time. Further tests must be made using large inoculums. In any event, the efficiency (ratio MTD/MED) of DCEP alone is so low (about 3 or less) that it is not likely to be a practical drug in human toxoplasmosis although the different host may affect its action. If DCEP proves to act synergistically with sulfadiazine then it may be of practical importance in enhancing the effect of that drug. If it is antagonized by PGA, interesting hypotheses with regard to the physiology of *Toxoplasma* may be raised.

Summary

Screening of compounds for antitoxoplasmic activity showed two members of the 2,4-diaminopyrimidine group to have effect. The most active compound was 2,4-diamino-5-(4'-chlorophenyl)-6-ethyl pyrimidine. Defining the effective dose as the dose permitting 10-day survival, this compound had a MED of 9 mg. percent in the diet as compared with 40 mg. percent for sulfadiazine under identical conditions.

The efficiency of the drug was low, as the MTD/MED ratio was about 3 or less. The cure rate following 14-day treatment was also low since only 9 of 33 mice (27 percent) given doses higher than the MED survived for 42 days.

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Since preparing this paper, it has come to the attention of the authors that Dr. W. A. Summers of the Indiana University Medical Center has tested some of the compounds reported in this paper with results parallel to those reported here. Dr. Summer's work will be separately published.

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International Tuberculosis Control Program

In a 4-year international tuberculosis campaign involving 22 countries on five continents, the United Nations International Children's Emergency Fund (UNICEF) and three Scandinavian relief agencies tested 37 million children and young adults and vaccinated nearly 17 million with BCG.

The Scandinavian associates—the Danish Red Cross, Norwegian Relief for Europe, and the Swedish Red Cross—started the work of tuberculosis control projects after World War II and were joined by the UNICEF in 1948. They have now withdrawn from the program after fulfilling their commitments.

Czechoslovakia, Poland, Hungary, and East Germany participated in the European phase of the program. Poland was highest in numbers of vaccinations with 2,535,026. Czechoslovakia was second, and East and West Germany, considered together, were third.

The UNICEF is continuing the international campaign and plans to test the entire child populations of Asia, Latin-America, and the Eastern Mediterranean countries. Five million children already have been tested in Ceylon, India, and Pakistan. The World Health Organization is responsible for the technical aspects of the program.

The results of the campaign will not be fully known until the children and young adults vaccinated have reached maturity. However, already there are some favorable indications in Poland, where very few of the persons vaccinated have contracted tuberculosis.