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THE ANTIVIRAL ACTIVITY OF ACRIDINES IN EASTERN EQUINE ENCEPHALOMYELITIS, RIFT VALLEY FEVER AND PSITTACOSIS IN MICE, AND LYMPHOGRANULOMA VENEREUM IN CHICK-EMBRYOS

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Previous reports (Hurst, Melvin, and Peters, 1952; Hurst, Peters, and Melvin, 1952) described the prophylactic and therapeutic activity of mepacrine against some of the smaller viruses infecting mice. This activity was not shared by a limited number of other acridines, some of which were fairly closely related to mepacrine, or by chloroquine or pamaquin in which the basic side-chain of mepacrine is attached to the quinoline nucleus. A later report (Hurst, Snow, and Roberts, 1955) dealt with the basiphil particles deposited in the liver and some other organs and tissues following administration of mepacrine, particles which had already been observed by Siegel and Mushett (1944) and by Fitzhugh, Nelson, and Calvery (1945). The details of formation and distribution of these particles differed somewhat in other animal species (guinea-pig, rat, rabbit, chick, monkey, and dog), and in a given species no correlation existed between their formation and the presence of a therapeutic effect against equine encephalomyelitis. The therapeutic effect was outstanding only in the mouse, and apparently lacking in several animal species.

Collaterally with these studies we examined other acridines for therapeutic activity, and for the ability to give rise to basiphil particles, in the mouse. Because of the effect of certain nitroacridines on the largest viruses (Eaton, van Allen, and Wiener, 1947; Hurst, 1948), we also examined a number of compounds for activity in psittacosis and lymphogranuloma venereum. A few were tested against Rift Valley fever.

METHODS

Since the effect of a single dose of mepacrine (10 mg./20 g.; but see footnote Hurst, Snow, and Roberts, 1955) given near the time of infection with equine encephalomyelitis virus is almost as good as that of twice-daily doses of the drug, and since we were seeking a degree of activity comparable to or greater than that of mepacrine, we normally administered a single oral dose of the various compounds 24 hr. before the virus. Where possible we gave 10 mg./20 g., but at times, owing to toxicity, we had to be content with smaller doses. Sometimes we studied also the effect of twice-daily oral doses over a period of 6 or more days. At times, with compounds known to be very sparingly soluble, we had recourse to intravenous injection; or, rarely, to dispersion in oil, instead of in an aqueous medium, in the hope of promoting absorption from the alimentary canal. Other details of technique, including the choice of mice, and the maintenance of virus, have been discussed in an earlier paper (Hurst, Melvin, and Peters, 1952).

The course of development of macroscopic coloration in the tissues of mice treated with mepacrine, and of histological changes in the liver and elsewhere, suggested that 48 hr. after administration of the compound was a suitable time for detecting "persistent" coloration and basiphil particles, and accordingly we examined treated, uninfected animals after this interval. Examination of frozen-dried tissue never revealed basiphil particles when they had not been detected after conventional methods of processing the tissues.

The experiments with lymphogranuloma and psittacosis employed viruses and techniques described previously (Hurst, Peters, and Melvin, 1950).

RESULTS

Equine Encephalomyelitis

The first indication that other acridines might possess therapeutic activity against equine encephalomyelitis came from discussions with Professor A. Albert, who very kindly made available two compounds for which he predicted activity. These compounds, "Nor-atabrine" [2-chloro-5-(4-diethylaminobutylamino)-7-methoxyacridine+2HCl+$3H_2O$] and "Acranil" [2-chloro-5-(3-diethylamino-2-hydroxypropylamino)-7-methoxyacridine dihydro-

chloride],* differ from mepacrine only in a slightly modified basic side-chain. Both, and especially acranil, appeared to be more toxic than mepacrine, in that they caused more loss of weight and condition in treated mice. Both, however, showed therapeutic activity against equine encephalomyelitis. Treatment with acranil led to an appreciable reduction in mortality associated with an increased mean-survival-time of the animals that died; the action of nor-atabrine was mainly confined to increasing survival-time. Both drugs caused "persistent" macroscopic coloration of the tissues, and the livers contained abundant basiphil particles which with acranil were larger and far more conspicuous than with mepacrine.

Other compounds since examined are listed in Table I. Here, to make the data from many experiments more manageable, we have expressed the therapeutic activity as a percentage of that of mepacrine *in the same experiment*. If treatment with mepacrine resulted in a sparing of 16 lives as compared with the untreated controls, and treatment with another compound a sparing of 8 lives, the latter was deemed to possess an activity of 50%. This method of computation does not take into consideration the mean period of survival of the animals which died, but major effects—60% or more—are almost always accompanied by a correspondingly increased mean-survival-period, while appreciably lesser effects are often not reproducible.

The following information will assist in evaluating the results of treatments with less than the normal dose of 10 mg./20 g. After a single oral dose of 10 mg./20 g. mepacrine given 24 hr. before virus, 0 to 3, or seldom 4 or more, mice die in a group of 20, whereas among the controls 16 to 19, or sometimes 15 or 20, animals die. A dose of 5 mg./20 g. in 11 consecutive experiments gave effects varying from 56 to 109%, with a mean of 89%; a dose of 2 mg./20 g. gave a mean of 64% in five experiments; and a dose of 1 mg. gave 36 and 31% in two experiments.

Table I shows that a number of acridines other than mepacrine possess considerable activity against equine encephalomyelitis in mice. In the order in which they appear in the table, these are 16,469, 16,468, 5,770, 10,064, 15,431, 17,465, 16,470, and 16,688. Where tested by administration of a range of doses, none appeared significantly more active than mepacrine. All these compounds produced "persistent" coloration of the tissues and gave rise to basiphil particles in the liver, usually to about the same extent as mepacrine. Sometimes, however, as with 16,468, macroscopic coloration was much more intense than with mepacrine, and the basiphil particles were far coarser and more numerous; in the liver cells they frequently attained a diameter of 2 μ, and in the Kupffer cells of 9 μ. Doses of this compound larger than 5 mg. caused extensive necrosis of the liver; the dead cells rarely contained any basiphil material, indicating both their rapid destruction and the primary involvement of the Kupffer cells noted previously (Hurst, Snow, and Roberts, 1955).

A second group of compounds (15,430, 16,570, and 16,527) apparently possessed less therapeutic activity, but coloured the tissues and produced basiphil particles. A third group (15,243, 16,372, 15,541, and 16,286), showing about the same apparent activity as the second group, seemed to differ in their inability to stain the tissues and to produce basiphil particles.

While, therefore, major activity seemed always to be accompanied by these pigmentary changes, lesser degrees of activity, if genuine, were not necessarily so associated. Conversely, with 17,359 an appreciable tendency to produce pigmentary change was not accompanied by a convincing therapeutic effect. The behaviour of 6,769 will be mentioned below.

None of a series of acridans or acridones possessed activity; neither did three dialkylamino-alkylamines nor several quinolines (including chloroquine, previously found devoid of activity by Hurst, Peters, and Melvin, 1952).

Rift Valley Fever

A few of these compounds were examined for activity against the virus of Rift Valley fever; against this infection acranil, 16,469, 16,470, and 16,688 showed activity; nor-atabrine, 16,468 and 5,770 were probably active; and 15,517, 16,472, 15,810, and 15,518 were inactive. These results agree well with those in equine encephalomyelitis.

Lymphogranuloma Venereum and Psittacosis

The maximum tolerated dose of many acridines given intravenously or intraperitoneally to mice is a mere fraction of that accepted orally, and sometimes is below that which experience would suggest as likely to be needed for therapeutic activity against equine encephalomyelitis. Similarly, many are poorly tolerated by the developing chick-embryo. Apparent lack of activity against lymphogranuloma virus in the embryo may therefore reflect only the inadequacy of the dose that can be administered. The following compounds showed no appreciable activity when a single dose was given $2\frac{1}{2}$ hr. following virus into the yolk-sac (the figures in parentheses

* In this, as opposed to earlier papers, we use the nomenclature recommended by the Chemical Society.

TABLE I

EXAMINATION OF VARIOUS ACRIDINES, ETC., FOR ACTIVITY AGAINST EASTERN EQUINE ENCEPHALITIS IN MICE

Single doses were given, or twice-daily treatment begun, 24 hr. before virus in groups of 20 mice. The results are expressed as percentages of those produced by 10 mg. mepacrine *in the same experiment* (for details see text). A single asterisk indicates a significant therapeutic effect at the $P=0.05$ level, as calculated by means of the tables of Loewenthal and Wilson (1939). A double asterisk indicates a significant effect at the $P=0.005$ or lower levels. The percentages needed to establish a significant difference from the effect of mepacrine varied, in the different experiments, from 33 to 50 ($P=0.05$) or 50–64 ($P=0.005$). (S) = increased mean period of survival of fatal cases exceeding that in controls by more than one day.

Cmpd. No.	Name of Compound	Dose (mg./20 g.) and Route	Therapeutic Effect (Mepacrine = 100)
	Acridines		
—	Acridine	20 oral	−6, 0
		20 × 2 oral	11
		20 × 1 i.v.	−13, 0
15,243	5-Benzylacridine	10 oral	60, 27 (S)
		10 ,, (in oil)	23
15,281	5-(2-Piperidinoethyl)-acridine dihydrochloride	10 oral	17, −20
—	5-Aminoacridine (" Aminacrine ")	1 ,,	17, 39, −21
		12 × 0·5 oral	−33, 17, 22, −14
16,256	5-[3-(2-Hydroxyethylamino)-propylamino]-acridine dihydrochloride	10 oral	0
		5 ,,	30
		2 ,,	7
18,006	5-(4-Diethylamino-1-methylbutylamino)-acridine dihydrochloride	5 ,,	13, −6
11,535	5-Acridone	4 i.v.	−13, −36
		12 × 2 i.v.	−13, −25
11,536	Acridan	10 oral	0
		1 i.v.	−6
15,244	5-Benzylacridan	10 oral	−10, 20
15,543	Sodium acridan-5-sulphonate	10 ,,	20, 0
15,468	1-Methoxyacridan	10 ,,	0
		1 i.v.	0
7,706	5-Amino-3-methoxyacridine	10 oral	22
15,372	5-[2-(2-Hydroxyethylamino)-ethylamino]-3-methoxyacridine dihydrochloride	10 ,,	−20,27 (S)
15,517	5-[2-(2:3-Dihydroxypropylamino)-ethylamino]-3-methoxyacridine dihydrochloride	10 ,,	−20, 13
		12 × 5 oral	20
16,469	5-(2-Diethylaminoethylamino)-3-methoxyacridine dihydrobromide	10 oral	100 (S)**
		5 ,,	88 (S)**
16,115	5-[3-(2-Hydroxyethylamino)-propylamino]-3-methoxyacridine dihydrochloride	10 ,,	17 (S)
16,468	5-(3-Diethylaminopropylamino)-3-methoxyacridine dihydrobromide	5 ,,	50 (S)*
		2 ,,	50 (S)*
		1 ,,	31 (S)
16,472	5-(2-Diethylaminoethylmethylamino)-3-methoxyacridine dihydrobromide	10 ,,	25
12,642	3-Methoxyacridan	10 ,,	13
		1 i.v.	0
16,287	3-Butoxy-5-[3-(2-hydroxyethylamino)-propylamino]-acridine dihydrochloride	5 oral	−29
17,236	2-Chloro-7-methoxyacridine	10 ,,	6
		1 i.v.	13
11,838	2: 5-Dichloro-7-methoxyacridine	10 oral	0
15,810	2-Chloro-5-*p*-dimethylaminophenyl-7-methoxyacridine	10 ,,	6, 0
17,381	2-Chloro-5: 7-dimethoxyacridine	10 ,,	15
		10 ,, (in oil)	0
		5 i.v.	−15
17,213	2-Chloro-5-ethoxy-7-methoxyacridine	10 oral	19
17,548	2-Chloro-7-methoxy-5-thiolacridine	10 ,,	−27
		12 × 10 oral	−18

TABLE I—*continued*

Cmpd. No.	Name of Compound	Dose (mg./20 g.) and Route	Therapeutic Effect (Mepacrine = 100)
17,754	S-(2-Chloro-7-methoxy-5-acridinyl)-aminothiuronium chloride	10 oral	−45
		12 × 2 5 oral	−18
17,534	S-(2-Chloro-7-methoxy-5-acridinyl)-thioglycollic hydrazide	10 oral	9
4,020	5-Amino-2-chloro-7-methoxyacridine	10 ,,	31, 22 (S), 0
11,722	2-Chloro-7-methoxy-5-methylaminoacridine acetate	10 ,,	−6, −36
		12 × 2 oral	−13, −17
11,721	2-Chloro-7-methoxy-5-*iso*propylaminoacridine acetate	20 oral	−50*
		12 × 2 oral	−6, −33
11,743	5-*n*Butylamino-2-chloro-7-methoxyacridine acetate	10 oral	−42
		12 × 2 oral	−33
16,740	2-Chloro-5-(2: 3-dihydroxypropylamino)-7-methoxyacridine	10 oral	0
16,525	N-(2-Chloro-7-methoxy-5-acridinyl)-β-alanine	10 ,,	−14
15,430	2-Chloro-5-[2-(2-hydroxyethylamino)-ethylamino]-7-methoxyacridine dihydrochloride	10 ,,	40, 33 (S)
16,372	2-Chloro-5-[2-(2: 3-dihydroxypropylamino)-ethylamino]-7-methoxyacridine dihydrochloride	10 ,,	20, 47 (S)*
16,897	2-Chloro-5-(2-benzylaminoethylamino)-7-methoxyacridine dihydrochloride	10 ,,	14
15,432	2-Chloro-5-[3-(2-hydroxyethylamino)-propylamino]-7-methoxyacridine dihydrochloride	10 ,,	60, 40*
16,895	2-Chloro-5-[3-(2-hydroxyethoxy)-propylamino]-7-methoxyacridine hydrochloride	10 ,,	−14
5,770	2-Chloro-7-methoxy-5-(3-piperidinopropylamino)-acridine dihydrochloride	10 ,,	86 (S)**, 64 (S)**
		5 ,,	93 (S)**
		2 ,,	57*
		1 ,,	29
4,160	2-Chloro-5-dimethylamino-7-methoxyacridine	10 ,,	−13, 0
		2 i.v.	0, 0
		12 × 2 i.v.	0
7,337	2-Chloro-5-piperidino-7-methoxyacridine	10 oral	
		2 i.v.	0, 0
		12 × 1 i.v.	−13, 0
15,156	2-Chloro-7-methoxy-5-*p*-methoxycarbonylanilinoacridine	10 oral	−30, 0
		12 × 5 i.p.	0
11,742	2-Diethylaminoethyl-*p*-(2-chloro-7-methoxy-5-acridinylamino)-benzoate dihydrochloride	20 oral	11, −25
		12 × 5 oral	17
15,187	*p*-(2-Chloro-7-methoxy-5-acridinylamino)-benz-(4-diethylamino-1-methylbutyl)-amide dihydrochloride	10 oral	40*, 10
10,064	2-Chloro-5-(4-diethylamino-1-methylbutylamino)-7-methoxyacridine di-*N*-oxide dihydrochloride (" Mepacrine di-*N*-oxide ")	10 ,,	65*, 88 (S)**
		5 ,,	50 (S)
		2 ,,	6 (S)
		1 ,,	0
18,959	2: 5-Dichloro-7-hydroxyacridine	10 ,,	−13
19,178	2-Chloro-5-(4-diethylamino-1-methylbutylamino)-7-hydroxyacridine citrate	10 ,,	13
1,616	2-Chloro-7-methoxyacridone	10 ,,	−20
13,622	2-Chloro-7-methoxyacridan	10 ,,	0
		2 i.v.	6
17,028	5-Amino-3-chloro-7-methoxyacridine	10 oral	25, 6 (S), 7
16,687	N-(3-Chloro-7-methoxy-5-acridinyl)-β-alanine	10 ,,	−14
15,431	3-Chloro-5-[2-(2-hydroxethylamino)-ethylamino]-7-methoxyacridine dihydrochloride	10 ,,	60, 66(S)**
15,518	3-Chloro-5-[2-(2: 3-dihydroxypropylamino)-ethylamino]-7-methoxyacridine dihydrochloride	10 ,,	11, −60*
		12 × 5 oral	−13
15,541	3-Chloro-5-[3-(2-hydroxyethylamino)-propylamino]-7-methoxyacridine dihydrochloride	5 oral	60, 33

TABLE I – *continued*

Cmpd. No.	Name of Compound	Dose (mg./20 g.) and Route	Therapeutic Effect (Mepacrine =100)
16,570	3-Chloro-5-(3-ethylaminopropyl-amino)-7-methoxyacridine dihydrochloride	5 oral	36 (S)
15,809	3-Chloro-7-methoxyacridan	10 ,, 1 i.v.	- 6 0
17,465	5-(4-Diethylamino-1-methylbutyl-amino)-3-methoxy-7-nitro-acridine	10 oral 5 ,, 2 ,, 1 ,,	87 (S)**, 82 (S)** 108 (S)** 64 (S)* 45
17,466	3-Amino-5-(4-diethylamino-1-methylbutylamino)-7-methoxy-acridine trihydrochloride	10 ,,	9 (S), 6
18,170	5-Chloro-3-methoxy-8-nitro-acridine	10 ,,	−12
19,198	5-(4-Diethylamino-1-methylbutyl-amino)-3-methoxy-8-nitro-acridine	5 ,,	0
17,689	5-Chloro-2: 3-dimethoxy-8-nitro-acridine	10 ,,	−27, −19
6,769	5-(3-Diethylamino-2-hydroxy-propylamino)-2: 3-dimethoxy-8-nitroacridine	5 ,, 12×2 oral 6×0·25 i.p.	25, −6 (S) 0 0
18,731	5-(4-Diethylamino-1-methylbutyl-amino)-2: 3-dimethoxy-8-nitro-acridine dihydrochloride	10 oral	−20
17,359	2-Chloro-5-(4-diethylamino-1-methylbutylamino)-7-*p*-nitro-benzyloxyacridine	10 ,, 5 ,, 12×1 oral	17, 0 (S) 9 (S) −9 (S)
16,286	2-Chloro-5-[3-(2-hydroxyethyl-amino)-propylamino] acridine dihydrochloride	10 oral	50, 40 (S)*
15,875	2-Chloroacridan	10 ,, 1 i.v.	−6 −6
15,876	3-Chloroacridan	10 oral 1 i.v.	6 0
10,997	3-Chloroacridone	10 oral 1 i.v.	0 6
16,527	2: 7-Dichloro-5-[3-(2-hydroxy-ethylamino)-propylamino]-acridine dihydrochloride	10 oral	36 (S)
16,741	1: 4-Dichloro-5-[3-(2-hydroxy-ethylamino)-propylamino]-acridine dihydrochloride	10 ,,	0
16,473	1: 3-Dichloro-5-(2-diethylamino-ethylamino)-acridine dihydro-bromide	10 ,,	50 (S)*, 0 (S), 17 (S)
16,470	1: 3-Dichloro-5-(3-diethylamino-propylamino)-acridine dihydrobromide	10 ,, 5 ,, 2 ,, 1 ,,	100 (S)**, 121 (S)** 121 (S)** 50 (S)* −20
16,688	1: 3-Dichloro-5-[3-(2-hydroxy-ethylamino)-propylamino]-acridine dihydrochloride	10 ,, 5 ,, 2 ,, 1 ,,	100 (S)**, 107 (S)** 79 (S)** 50 (S)* 0
18,044	1: 3-Dichloro-5-(4-diethylamino-1-methylbutylamino)-acridine dihydrobromide	2 ,,	0 (S), 0
10,996	1: 3-Dichloroacridone	10 ,,	0
15,698	3: 7-Dimethylacridan	10 ,, 2 i.v.	19, −13 6, −7
17,006	Diacriflavine	10 oral 2·5 ,,	−6 0, 0
	Euflavine	5 ,, 12×0·5 oral	11, 17, 14 −33, 6, 17,
	Proflavine		0
17,650	Polymer from proflavine dihydro-chloride and hexamethylene-*bis*-dicyandiamide	10 oral	−27
	Quinolines		
15,157	7-Chloro-4-*p*-carboxyanilino-quinoline	12×2 i.p.	0
16,569	7-Chloro-4-[3-(2-hydroxyethyl-amino)-propylamino]-quinoline	10 oral	0
	Chloroquine	10 ,, 12×1 oral 12×0·5 i.p.	0 −11, −33 −11, 7, 24

TABLE I – *continued*

Cmpd. No.	Name of Compound	Dose (mg./20 g.) and Route	Therapeutic Effect (Mepacrine =100)
5,120	4-(3-Diethylaminopropylamino)-6-methoxy-3-methylquinoline dihydrochloride	10 oral 12×2 oral	−20 −13
5,372	4-(3-Diethylaminopropylamino)-2: 3-dimethylquinoline sulphate	10 oral	40 (S)*, 11, 10, 19
16,965	4: 4′-(Ethylenedioxy)-*bis*-7-chloroquinoline	5 ,,	0
16,966	7-Chloro-4-(2-ethoxyethoxy)-quinoline	10 ,,	−6
	Amines		
3,857	4-Diethylamino-1-methylbutyl-amine	5 ,,	−20
16,917	*N*-(4-Diethylamino-1-methyl-butyl)-guanidine sulphate	10 ,,	0
17,104	*N*-(4-Diethylamino-1-methyl-butyl)-benzylamine	10 ,, 5 ,,	25, 6 −14

are the larger doses (in mg.) employed; in addition each compound was tested at one-quarter or one-third of this dose):

7,337 (0.2), 10,064 (0.5), 11,742 (0.2), 15,156 (0.5), 15,187 (1.25), 15,243 (10), 15,244 (10), 15,281 (2.5), 15,372 (0.1), 15,430 (0.5), 15,431 (0.5), 15,541 (0.2), 16,115 (0.05), 16,256 (0.05), 16,286 (0.05), 16,527 (0.05), 16,570 (0.5), 16,687 (1.25), 16,740 (0.5), 16,741 (0.5), 17,359 (0.2), 17,465 (0.2), 17,466 (1.25), 17,534 (0.1), 17,548 (0.05), 17,689 (2.5), 17,754 (0.05), 18,006 (0.2), 18,044 (2.5), 18,170 (0.01), 18,959 (0.5), 19,178 (0.5), 19,198 (0.5).

In all tests mepacrine itself was included and was always without appreciable effect. However, one of the compounds listed above (19,198) possessed definite activity in the mouse but none in the chick-embryo.

Of compounds available in sufficient quantity for test against psittacosis in mice, the following showed no activity when given at the dose stated, twice daily for 12 days, beginning 4 hr. before virus:

7,337 (5 mg. oral), 11,742 (5 mg. oral), 15,156 (5 mg. i.p.), 15,430 (5 mg. oral), 15,431 (2 mg. oral), 16,687 (5 mg. oral), 17,466 (2 mg. oral), 17,754 (2 mg. oral), 18,959 (5 mg. oral), 19,178 (2 mg. oral).

Euflavine, proflavine, and mepacrine were also inactive. As shown in Table II, three compounds had considerable activity. Compound 6,769—which, as "Nitroakridin 3582," was previously shown by Eaton, van Allen, and Wiener (1947), and by Hurst (1948), to possess therapeutic properties against the largest viruses—was now administered orally and in larger doses than heretofore. Compound 18,731 was a similar compound with the mepacrine side-chain, and 19,198 an $-NO_2$ analogue of mepacrine itself. Of these, the second compound was less toxic than the others and appeared to be the best, both when judged by the mortality in treated animals and by the clinical condition of the mice during the period of therapy. All these compounds were active when dosing was deferred until 48 hr. after infection, and with 18,731 the clinical condition of the mice was nearly as good

TABLE II

ACTION OF THREE NITROACRIDINES AGAINST PSITTACOSIS IN MICE

Virus was given intraperitoneally, drug orally. The figures in parentheses are the mean periods of survival of fatal cases in days. Compound 6769 is identical with "Nitroakridin 3582" prepared originally by A. G. Hoechst, Main.

No.	Name	Oral Dose (mg./20 g., b.i.d.)	Time of First Dose	Deaths in 10 Mice	
				(i)	(ii)
—	No drug given	—	—	9 (6·1)	9 (7·3)
—	Aureomycin	2	48 hr. after virus	0	0
6,769	5-(4-Diethylamino-2-hydroxypropylamino)-2: 3-dimethoxy-8-nitroacridine dihydrochloride	2	4 hr. before virus	1 (12·0)	2 (16·0)
		2	48 hr. after virus	—	2 (11·0)
18,731	5-(4-Diethylamino-1-methylbutylamino)-2: 3-dimethoxy-8-nitroacridine dihydrochloride	4	4 hr. before virus	0	0
		4	48 hr. after virus	—	0
19,198	5-(4-Diethylamino-1-methylbutylamino)-3-methoxy-8-nitroacridine	1	4 hr. before virus	0	3 (16·5)
		1	48 hr. after virus	—	3 (14·8)

as in those receiving aureomycin. With diminishing dosage, however, the activity of 18,731 fell off very much more rapidly than did that of aureomycin.

Under the conditions of treatment in Table I, 18,731 and 19,198 produced no "persistent" coloration of the tissues and no basiphil particles were detected in the liver 48 hr. after the dose; as we have seen, they were inactive against equine encephalomyelitis. The behaviour of 6,769 was slightly different. Forty-eight hr. after a single dose we noted no "persistent" coloration, but on histological examination a few basiphil flecks were present. Four hr. after the last of five twice-daily doses, however, the tissues were faintly yellow and abundant basiphil particles were seen in the liver and Kupffer cells; again only traces of the particles remained 48 hr. later. Apparently, therefore, this compound gives rise to basiphil material which is less durable than that produced by mepacrine. On retesting, both with a single large dose and with repeated smaller intraperitoneal doses (Table I), we confirmed our previous finding that the drug is not active against equine encephalomyelitis (Hurst, 1948). The factor determining activity against the largest rather than the smaller viruses is not the presence of the $-NO_2$ group, as appeared likely from the experiments of Eaton, van Allen, and Wiener, because 17,465, in which the $-NO_2$ group occupied position 7 instead of position 8 of the acridine nucleus, was quite highly active against equine encephalomyelitis. Although our experience with lymphogranuloma and psittacosis is clearly limited, we have not yet encountered a compound in which activity has been present simultaneously for both types of virus.

SUMMARY

1. Of 83 acridines examined, some showed therapeutic activity against equine encephalomyelitis in mice. The activity was never significantly greater than that of mepacrine. Major activity was always associated with "persistent" coloration of the tissues by the drug, and by the formation of basiphil particles. The converse did not hold. Minor apparent activity might or might not be associated with these phenomena. Three dialkylaminoalkylamine and seven quinoline derivatives had no beneficial effect.

2. The results of testing a few compounds against Rift Valley fever in mice agreed well with those in equine encephalomyelitis.

3. Many acridines are very poorly tolerated by the chick-embryo and cannot be administered in doses likely to be effective against lymphogranuloma venereum infections; 33 compounds were inactive against lymphogranuloma venereum in the yolk-sac.

4. Three nitroacridines were considerably active against psittacosis in the mouse, but were inactive against equine encephalomyelitis. The factor determining therapeutic activity against the largest or the smaller viruses is not the $-NO_2$ group, because one nitroacridine was active against equine encephalomyelitis.

5. We have not yet found a compound which is active against both small and large viruses.

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