AN ANTIVIRAL SUBSTANCE FROM PENICILLIUM FUNICULOSUM

II. Effect of Helenine upon Infection in Mice with Semliki Forest Virus

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In order to learn whether or not the therapeutic effect of helenine is specific and limited to activity against Columbia SK encephalomyelitis virus, or whether it might have a broader therapeutic spectrum and exert its action against other viruses, a study was undertaken of its activity against Semliki Forest virus. This agent was selected because, like the SK virus, it is lethally infective when given to mice, either subcutaneously or intraperitoneally, and in adequate dosage can be expected to kill every animal. Furthermore, the period of approximately 3 days between infection and illness allows sufficient time for a therapeutic agent to exert its effect. In addition, so far as known, the virus is not pathogenic for man and hence is safe for use in large scale therapeutic experimentation.

Materials and Methods

Virus.—The Semliki Forest virus used in these experiments was initially isolated by Smithburn and Haddow (1). For present purposes it was obtained from Dr. W. F. Friedewald of Atlanta, Georgia. It is lethally pathogenic for white mice of the Carworth Farms CFW strain in high titre when administered either subcutaneously or intraperitoneally. In the present experiments, infections to be treated with helenine were induced by the subcutaneous inoculation, under the loose skin of the back, of 0.5 cc. of a dilution of virus containing from 10 to 1000 times the amount that regularly killed all untreated control animals. In most of the experiments this amounted to a 1×10^{-5} to 1×10^{-7} dilution of infected mouse brain. Pools of brains from about 10 infected mice, harvested when some were dead, and the remainder showed characteristic signs of infection, were prepared in 5 per cent suspension in buffered saline (pH 7.3) and divided among a number of screw-capped vials for storage frozen under CO_2 until ready for use. Kept in this way the virus retained an adequate potency and a single batch was sufficient for 3 to 4 months of experimental work. Dilution of the virus suspension to the concentration desired in an individual experiment was made just prior to use and the diluted virus was kept in an ice bath during the time of its administration.

All of the helenine preparations tested against Semliki Forest virus were administered intraperitoneally usually in divided doses 3 and 24 hours after infection. In this way there was little chance that the helenine would exert any direct *in vitro* effect on the subcutaneously administered virus.

Tests of mold preparations for antiviral activity against Semliki Forest virus were conducted in very much the same manner as those previously outlined for SK virus (2).

Mice to be used were placed 6 to a cage, and control or treated groups comprised from 1 to 5 such cages; that is, from 6 to 30 animals per group. Mice dying during the first 48 hours after infection were not included in the experimental results but rather were ascribed as toxic deaths. Mice dying from the 3rd day after infection onward were considered to have succumbed of Semliki Forest virus infections. 10 days was selected as the period of observation since it has been our experience that mice living this long were likely to survive indefinitely.

Deaths were recorded in the same manner as for the SK virus experiments. Dead animals were collected twice daily and, for statistical purposes, the deaths were grouped for each 24 hour period recording the deaths found at the afternoon reading with those of the following morning. Mice surviving beyond the 10th day were, for purposes of calculating the survival index, scored as having succumbed on the 10th day.

A statistical evaluation of the results on the same basis as that employed in the experiments with SK virus (2) was complicated by the large numbers of animals, infected with Semliki Forest virus, that were saved by treatment with helenine. While the survival indexes (SI) were calculated as in the SK virus experiments they are not believed to represent the therapeutic efficacy of given preparations as accurately in the case of Semliki Forest virus as they did with SK virus infections. A more correct evaluation of the results in experiments yielding so many survivors seemed to be on the basis of actual survivors resulting from treatment, and in most of the tables included in the present paper both survival indexes and number of survivors will be given.

Because the tests were performed with groups of 12, 18, or 24 animals regularly it was possible to simplify their statistical evaluation. Dr. W. H. Ott has calculated the minimum significant differences between survival or mortality rates in groups of 12, 18, or 24 animals each (at 5 per cent probability of such differences occurring due to chance alone) to be 6 animals in the case of groups of 12, 7 animals in the case of groups of 18, and 9 animals in the case of groups of 24 (3). Thus, to take an example, if in groups of 18 treated and 18 untreated control mice, 2 control mice survived, then 9 treated mice would have to be saved to achieve a minimum significant result and, if 5 of the 18 control mice survived, then 12 of the 18 treated mice would have to be saved. This difference of 7 animals up and down the series would thus constitute the minimum significant difference for groups of 18 animals.

Effect of Helenine upon Semliki Forest Virus Infection in Mice

In a preliminary experiment 5 groups of 24 mice each were infected subcutaneously with Semliki Forest virus. One control group was injected intraperitoneally with sterile culture medium 3 and 24 hours after infection while the other 4 groups were similarly injected with various preparations of helenine. The results are shown in Table I.

As shown in Table I, 2 of 24 control mice survived, while of the groups treated with SPS (supernatant fluid of pellicles fragmented in a Waring blendor (2)) 1 had 7 and the other 9 survivors with survival indexes respectively of 1.36 and 1.42. The 2 groups treated with acetone precipitates of the SPS preparations reconstituted to double the initial SPS concentrations, each had 15 survivors and survival indexes of 1.67 and 1.65. The results obtained in the groups treated with SPS were thus significant when calculated on the basis of survival indexes but not when considered on the basis of number of survivors. The results obtained with acetone-precipitated material on the other hand were highly significant, when calculated either on the basis of survival index or number of survivors.

This experiment made it quite clear that helenine exerted a favorable therapeutic effect upon Semliki Forest virus, just as it was known to do in the case of SK virus. Further, since the amount of helenine in the acetone-

precipitated material was approximately double that in the SPS, the superior therapeutic result achieved by the former suggested a progressively favorable dosage response to increasing amounts of helenine in Semliki Forest virus

TABLE I

Effect of Helenine upon Semliki Forest Virus Infection in Mice

Culture No.	Therapeutic activity against Semliki Forest Virus			
	SPS*		SPS AP 2 ×‡	
	Survivors	Survival index	Survivors	Survival index
BC 26	7/24§	1.36	15/24	1.67
BC 26A	9/24	1.42	15/24	1.65
Controls, average days survived = 5.3	2/24			

* SPS, supernatant fluid of pellicles fragmented in a Waring blendor (2).

‡ SPS AP 2 ×, Acetone precipitate of SPS redissolved in an amount of distilled water equal to one-half the volume of SPS from which precipitated.

§ $\frac{\text{Surviving mice}}{\text{No. of mice in group}}$.

TABLE II

Role of Size of Infecting Dose of Virus on Therapeutic Effect Achieved by Helenine against Semliki Forest Virus Infections in Mice

Dilution of infected brain	No. of survivors in each group of 12 mice		
	Controls	Treated*	Survival index
1×10^{-5}	0	0	1.19
1×10^{-6}	0	6	1.39
1×10^{-7}	0	10	2.22
1×10^{-8}	5	12	1.33
1×10^{-9}	12	12	1.0
1×10^{-10}	12	12	1.0

* Treated intraperitoneally with acetone-precipitated helenine 3 and 24 hours after subcutaneous infection.

infections. The subject of dosage response will be considered in greater detail later in the experiments to be presented in Table IV.

Role of Size of Infecting Dose of Virus on the Therapeutic Effect Achieved by Treatment with Helenine

In our experiments, both with SK virus and with Semliki Forest virus, we have endeavored to use an infecting dose lying somewhere between 10

and 1000 times that which will kill all or almost all the untreated control mice. In order to learn whether the apparent therapeutic effectiveness of helenine against Semliki Forest virus might be modified by variation in the initial infecting dose of virus, experiments of the type outlined in Table II have been carried out.

In the experiment shown in Table II, 6 groups of 24 mice each were infected subcutaneously with 0.5 cc. doses of Semliki Forest virus diminishing by progressive ten-time dilutions. One-half of the mice infected with each virus dilution were injected intraperitoneally with sterile medium 3 and 24 hours after infection and designated as controls. The other half were treated intraperitoneally with an acetone precipitate of SPS BC 48–49 3 and 24 hours after infection. This acetone precipitate had been reconstituted in distilled water to the volume of the original SPS from which derived.

As can be seen from the results presented, helenine exerted a favorable therapeutic effect against the infections produced by the 10^{-6}, 10^{-7}, and 10^{-8} dilutions of Semliki Forest virus. The results obtained at these 3 dilutions were significant whether calculated on the basis of survival indexes or actual numbers of survivors. At the 10^{-5} dilution the virus killed all of both the treated and untreated animals with a survival index of 1.19 for the treated group which was just under the minimum for significance. The 10^{-9} and 10^{-10} dilutions of virus failed to kill any of the control animals and hence had no application in the experiment except to indicate that the limit of dilution of the virus had been reached.

Comparison of the Antiviral Activities of the Fluid Portions and Pellicles of Stationary Cultures of P. funiculosum

Although it had been clearly shown (2) that the pellicles of *P. funiculosum* were considerably more active against SK virus than the fluid portions of cultures it seemed worth while to establish that a similar situation held in the case of Semliki Forest virus. Several tests of the comparative therapeutic activity of the SPS with the fluid portions of cultures were made and the results of one such experiment are shown in Table III.

As shown in Table III, the fluid portions of stationary cultures of *P. funiculosum* saved only 1 animal out of 2 groups of 18 mice infected with Semliki Forest virus. Despite this poor showing on the basis of saving lives, both fluid preparations yielded significant survival indexes; one 1.29 and the other 1.44. The two SPS on the other hand showed very high survival indexes, 1.81 and 1.94, and, in addition, each preparation saved a significantly large number of mice, 8 and 10 out of the 2 groups of 18. The results obtained with the acetone precipitates of the two SPS reconstituted to the original volume of SPS from which they were derived roughly equalled those with the SPS. Diluted 4 times, these acetone precipitates, while considerably less active than when undiluted, still possessed some therapeutic activity, judged on the basis of survival indexes, and even at this dilution appeared to be more effective than were the culture fluids.

It seems clear from this and similar experiments carried out with Semliki Forest virus that there is much more helenine in, or in intimate association with, the cellular constituents of the pellicles of stationary cultures than there is in the culture fluid itself. These findings with Semiliki Forest Virus, therefore, parallel those with SK virus (2).

Dosage Response to Helenine

An indication that mice infected with Semliki Forest virus exhibit a dosage response to helenine was suggested by the experiments outlined in Tables I and III. It seemed of interest to determine whether, as it did with SK virus,

TABLE III

Comparative Antiviral Activity of Fluid Portions and Pellicles of Stationary Cultures of P. funiculosum against Semliki Forest Virus Infections in Mice

Culture No.	Activity against Semliki Forest virus							
	Fluid		SPS		SPS AP 1 ×		SPS AP 0.25 ×	
	Survivors	SI	Survivors	SI	Survivors	SI	Survivors	SI
56	0/18*	1.29	8/18	1.81	6/18	1.71	2/18	1.77
57	1/18	1.44	10/18	1.94	14/18	2.07	2/18	1.53
Controls, average days survived = 4.3.....	0/24							

* $\frac{\text{Surviving mice}}{\text{No. of mice in group}}$.

the helenine therapeutic effect would tend to "plateau" with large doses. The experiments outlined in Table IV were carried out to learn whether this was the case.

In the experiments recorded in Table IV, two series of tests of acetone precipitates of SPS in dosages ranging from 11 to 0.55 mg. were conducted. The two series were identical except for the fact that the reconstituted precipitate had in one case been filtered through a Seitz pad, while in the other the preparation was not filtered prior to use. In addition, a third series of tests with an acetone precipitate of SPS in dosages ranging from 5.72 to 0.022 mg. was carried out.

Based on a consideration of the survival indexes, both of the first two series of tests gave therapeutically significant results at all doses tried and, as in the experiments with SK virus, there was a plateauing of effect at the larger doses. In the third series a definite break in the therapeutic effect came between a dosage of 0.36 and 0.09 mg.

Judged on the basis of mice saved by treatment the results were not as

TABLE IV

Response to Various Doses of Helenine of Mice Infected with Semliki Forest Virus

Acetone precipitate of SPS culture	Total dose*	Result of treatment	
		Survivors	Survival index
	mg.		
BC 56–59 unfiltered	11.0	11/18‡	1.88
	5.5	6/18	1.71
	2.75	6/18	1.73
	1.37	5/18	1.52
	0.55	5/18	1.48
BC 56–59 Seitz filtered	11.0	12/18	1.85
	5.5	10/18	1.86
	2.75	10/18	1.76
	1.37	8/18	1.74
	0.55	2/18	1.33
Controls, average days survived = 5.0..		0/24	
BC 63	5.72	8/12	1.69
	1.43	3/12	1.29
	0.36	1/12	1.39
	0.09	0/12	0.99
	0.022	0/12	0.87
Controls, average days survived = 5.4..		2/24	

* Administered intraperitoneally in two equal amounts 3 and 24 hours after subcutaneous infection with Semliki Forest virus.

‡ $\frac{\text{Surviving mice}}{\text{No. of mice in group}}$.

TABLE V

Influence of Time of Administration of Helenine on Its Therapeutic Efficacy against Semliki Forest Virus

Time of treatment*	Survival index	No. of mice surviving of 24
3 hours before infection.....	1.84	15
3 " after "	1.62	13
6 " " "	1.46	10
12 " " "	1.21	10
18 " " "	1.24	9
24 " " "	1.10	3
Controls, average days survived = 5.1.....		1

* Single intraperitoneal injection of 5.5 mg. dose of acetone precipitate SPS culture BC 56–59 (Seitz filtered).

clear cut. Based on the first series of tests with unfiltered acetone-precipitated material only the 11 mg. dose saved a significant number of animals. The results with filtered acetone precipitate were somewhat better and here the 1.37 mg. dose gave a significant number of survivors. In the third series of tests, also with unfiltered materials, 5.72 mg. saved a significant number of animals while 1.43 mg. did not. Even on the basis of survivors, however, a plateauing of effect at higher dosage was apparent.

The Influence of Time of Administration of Helenine on Its Therapeutic Efficacy against Semliki Forest Virus

Preliminary experiments with Semliki Forest virus infections in mice indicated that, as previously found in the case of SK virus (2), single doses of helenine large enough to fall within the plateau zone of maximum effect, given 3 hours after infection, exerted as favorable a therapeutic effect as two such doses given 3 and 24 hours after infection. In order to learn how long after infection a single large dose of helenine would exert a therapeutic effect, groups of 24 mice were treated intraperitoneally at various intervals before or after infection subcutaneously with a surely fatal dose of Semliki Forest virus. The results are shown in Table V.

From the experiment outlined in Table V, it can be seen that helenine, given either 3 hours before or 3 hours after infection, exerted a good therapeutic effect against Semliki Forest virus. There was some falling off in effect when treatment was delayed 6 and 12 hours. Even at 18 hours, however, helenine exerted a significant therapeutic effect. Treatment delayed 24 hours was not significantly effective in therapeutically influencing the virus infection. These results parallel very closely those gotten in similar experiments with SK virus (2). As in the case of SK virus also, treatment after obvious signs of infection are apparent is without effect.

The Effect of Modification of Medium on the Production of Helenine Active against Semliki Forest Virus

In the case of both SK and Semliki Forest virus infections, enrichment of the medium by increasing the amounts of glucose and yeast extract, or aging of the pellicle mash before final processing to SPS, did not increase the yield by *P. funiculosum* of helenine. Furthermore, gelatin could be substituted in the medium for yeast extract and various sugars for glucose without materially altering the therapeutic effectiveness, against either virus, of the helenine produced. The results of a typical experiment in which media modified in various ways was employed to produce the helenine tested against Semliki Forest virus are shown in Table VI.

As shown by the findings recorded in Table VI, 3 of the 4 cultures tested had therapeutic activity in their fluid portions when judged on the basis of survival indexes. The only inactive broth was that from the media in which

maltose had been substituted for glucose (MY). All four of the SPS were active on the basis of survival indexes and the two containing maltose (MY and MG) on the basis of number of survivors. The helenine activity of all four SPS, regardless of the media on which produced, could be precipitated with acetone, and, as in similar experiments with SK virus, the amount of solid material precipitable with acetone was less from SPS derived from media containing gelatin than from that containing yeast extract. The conclusion to be drawn from the results outlined is that gelatin can be substituted

TABLE VI

*Production of Helenine in Media Modified in Various Ways—Therapeutic Effect Tested against Semliki Forest Virus**

Culture No.	Medium	Survivors and survival indexes						
		Broth		SPS		SPS AP 1 ×		Acetone precipitable solids per cc. SPS
		Survivors	SI	Survivors	SI	Survivors	SI	
								mg.
BC 50	DY‡	2/12§	1.46	4/12	1.84	6/12	1.93	4.0
BC 51	DG	4/12	2.05	5/12	1.67	3/12	1.78	1.57
BC 52	MY	0/12	1.19	7/12	1.92	6/12	1.88	4.5
BC 53	MG	1/12	1.47	8/12	2.11	2/12	1.62	2.47
Controls, average days survived = 4.3..				0/30				

* Infected subcutaneously and treated intraperitoneally 3 and 24 hours after infection.

‡ DY, regular medium containing dextrose and yeast extract; DG, regular medium in which gelatin was substituted for yeast extract; MY, regular medium in which maltose was substituted for dextrose; MG, regular medium in which maltose and gelatin were substituted for dextrose and yeast extract.

§ $\frac{\text{Mice surviving}}{\text{No. of mice in group}}$.

for yeast extract and maltose for glucose in media in which helenine, active against Semliki Forest virus, is produced.

Effect of Helenine upon Neuroinvasiveness of Semliki Forest Virus

In the work with SK virus (2) evidence was presented indicating that helenine had an *in vivo* action either on the rate of multiplication of the virus or on its rate of invasion of the central nervous system of infected mice. In order to learn whether it had a similar action on Semliki Forest virus an experiment like that done with SK virus was conducted.

In this experiment 62 mice were infected subcutaneously with an ordinarily fatal dose of Semliki Forest virus. 23 of these were set aside as controls and were given 2 intraperitoneal injections of sterile medium 3 and 23 hours after infection. The remaining 39 animals were

injected intraperitoneally with a known effective preparation of helenine as an acetone precipitate 3 and 23 hours after infection. 24 hours after infection and thereafter at intervals of 24 hours for a total of 4 days in the case of the controls and 5 days in the case of the treated mice several animals of each group were sacrificed by cervical fracture. The control mice and 1 treated mouse that died on the 4th day were included in the test along with those that were sacrificed on the 4th day.

The brain of each mouse was removed aseptically promptly after sacrifice or death, ground in a mortar and suspended in buffered saline (pH 7.3) to make a 5 per cent suspension by wet brain weight. A portion of each brain suspension was injected intraperitoneally in 0.5 cc. dosage into 3 or 4 mice to test for the presence of virus and the remainder was stored frozen in the CO_2 box in screw-capped vials for possible later titration of virus content. If all the mice in a test group lived and appeared normal at the end of a 10 day period of observation the brain under test in that group was considered to have been free of virus. On the other hand, death of all mice in a test group with signs typical of Semliki Forest virus infection was considered evidence that the brain under test had contained virus. An approximate titration of the amount of virus present was made in some cases by injecting groups of 3 or 4 mice intraperitoneally with progressive tenfold dilutions of the stored frozen virus. The virus titre was considered to be the greatest dilution of infected brain killing all the mice in a group. The result obtained in testing the brains of treated and control mice for the presence of virus is recorded in Table VII.

The findings outlined in Table VII indicate a striking difference in virus content between the brains of untreated and treated mice.

Of the series of untreated control mice sacrificed from 1 to 4 days after infection or dead on the 4th day, the brain of only 1 was free of virus. This animal had been sacrificed 24 hours after infection and to judge from the result, virus had not yet reached its brain from the site of subcutaneous inoculation. The brains of all the remaining 16 control mice contained virus in varying amounts.

In the series of mice treated with helenine, on the other hand, virus was not demonstrably present in the brains of any of those killed 1 day after infection. Of the 7 treated mice killed 2 days after infection the brains of 2 contained virus in the same relatively low titre as shown by the brains of control animals killed on the corresponding day. The brains of the other 5 treated mice killed on the 2nd day were free of virus. Of the 7 treated mice killed 3 days after infection, virus was present in the brain of only 1 animal. 1 treated mouse was found dead 4 days after infection and 6 others were sacrificed. Virus was present in the brain of the mouse that died but not in the brains of any of the 6 animals sacrificed. 4 treated mice were sacrificed on the 5th day after infection. Virus was present in the brain of 1 and not in the brains of the other 3.

Only 17 of the 23 control mice in the experiment are recorded in Table VII. Of the remaining 6 animals, 1 died on the 3rd, 3 on the 4th, 1 on the 6th, and 1 on the 7th days after infection. Of the 10 treated mice in the experiment not recorded in the table, 1 died on the 4th, 1 on the 5th, 2 on the 6th, and 1 on the 7th day after infection, while the remaining 5 survived the 10 day period of observation.

From the findings presented it would appear that treatment of Semliki Forest virus infections with helenine exerts either a marked delaying effect upon the neuroinvasiveness of the virus or an actual curative effect on the virus infection itself. That the effect is probably a curative one is suggested by the irregular distribution, from the standpoint of time with regard to

TABLE VII

Presence of Semliki Forest Virus in the Brains of Treated and Control Mice Sacrificed or Dead at Various Times after Subcutaneous Infection

Control mouse No.	Time after infection	Virus in brain	Virus titre
	days		
1	1	Present	Undetermined
2	1	Absent	
3	1	Present	Undetermined
4	2	"	1×10^{-3}
5	2	"	1×10^{-3}
6	2	"	1×10^{-3}
7	2	"	Undetermined
8	2	"	"
9	2	"	"
10	3	"	1×10^{-5}
11	3	"	1×10^{-5}
12	3	"	1×10^{-8}
13	3	"	Undetermined
14	4	"	1×10^{-9}
15	4 died	"	1×10^{-5}
16	4 "	"	1×10^{-6}
17	4 "	"	Undetermined
Treated mouse No.			
1	1	Absent	
2	1	"	
3	1	"	
4	1	"	
5	2	Present	$<1 \times 10^{-3}$
6	2	"	1×10^{-3}
7	2	Absent	
8	2	"	
9	2	"	
10	2	"	
11	2	"	
12	3	Present	1×10^{-4}
13	3	Absent	
14	3	"	
15	3	"	
16	3	"	
17	3	"	
18	3	"	
19	4 died	Present	Undetermined
20	4	Absent	
21	4	"	
22	4	"	
23	4	"	
24	4	"	
25	4	"	
26	5	Present	Undetermined
27	5	Absent	
28	5	"	
29	5	"	

infection, of the cases in which virus was demonstrable in the brains of treated animals. Excluding treated mouse 19 which died, virus was found in the brains of 2 mice on the 2nd day, 1 on the 3rd day, and 1 on the 5th day after infection while it was absent in the brains of the remaining 24 treated mice sacrificed from 1 to 5 days after infection. This irregular distribution of brains containing virus would suggest rather that the virus infection had been cured in the majority of the treated mice than that the agent had merely been delayed in its invasion of the central nervous system, as had clearly been the case with SK virus (2). It seems quite possible, comparing these Semliki Forest virus experiments with similar ones with SK virus (2), that the mode of therapeutic action of helenine on the two viruses may be quite different or that helenine is considerably more effective quantitatively against Semliki Forest virus than it is against SK virus.

DISCUSSION

Much of the work with Semliki Forest virus reported in this paper is confirmatory of similar experiments with the SK virus outlined in an earlier paper (2) and would appear to indicate that the therapeutic effect exerted by helenine may be directed against members of an as yet undefined group of viruses rather than against only one specific agent. In this respect it behaves similarly to the established antibiotics, all of which influence infection with several to many species of bacteria of diverse types and pathogenic activities.

Against Semliki Forest virus helenine exerted a therapeutic effect that was somewhat more clear cut, from the standpoint of apparent cures, than was the case in SK virus infections, in which its therapeutic effect was, in most instances, largely manifested only by a prolongation of life. At first sight this might seem to indicate that the mechanism by which helenine acted was different in these two virus infections, and this possibility cannot be ruled out with certainty from the information available. However, it might equally well be that the differences in therapeutic effect were entirely quantitative, and this would seem the more likely case from the information presented in an accompanying paper (4) in which comparative therapeutic tests against the two viruses are described. The action of helenine against Semliki Forest virus would seem to have been a twofold one; first of delaying or preventing the entrance of the virus into the central nervous system, and second, of destroying the virus, frequently in such a manner as to prevent the acquisition of any viral immunity by the cured host. In the case of SK virus, on the other hand, only the first phase of helenine effect was usually observed, namely that of delaying the invasion of the central nervous system. The second phase, actual destruction of SK virus, was achieved with relative infrequency. The differences in end-result, prolongation of life in most cases

of SK virus infections and actual cures in many cases of Semliki Forest virus, probably reflect certain differences in the pathogenic characters and properties of the two viruses more than they do actual qualitative differences in the therapeutic effect of helenine against the two agents.

As was the case with SK virus infections, helenine appeared to exert little if any therapeutic effect in mice suffering massive infections with Semliki Forest virus. The most significant results were obtained in treating infections caused by from 10 to 100 surely fatal doses of the virus. For optimal therapeutic effect the helenine had to be given within 12 hours of the time of infection though some effect is apparent when treatment has been delayed for 18 hours. Plateauing of effect with large doses of helenine was observed in Semliki Forest virus infections, just as it was with SK virus, and in order to demonstrate a dosage response it was necessary to use relatively small amounts of helenine well below the maximum tolerated dose.

Modification of the medium in which helenine is produced, variations in the methods by which it is extracted from pellicles, and any of the other dodges tried to enhance its yield by *P. funiculosum* gave results with Semliki Forest virus that very closely paralleled those obtained with SK virus. These findings indicate that the substance found to be therapeutically active against Semliki Forest virus was in all likelihood the same one that had been found effective against SK virus. There is nothing to indicate that our results were due to two different materials produced by *P. funiculosum*, one active against SK and the other against Semliki Forest virus. So far as can be told from our data, helenine, a single entity, was responsible for the therapeutic action against both viruses.

SUMMARY

Helenine exerts a therapeutic effect against Semliki Forest virus infections of mice. Cures, that is to say the survival of treated animals, were more frequently observed in Semliki Forest virus infections than they were in SK virus infections. It is believed that this difference in end-result probably represented only a quantitative difference in the therapeutic effect of helenine against these two viruses and not a qualitative difference in its mechanism of therapeutic action.

The findings reported in this paper with regard to the treatment of Semliki Forest virus infections with helenine parallel very closely those described in an accompanying paper which deals with the action of helenine on SK virus infections.

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