AN ANTIVIRAL SUBSTANCE FROM PENICILLIUM FUNICULOSUM

III. General Properties and Characteristics of Helenine

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This paper presents some of the general properties and characteristics of helenine. These properties will be considered, using therapeutic activity against Columbia SK encephalomyelitis virus infections in mice as the criterion for helenine activity (1). It will be assumed that the therapeutic activity of various crude or partially purified preparations results from the presence in them of helenine. Loss of therapeutic activity of the various preparations resulting from some of the procedures to be described will be considered to result from alteration or destruction of helenine.

GENERAL PROPERTIES

Keeping Qualities of Helenine.—Therapeutic activity is gradually lost by preparations of helenine kept at room temperature. Similar preparations stored in the refrigerator at or near 5°C. maintain their therapeutic activity considerably longer and some preparations have maintained their full original activity for periods of a month or longer. Sterile filtrates of active preparations have appeared to be more stable than unfiltered non-sterile materials, suggesting that bacterial decomposition may play a role in helenine inactivation.

Stored frozen under solid CO_2 helenine preparations have remained active for relatively long periods of time as indicated by the findings recorded in Table I.

Crude SPS preparations (supernatant fluid of pellicles fragmented in a Waring blendor (1)) remained apparently fully active for periods of 136, 143, and 314 days. Acetone precipitates of crude SPS preparations, reconstituted in water, retained their therapeutic activity for periods of 119, 127, and 145 days. Materials stored for longer periods have not been tested for retention of activity but it seems likely that, at the temperature of solid CO_2, helenine will remain stable indefinitely.

Filtrability of Helenine.—Because of the viscous nature of SPS preparations and their reconstituted acetone precipitates, filtration of helenine is a very slow and tedious process. However, to obtain sterile preparations for crucial therapeutic tests in experimental animals, filtration through Seitz pads presented the most dependable means of sterilization without inactivation or loss of material.

As initially prepared by homogenizing SPS acetone precipitates with water in a Waring blendor a turbid and very viscous preparation results. Such preparations are not suitable for filtration through Seitz pads. If, however, such turbid preparations are frozen slowly by placing them for 12 to 24 hours in the freezing compartment of an ordinary electric refrigerator and then thawed without agitation, a flocculent precipitate of viscous material is observed floating in completely clear fluid. If this fluid is carefully poured through a gauze pad the flocculent precipitate is retained by the gauze and the filtrate is a clear amber fluid

which, while still viscous, is now slowly filtrable under pressure through a Seitz pad. The flocculent precipitate, which on a dry weight basis is very small, is completely devoid of therapeutic activity. Strangely, rapid freezing in the CO_2 box will not produce a precipitate and slow freezing appears to be the essential step.

TABLE I

Keeping Qualities of Helenine Stored Frozen under CO_2

Culture No.	Preparation	Storage	Survival index*
Pooled 196–200	SPS AP 2 ×‡	Frozen 145 days	1.50
BC 25A	SPS AP 2 ×	Fresh	1.53
BC 25A	SPS AP 2 ×	Frozen 127 days	1.65
BC 25 and 26	SPS AP 2 ×	" 119 "	1.58
Pooled 173 and 174	SPS§	Fresh	1.80
" " " "	SPS	Frozen 314 days	1.65
BC 24	SPS	" 143 "	1.67
BC 25A	SPS	Fresh	1.52
BC 25A	SPS	Frozen 136 days	1.77

* Tested in groups of 12 mice infected with SK virus.

‡ SPS AP 2 ×, acetone precipitate of SPS concentrated 2 times.

§ SPS, supernatant fluid of pellicles fragmented in a Waring blendor (1).

TABLE II

Filtrability of Helenine through Seitz Pads

Preparation	Unfiltered	Filtered
Acetone precipitate	Survival index*	Survival index*
4XPDQ	1.75	1.55
3XPDQ	1.64	1.60
SPSBC 56–61	1.53	1.70
SPSBC 56–59	1.87	1.75
SPSBC 48–55	1.57	1.52

* Tested in groups of 12 mice infected with SK virus.

Filtration of the gauze filtrate through a Seitz pad results in no loss of either solids or therapeutic activity. The comparative activities of several Seitz-filtered preparations with their corresponding gauze filtrates are given in Table II.

The small discrepancies in the survival indexes between comparable Seitz-filtered and unfiltered preparations recorded in the table are within the normal range of variation for tests in which therapeutic activity is determined in groups of 12 mice and it is believed that the results indicate that sterilization by Seitz pad filtration results in no loss or inactivation of helenine.

Failure of Helenine to Dialyze.—Dialysis of either SPS preparations or their reconstituted acetone precipitates against repeated changes of cold distilled water in the refrigerator resulted in no detectable loss of helenine activity as indicated by the results of a typical experiment recorded in Table III.

In comparing preparations for therapeutic activity before and after dialysis corrections were made for the change in volume of the dialyzed materials by correspondingly varying the fluid volume administered therapeutically to the mice. It is believed that the small discrepancies in the survival indexes between dialyzed and non-dialyzed preparations are within the range of variation of the test as conducted and that the results indicate that helenine is not dialyzable under the conditions employed.

The Effect of Freeze-Drying on Helenine.—During the course of the study of helenine we frequently wished to dry active material without losing its therapeutic activity. Our usual method was to dry under vacuum from the frozen state and we early found that SPS preparations dried in this manner possessed full activity when subsequently reconstituted with water and tested therapeutically against SK virus infections in mice. However, the similar drying of semipurified preparations of helenine, notably those reconstituted in water after acetone precipitation, resulted in some loss of therapeutic activity during the process. That this loss was associated with the drying and not the freezing part of the process was indicated by the fact that acetone-precipitated helenine can be frozen repeatedly without

TABLE III

Failure of Helenine to Dialyze

Preparation	Before dialysis Survival index*	After dialysis Survival index*
SPSBC 90	1.51	1.51
SPSBC 90 (AP2 ×)‡	1.75	1.60
SPSBC 91	1.30	1.22
SPSBC 91 (AP2 ×)	1.47	1.66

* Tested in groups of 12 mice infected with SK virus.

‡ AP2 ×, acetone precipitate concentrated two times.

loss and that the best way to retain activity for long periods of time is by storage frozen in the wet state.

The fact that SPS preparations could be freeze-dried without loss suggested that something in such preparations, carried over from the medium, was capable of protecting helenine during the drying process. Preliminary experiments indicated that broth of the type utilized by *P. funiculosum* in producing helenine, when added to acetone-precipitated helenine prior to freeze-drying, protected it against the loss of activity. A further series of tests of the capacity of individual constituents of the broth to protect against loss of activity during freeze-drying have been carried out. The results of some of these tests are recorded in Table IV.

As shown in Table IV, none of the four SPS preparations lost activity as a result of freeze-drying from their natural state (water) whereas all of the acetone-precipitated preparations that were freeze-dried from water lost variable amounts of activity during the process. The remaining preparations shown in Table IV, all of them acetone precipitates of SPS preparations, were freeze-dried either from broth, a solution of the salts of the broth, a 4 per cent solution of dextrose, or a 1 per cent solution of yeast extract. These concentrations of dextrose and yeast extract were selected because they corresponded to the concentrations of these constituents in the broth and the salts too, were, of course, a solution of the concentrations ordinarily used in the broth.

The results shown in Table IV indicate that acetone-precipitated helenine can be dried from broth solution without appreciable loss of activity. Dried from a solution of broth

TABLE IV

The Effect of Freeze-Drying under Varying Conditions on the Therapeutic Activity of Helenine

Preparation	State	Activity of original fluid preparation	Therapeutic activity expressed as survival indexes* against SK poliomyelitis virus of preparation dried from				
		Survival index*	Water	Nutrient medium	Salts of medium	4 per cent dextrose	1 per cent yeast extract
BC 17	SPS	1.43	1.60				
BC 17	SPS AP 5 ×‡	1.81	1.24				
BC 25A	SPS	1.52	1.47				
BC 25A	SPS AP 1 ×	1.53	1.36				
BC 26	SPS	1.48	1.59				
BC 26	SPS AP 1 ×	1.42	1.19				
BC 26B	SPS	1.52	1.56				
BC 26B	SPS AP 1 ×	1.43	1.22				
4XPDQ(A)	SPS AP 2 ×	1.75	1.03			1.61	
4XPDQ (B)	SPS AP 2 ×	1.71	1.43		1.34	1.60	1.75
BC 90	SPS AP 2 ×	1.74	1.39		1.44	1.81	1.77
BC 64	SPS AP 2 ×	1.64	1.32		1.53	1.43	1.56
BC 56–62	SPS AP 2 ×	1.64	1.19	1.82	1.52		
BC 62	SPS AP 2 ×	1.66	1.40	1.59			
BC 56–59	SPS AP 2 ×	1.92	1.57	1.65	1.56		
BC 60	SPS AP 2 ×	1.61	1.25				
BC 61	SPS AP 2 ×	1.86	1.22				
BC 62	SPS AP 2 ×	1.86	1.30	1.78	1.48		
BC 48–55	SPS AP 1 ×	1.57	1.24				
BC 48–55	Seitz filtrate of above	1.52	1.36				

* Tested in groups of 12 to 30 mice infected with SK virus.

‡ SPS AP 5 ×, acetone precipitate of SPS concentrated 5 times.

TABLE V

Inactivation of Helenine by Heat

Preparation	Heated	Activity against SK virus Survival index*
SPSBC25A	No	1.52
SPSBC25A	Boiled 3 min.	1.24
SPSBC25 AP 1 ×	No	1.53
SPSBC25 AP 1 ×	Boiled 3 min.	1.27
SPSBC56–59 AP 2 ×	No	1.81
SPSBC56–59 AP 2 ×	Autoclaved, 15 lbs. 15 min.	1.05

* Tested in groups of 18 or 30 mice.

salts there appeared to be some loss of activity but probably less than that suffered during drying from water. Both 4 per cent dextrose and 1 per cent yeast extract appeared to exert a fairly good protective effect against the loss of activity by acetone-precipitated helenine

during the drying process. For routine purposes we have selected 4 per cent dextrose as the vehicle of choice for preserving the therapeutic activity of acetone-precipitated helenine during the freeze-drying process.

Inactivation of Helenine by Heat.—Helenine in an Erlenmeyer flask lost much of its activity when held for 3 minutes in a boiling water bath. It was completely inactivated by autoclaving for 15 minutes at 15 pounds' pressure. The results are recorded in Table V.

Interference in the Development of Viral Immunity by Treatment with Helenine

Mice infected with either SK virus or Semliki Forest virus and saved by treatment with helenine may or may not be immune subsequently to infection with the virus to which they were previously exposed. If, as rarely happens in the case of SK virus, a relatively large percentage of the mice have been saved, the survivors will usually succumb to a later exposure to the virus. If, on the other hand, only a small proportion of the mice have been saved by treatment with helenine, these will usually be solidly resistant to infection on later exposure to the virus. The outcome of the test for immunity of mice saved from SK virus infection cannot, however, be predicted with certainty.

In the case of Semliki Forest virus infection, the presence or absence of immunity in mice saved by treatment appears also to be somewhat dependent upon the size of the initial infecting dose of virus. If the mice have been saved by helenine, following a small but surely fatal dose of virus, the survivors are not likely to be immune to fatal infection upon later exposure to virus. If, on the other hand, the dose of virus from which the mice have been initially saved is a relatively large one, then the survivors are usually solidly immune upon subsequent exposure to virus.

Although, as just pointed out, it is not possible to predict with certainty whether or not mice saved from death by treatment with helenine will or will not be subsequently immune to viral infection, the same uncertain situation does not hold in the case of mice saved by treatment with specific immune serum. Mice infected subcutaneously with either SK or Semiliki Forest virus can all be saved if potent specific virus-neutralizing serum is administered intraperitoneally to them 3 or 4 hours after infection. Mice saved in this way by treatment with antiserum prove almost uniformly to be solidly immune when later exposed to viral infection of the same type. The virus-neutralizing serum used in the present experiments was obtained from swine recovered from intracerebral infection with SK virus or Semliki Forest virus. Both of these agents produce a febrile, usually non-fatal encephalitis in swine when given intracerebrally.

If mice infected with either SK or Semliki Forest virus are treated with both helenine and specific virus-neutralizing serum the therapeutic result achieved is similar to that obtained by treatment with the serum alone; that is, all or most of the treated mice survive. However, the situation as regards

the development of immunity is quite different as indicated by the results of the experiment recorded in Table VI.

As shown in Table VI, 120 mice in four groups of 30 each were infected with SK virus. 5 of the group treated with helenine, all of those treated with serum, and 24 of the group treated with the combination of helenine and serum survived a dose of virus that killed

TABLE VI

Interference by Helenine with the Development in Mice of Immunity to SK and Semliki Forest Viruses

Infection subcutaneously with	Initial treatment*	Survivors	Result of test for immunity‡	
			Virus	Survivors
SK virus	Helenine	5/30§	SK virus	Not tested
	Anti-SK serum	30/30		30/30
	Both of above	24/30		13/24
	Controls	1/30	New controls	2/30
Semliki Forest virus	Helenine	11/24	Semliki Forest virus	9/11
	Anti-Semliki serum	24/24		20/24
	Both of above	24/24		7/24
	Controls	1/12	New controls	0/6

* The SK virus-infected mice were treated intraperitoneally with helenine 3 and 24 hours after infection. The anti-SK serum was administered in two doses of 0.25 cc. each 3 and 24 hours after infection. The group of mice treated with both helenine and anti-SK serum received the former approximately 15 minutes before the latter. The Semliki Forest virus-infected mice were treated intraperitoneally with a single dose of helenine 4 hours after infection. The anti-Semliki serum was administered in a single 0.25 cc. dose 4 hours after infection. The group of mice treated with both helenine and anti-Semliki serum received the former approximately 15 minutes before the latter.

‡ Test for immunity to SK virus made 15 days after initial infection. Test for immunity to Semliki Forest virus 20 days after initial infection. Challenge dose of virus given subcutaneously.

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

29 of 30 control mice. The reason for the loss of 6 mice in the group treated with the combination of helenine and serum is not clear, but it seems likely that the administration of the two materials may have resulted in a fatally toxic reaction in a few animals that neither material alone elicited.

In testing the surviving mice for immunity to SK virus 15 days after their initial infection it was found that, while all 30 of those that had been treated with serum alone were immune, 11 of the 24 treated with both helenine and serum were not immune and succumbed to reinfection with SK virus.

Obviously helenine in some manner prevented the virus given in the initial infection from eliciting as effective an immune response as it did in mice treated with virus-neutralizing serum alone.

The findings in the case of the Semliki Forest virus infections in mice paralleled quite closely those obtained with SK virus. Helenine saved 11 of the 24 mice to which it was given while the serum, alone and in combination with helenine, saved all the 24 mice in each group.

In testing the surviving mice for immunity to Semliki Forest virus 20 days after their initial infection it was found, as in the case of the SK virus mice, that a quite solid immunity had been conferred on the group that had been treated with serum alone. Of this group 20 of the 24 animals were found to have become immune. Of the group of 11 that had been saved by treatment with helenine alone, 9 proved immune to the challenge infection. The group that had been treated with both helenine and antiserum showed evidence however, of only a poor immunity, and of the 24 mice saved by the combined treatment 17 proved not to be immune.

TABLE VII

Partial Composition of an Acetone Precipitate of SPS

Determination	Found
	*per cent**
Nitrogen—total (Kjeldahl)	1.28, 1.30
Phosphorous total	0.26, 0.27
Phosphorous as free phosphate	0.20, 0.22
Phosphorous bound—by difference	0.06, 0.05
Polysaccharides	49.0, 54.0
Reducing sugars, calculated as glucose	6.1, 6.1
Pentose sugars, calculated as ribose	6.9, 7.1

* Calculated on basis of solids present in sample.

It seems likely that the interference by helenine with the elicitation of an immune response, occurring as it does with two unrelated viral infections, represents a truly reproducible and probably significant phenomenon, one suggesting a possible explanation of the means by which helenine exerts its therapeutic effect. This will be discussed more fully further on.

Some Properties of an Acetone Precipitate of SPS[1]

The preparation whose analysis is to be outlined was a reconstituted acetone precipitate of SPS 56–61 that had been filtered through a Seitz pad. It had been reconstituted in distilled water to one-half the volume of the original SPS from which it had been derived and hence had two times the concentration of helenine of the original SPS. Tested against SK virus infection in mice it was therapeutically quite active, giving a survival index of 1.70.

The solution for chemical tests was clear and amber colored, had a pH of 6.1, and contained 10 mg. of acetone-precipitated material per ml. It gave a positive Molisch test for

[1] These determinations were made by Dr. K. Folkers and Dr. R. L. Peck.

carbohydrate and a positive ninhydrin test. No precipitate was formed on addition of trichloracetic acid to a final concentration of 10 per cent in a 10 mg. per ml. solution of the sample, indicating the absence of significant amounts of protein and the absence of significant amounts of nucleic acids. In the ultraviolet, a 1 mg. per ml. solution in water showed an absorption maximum at 2600 A, E per cent of 5.9, which may indicate the presence of small amounts of nucleotides or nucleosides.

Various chemical determinations are outlined in Table VII.

It is realized that with no knowledge as to the relative amount of pure helenine represented in the acetone-precipitated material, the results of the chemical tests are difficult to interpret. However, the high percentage of polysaccharides found suggests that this class of substances should be kept prominently in mind in any surmise, at this stage of the investigation, as to the character and nature of helenine.

RECAPITULATION AND DISCUSSION

Although the experiments described scarcely touch upon the chemical nature of helenine they do present some of its general properties and characteristics. The substance is not notably unstable if refrigerated under sterile conditions, and can be kept without serious loss in activity for relatively long periods of time when stored frozen at the temperature of solid CO_2. Acetone precipitation of helenine from the crude SPS preparations does not apparently alter its keeping qualities so long as it is reconstituted in water and stored frozen under solid CO_2.

Filtration of helenine through Seitz pads is interfered with by the turbidity and viscosity of reconstituted acetone-precipitated preparations. However, slow freezing and thawing of such preparations brings down an inactive flocculent precipitate which can be removed by filtration through gauze leaving a clear amber fluid which contains all of the helenine activity. This clear fluid can then be filtered through Seitz pads without loss of helenine activity.

Although helenine is apparently readily filterable it does not pass a dialyzing membrane. Either in the crude SPS or after precipitation by acetone it remains within the bag when dialyzed against cold distilled water.

The effect of freeze-drying upon helenine activity is determined largely by the vehicle from which the drying is effected. Crude SPS can be freeze-dried without apparent loss of activity while acetone-precipitated helenine, reconstituted in distilled water, is frequently almost completely inactivated by freeze-drying. On the other hand, acetone-precipitated helenine, reconstituted in broth medium, does not lose activity on being freeze-dried. Of the various constituents of the medium that might be responsible for this protective effect, glucose and yeast extract appear to be more effective individually than do the salts of the medium.

Heating exerts a deleterious effect on helenine. Exposure for 3 minutes to the temperature of a boiling water bath markedly decreases its activity and autoclaving for 15 minutes at 15 pounds' pressure destroys it entirely.

Determinations of some of the chemical properties of active but crude preparations containing helenine have been made but these are of little or no value in arriving at a knowledge of the chemical make-up of helenine because we have no means of determining the relative amount of pure material contained in the preparations studied. The percentage of polysaccharide found was high but this may or may not indicate the importance of this class of substance in the make-up of helenine.

Although the complete mechanism by which helenine acts in achieving a therapeutic effect against the SK and Semliki Forest viruses cannot be decided from the information at hand, some phases of that information suggest possible explanations for at least portions of the mechanism of its action. The role of helenine either in inhibiting SK virus multiplication or delaying its neuroinvasiveness has been pointed out in an earlier paper (1). Subsequent work, especially that given in the present paper, has made possible additional surmises concerning the mode of action of helenine and some of these will be briefly discussed.

The achievement of maximum therapeutic action with relatively small doses and the "plateauing" of effect would suggest that helenine acts only indirectly through some antiviral function of the host itself. The fact that very large doses or repeated doses frequently do not improve the therapeutic result obtained with smaller, optimal doses of helenine can perhaps be best explained on the basis of a "triggering" function for helenine—a heavy pull on the trigger of a gun does not fire it any more completely or powerfully than does a small but adequate pull.

Whether helenine acts directly on the virus or exerts its effect through triggering an antiviral function of the host, a noteworthy finding in animals saved by helenine therapy has been the irregularity of subsequent immunity. Ordinarily, in the cases of the two viruses with which our experiments have been largely conducted, SK and Semliki Forest viruses, the few animals that survive untreated infections are solidly immune. However, mice that have been saved from either virus by treatment with helenine are frequently found to be still fully susceptible. This is especially true if the infecting dose of virus from which they have been saved was a small one. When the test has been a more severe one and the infecting dose of virus larger, then more of the mice saved by helenine therapy will be found subsequently immune. The suggestion from such results is that helenine therapy against minimal doses of virus exerts its effect, directly or indirectly, on the virus, destroying not only its infectivity, but its antigenicity as well. In the case of larger doses of virus it probably exerts the same effect against a portion of the virus.

If the affected portion of the virus is great enough, and the unaffected portion small enough, the animal survives but acquires immunity by virtue of exposure to the unaffected portion of virus.

In contrast to the irregular immunity found in animals saved by helenine therapy, those saved by treatment with specific virus-neutralizing serum are quite regularly immune on subsequent challenge. This difference in end-result indicates that the mechanism by which helenine exerts its therapeutic effect is not the same as that by which the antiviral serum acts. Though both prevent the virus from progressing to a fatal termination, the mechanism by which the antiviral serum acts is such that the antigenicity of the virus is not destroyed. The mechanism by which helenine acts upon the virus in arresting its progress, on the other hand, is one involving destruction of its antigenicity. This explanation presupposes eventual action of the two substances on the virus itself. If the assumption is made that the effect of both is not on the virus itself but on some stage in the cycle of virus multiplication then it would appear that the helenine effect takes place at an earlier stage than does that of the antiserum—a stage before the developing virus has acquired the capacity to elicit an immune response.

Some support for this second assumption is given by the results obtained in experiments in which viral infections were treated with both helenine and specific antiviral serum. In these the therapeutic results achieved resembled those got by treatment with the serum alone. However, though all the animals were saved by the combined therapy, they developed only a poor immunity in contrast to the almost perfect immunity achieved by animals treated with serum alone. This finding suggested that the helenine effect superseded the antiserum effect, yielding the end-result frequently seen in animals cured by helenine alone, namely, cure without subsequent immunity. This explanation would be more compatible with the view that helenine exerts its therapeutic effect, either directly or indirectly, by acting to interfere with some stage of virus multiplication, than that it acts upon the fully developed virus itself, and that this action precedes that at which the antiserum exerts its effect.

The results got by combined therapy with helenine and antiserum do not, of course, categorically rule out the possibility that both agents act directly on the virus itself; the helenine effect being to destroy it in such a way as to render it non-antigenic and the antiserum acting without destruction of viral antigenicity. Under such an explanation the therapeutic end-result expected would be the perfect one ordinarily achieved by treatment with serum alone. However, a portion of the cures would have been effected by helenine with destruction of viral antigenicity and the remainder, that percentage of animals ordinarily not cured by helenine alone, would have been achieved by the antiserum without destruction of viral antigenicity. Those mice then in which the virus had been destroyed by the helenine effect would subsequently

prove to be still susceptible while the remaining mice in which helenine had been incompletely effective and whose virus had been ultimately acted upon by antiserum would prove immune on subsequent challenge with virus.

Three products of microbial metabolism have in recent years been reported to exert a therapeutic effect against certain of the viruses not ordinarily affected by the commercial antibiotics. One of these is the polysaccharide substance of Horsfall and McCarty (2), derived from various bacterial species and active against the pneumonia virus of mice; another is an impurity in certain commercial grades of penicillin found by Groupé and Rake (3) to have an *in vitro* antiviral effect against some of the pox viruses; and the third is the antiviral substance recently reported by Powell and his coworkers (4) to be active against MM and Semliki Forest virus. While helenine has not been tested for activity against the pneumonia virus of mice, or any of the pox viruses, there is little to make one suspect an identity between it and either of the first two substances mentioned. The polysaccharide of Horsfall and McCarty, unlike helenine, has no therapeutic activity when administered intraperitoneally, and is effective against pneumonia virus of mice only when given intranasally. The material of Groupé and Rake exerts its effect upon the pox viruses only when incubated *in vitro* with them prior to inoculation into embryonated eggs. So far as we have been able to determine, in the case of helenine, it does not exert an *in vitro* effect upon the viruses against which it is active and certainly *in vitro* incubation with virus is not an essential method for observing its effect. While neither the polysaccharide substance nor the penicillin impurity, so far as can be told from the published reports, has been tested for activity against either SK or Semliki Forest virus, their mechanism of action against viruses upon which they do exert an effect is so different from the means by which helenine appears to act that there seems little likelihood that either substance is related in any direct way to helenine. Furthermore, helenine is destroyed by heating whereas both the other materials are relatively thermostable.

That helenine is distinct from the antiviral substance of Powell and his coworkers can be stated with much less certainty. In fact, much of what Powell's group have written in their preliminary publication on their antiviral substance suggests strongly that helenine may be related to it very closely indeed, and may even be the same substance. The closeness of the relationship between these two antiviral substances, both of them derived from penicillia, or their actual identity, may perhaps become evident on publication of the complete findings of Powell's group.

SUMMARY

Helenine is moderately stable in solution at refrigerator temperature and can be kept for long periods of time without evident loss of activity if stored

frozen at the temperature of solid CO_2. It is filterable through a Seitz pad but not dialyzable. Crude SPS preparations of helenine do not lose activity when dried from the frozen state. Some conditions are described, however, which influence the preservation or inactivation of acetone-precipitated helenine when freeze-dried. Helenine is partially inactivated by exposure for 3 minutes to the temperature of a boiling water bath and is completely inactivated by autoclaving at 15 pounds' pressure for 15 minutes.

The data presented suggest that helenine acts, either directly or by triggering some mechanism of the host itself, to destroy virus by a process which renders the latter non-antigenic. This effect may be exerted by action upon the virus itself or by interference with some stage in the developmental cycle of the virus.

While the chemical nature of helenine is not known, the presence of a large proportion of polysaccharide in crude active preparations might suggest the possible importance of this class of substance in helenine activity. It is believed that helenine differs from the polysaccharide reported by Horsfall and McCarty and the penicillin impurity reported by Groupé and Rake to be active against certain viruses. It may be related, however, to the antiviral substance recently reported by Powell and his co-workers.

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