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SECOND CONFERENCE ON ANTIVIRAL SUBSTANCES*

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INTRODUCTORY REMARKS

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In December, 1964 the New York Academy of Sciences sponsored the first conference on Antiviral Substances that now in retrospect seems to have been a turning point into the antiviral era. At that time no less than three drugs were fully established to have a worthwhile influence on viral disease in man. Until that time, many had believed that a chemical attack on viral disease was not possible and vaccination was the only acceptable approach. In the last 54 months there has been a noticeable increase in interest and, what is more important, a substantial increase in effort in the antiviral area, with continuing disenchantment with the vaccine approach. I think it is reasonable to say that of those interested in solving the problem of viral disease most believe chemotherapy must be the wave of the future just as it was 35 years ago in bacterial diseases.

At our first conference, four and a half years ago, research on interferon had not been included. Although many fundamental studies had been done on interferon, it seemed at the time that little progress had been made toward possible use of this knowledge in human viral disease. There is now, however, considerable practical interest. Interferon will, therefore, as it should, take its place as another aspect of the antiviral field.

We have before us at this conference some significant questions. Will interferon or its chemical induction be a practical method to alleviate viral disease? What direction should antiviral studies take to be most productive? Is chemoprophylaxis a logistically practical approach? How readily can true chemotherapy be achieved? What indeed will the basic studies of antiviral agents contribute to the overall solution of the viral disease problem? Hopefully we can find some answers to these and many more questions that may well determine the destiny of the antiviral field for some time to come. We cannot any longer hold to the outworn and sentimental concepts. New ground was broken four and one half years ago and some rewards were harvested. At this conference we shall reaffirm the often unrecognized progress of the past and again further prove that what many said could not be done has been and is being done.

The contribution and interest of the New York Academy of Sciences in the antiviral field has been immeasurable. They took a gamble in December, 1964 and it resulted in a significantly successful conference. Again they have been extremely generous and cooperative in supporting this Second Conference on Antiviral Substances. There is some considerable doubt, however, that a conference of this size and scope could ever have been produced without the generous financial support of 27 commercial firms both here and overseas. It perhaps is some measure of the significance of the subject that so many were so willing to invest so much to see this conference come to pass.

On behalf of Dr. Stinebring and myself I therefore welcome you, encourage you to actively participate in the discussions and wish you a highly productive four days.

Evaluation of Amantadine Hydrochloride in the Treatment of A_2 Influenzal Disease

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The therapeutic utilization of antibiotics resulted in dramatic relief from the ravages of bacterial infections. As clinicians involved in the management of patients with viral infections we look forward to similar drugs useful in the control of such illnesses. Few viral infections presently prevalent in this country are life threatening; those previously dreaded such as polio and the childhood exanthemata (not necessarily benign) have been contained through the use of effective vaccines. This approach may be impractical with the very common respiratory viral diseases which are initiated by a constellation of individual viral agents. Although, immunoprophylaxis may be useful for selected agents that cause the more serious infections. Unfortunately, vaccines developed against influenza virus have several shortcomings and have not yet provided ultimate protection. Thus, it is against this array of respiratory viruses, responsible for significant morbidity, that a chemotherapeutic or chemoprophylactic drug would be useful. It is the purpose of this paper to summarize the data indicating a therapeutic effect of one drug, amantadine hydrochloride in the management of infections caused by influenza viruses of the A_2 variety.

Promise of potential usefulness of this antiviral agent was first entertained from results in tissue culture, eggs and mice some years ago.(1,2) The information from these studies plus many additional experiments have provided reliably prognostic value as to whether an effect will be attained in man.(3) Thus there does appear to be correlation between sensitivity to amantadine as demonstrated in eggs, tissue culture or mice and subsequent effectiveness of the drug in man against particular influenza strains. The prophylactic use of amantadine in volunteers with induced A_2 infections demonstrated first that incidence and severity of illness was reduced and serological evidence of infection was impaired in drug treated individuals. These studies implied that the drug was protecting susceptible cells from virus. The known action of amantadine is not virucidal but rather viral penetration through the cell membrane is prevented. (4) The presence of the drug prior to initiation of disease could protect many cells reducing the viral population and thereby aborting infection and hindering the production of antibodies. Whether drug given after infection had started could impede progression of disease remained to be elucidated. Early experiments conducted by Jackson, 1965, were designed to test efficacy of drug given 1/2 hour prior to or 3 hours after virus inoculation (5). The attenuated vaccine strain A_2 type employed in these studies was not inhibited in man when drug was administered at approximately the time of challenge. In contrast drug given 20 hours prior to challenge was extremely effective in preventing infection. Additional studies conducted in volunteers

using only susceptible individuals - that is men with low or absent titers of homologous circulating antibody to a virulent challenge strain of A_2 virus, confirmed the drug's prophylactic usefulness (6). These encouraging results prompted therapeutic trials to further test the action of the drug once infection had already become manifest. Early in January, 1968 A_2 influenza was documented in several areas of the country. Following a uniform protocol, studies were conducted in Virginia, Maryland, Texas and Missouri in prisoners ill with a influenza-like illness. A small number of patients from a Masonic home in St. Louis, Missouri were also enrolled. Febrile men who by history were ill less than 48 hours were asked to volunteer. Drug was assigned in a double-blind fashion and each volunteer received either 100 mgm amantadine or lactose as a placebo twice a day for 10 days. Physicians admitted patients to a hospital ward where they were kept until discharge. Temperature recordings were obtained every four hours and findings were recorded on check sheets listing common signs and symptoms of influenza. Examinations were conducted twice daily or at least once a day after the acute illness had abated. In each study serum specimens were obtained on admission and day 21. Throat and nasal swabs for virus isolation were obtained in all studies at least twice during the first 3 hospital days. In the 2 Texas prisons we made a concentrated effort to determine effect of amantadine on virus shedding and swabs were obtained daily for five days.

TABLE I

Mean time of start of study medications after onset of influenza A_2 illness

	"Symmetrel" Groups		Placebo Groups	
	Hrs.	No.	Hrs.	No.
Walls, Texas	24.2	23	22.0	20
Wynne, Texas	23.7	17	21.8	17
Jessup, Maryland	19.6	12	22.7	15
Richmond, Virginia	15.6	21	15.0	28
Missouri State	20.7	12	24.9	8
Masonic Home (Mo.)	12.4	9	8.1	15
Total		94		103

The analysis of the results of the combined studies involved mainly clinical responses, temperature, antibody response and virus shedding. Only individuals with confirmed influenza either by four fold or greater rise in antibody titer or repeated virus isolation, were included. The first table outlines the areas where these studies were conducted and in addition the time interval between onset of illness und initiation of drug treatment. The figures represent only those men with confirmed influenza infection. Note the comparable time intervals for placebo and amantadine within the population groups.

TABLE II

Group* mean admission temperatures (F°)

	"Symmetrel"	Placebo
Walls Prison	100.9	101.3
Wynne Prison	100.9	101.1
Jessup Prison	101.3	101.9
Richmond Prison	101.5	101.0
Missouri State Prison	100.3	99.6
Masonic Home	100.4	100.4

*For study subjects with laboratory confirmation of influenza A_2

The second table lists the group mean temperature recordings at the time of admission to the study. The greatest mean variation between placebo and amantadine group was 0.7°F. The subsequent clinical response of these groups has been analyzed to determine whether amantadine had a beneficial effect. Our studies in the 2 Texas prisons and the Maryland prison convinced us that post treatment illness fell mainly into 2 categories. One in which there was a rapid clinical improvement and a second in which the response was delayed. Criteria were therefore established to catagorize the majority of these cases and to account for those falling in between the two groups. Figure 1 lists the criteria for rapid, medium and slow resolvers and depicts the number of individuals from the 6 clinical trials that fitted into each group. There is a statistically significant difference between the rapid resolver group and the slow resolvers favoring amantadine effect. 51% of the patients receiving this drug had a rapid response compared to 13.6% of the placebo group.

Analysis of one objective parameter of this complex, fever was carried out in several fashions. Figure 2 is a bar graph showing the number of individuals who had short lived temperature elevations compared to those who had slower defervescence. The results were identical to the preceding figure since fever was one of the main criteria utilized.

The flowing mean temperatures shown in Figure 3 more clearly demonstrate the comparison of temperature responses between the two drug groups in the individual studies. The time intervals at the bottom of five of the 6 graphs indicate the period when there was a statistically significant difference between amantadine or placebo groups. These intervals are shown again on Table 3. Note in the Maryland prison group, Jessup, only one very late period could be shown to be significantly different. Reasons for this exception to the trend in the other study locations are unknown.

The durations of fever of 99°F or greater from onset of illness are shown in Table 4. The expected 3 days of fever due to influenzal disease was apparent in the control group. Treatment with amantadine appeared to shorten the temperature course by about 1 day. The differences between amantadine and placebo groups were significantly different in the four

TABLE III

Moving group averages of body temperature during the 5-days from onset of dosing

Study Units	Times of significant differences*
Walls Prison	33 - 90 hours
Wynne Prison	0 - 85 hours
Jessup Prison	80 hours
Richmond Prison	24 - 90 hours
Missouri Prison	28 - 108 hours
Masonic Home	17 - 70 hours

* Between the drug-dosed and placebo-dosed groups for subjects with serologically confirmed influenza.

TABLE IV

Duration (hrs.) of fever of $\geq$ 99°F from onset of influenza illness

	"Symmetrel" Group	Control Group	p-value
Richmond Prison	60.9	80.1	<0.05
Wynne Prison	49.8	82.1	<0.02
Walls Prison	65.1	88.3	<0.01
Jessup Prison	66.0	92.0	<0.05

TABLE V

Duration (hrs.) of fever $\geq$ 100°F from onset of influenza illness

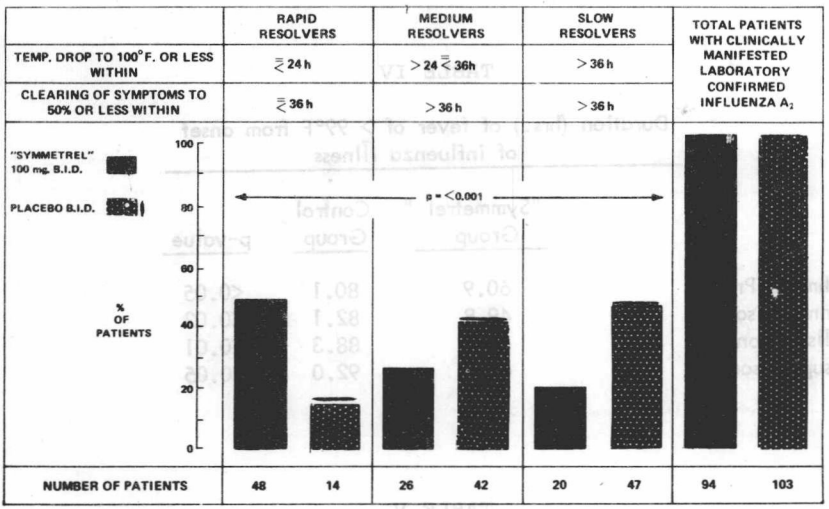
	"Symmetrel" Group	Control Group	p-value
Richmond Prison	41.7	63.3	<0.02
Wynne Prison	38.5	68.8	<0.01
Walls Prison	53.5	68.4	ns
Jessup Prison	48.0		ns

prisons analyzed using 99°F as the cut-off point. However when 100°F was established as the baseline for fever, the results were less clear cut. (Table 5) The trend in all four was for less fever in the amantadine groups but the results were only significant in one Texas and the Virginia prisons.

Symptoms and signs were recorded twice daily during the early phases of each study. In a prisoner population such subjective reactions are difficult at times to interpret. Quantitation of symptoms is frequently difficult in any population group. Duration of symptoms following influenzal disease has been shown by Imboden and colleagues to often be a function of the personality involved and not the illness itself (7). Nevertheless, when symptoms and signs were analyzed in the individual groups, differences were seen. As shown previously a 50% reduction in symptom score was incorporated in the criteria for clinical response, rapid, medium or slow resolution. In these prison

FIGURE 1

CURATIVE EFFECT OF "SYMMETREL"® (AMANTADINE HCl)
COMPOSITE OF 6 DOUBLE BLIND, PLACEBO CONTROLLED CLINICAL TRIALS



populations the trend was for recorded signs and symptoms to persist for a shorter period of time in the amantadine treated than in the placebo groups. In each location a few signs or symptoms were found to be significantly more persistent in the placebo group.

Antibody Response. The effect of prophylactic amantadine on antibody response has been to reduce the number of individuals who had four fold or greater antibody responses and also to reduce the geometric mean neutralizing antibody titer. In the latter instance this was assumed to be due to reduction in antigenic mass or perhaps prevention of viral multiplication which is

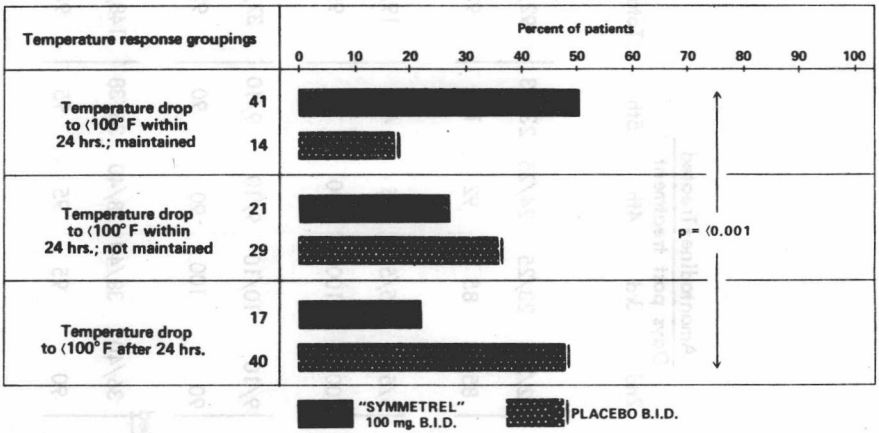
TABLE VI

Shedding of A2 Influenza Virus
(Walls and Wynne Prisons)

Placebo-Treated						Amantadine-Treated					
	Days post treatment						Days post treatment				
1st Before Treatment	2nd	3rd	4th	5th	Total	1st Before Treatment	2nd	3rd	4th	5th	Total
Rapid Resolvers											
4/6	6/6	5/6	6/6	6/6	23/24	19/25	22/25	23/25	24/25	23/23	92/98
%67	100	83	100	100	96	69	85	85	92	100	93
Medium Resolvers											
10/12	10/12	12/12	11/12	11/11	44/47	4/5	5/5	5/5	5/5	4/5	19/20
%83	83	100	92	100	94	80	100	100	100	80	95
Slow Resolvers											
16/19	12/19	16/19	16/19	16/19	60/76	10/10	9/10	10/10	9/10	9/10	37/40
%84	63	84	84	84	79	100	90	100	90	90	93
Rapid, Medium, Slow Combined											
30/37	28/37	33/37	33/37	33/36	127/147	33/40	36/40	38/40	38/40	36/38	148/158
%81	76	89	89	92	86	83	90	95	95	95	93

FIGURE 2

Defervescence



apparently needed for antibody stimulation. In the therapeutic studies included in this report no significant differences in antibody measurements were noted between placebo and amantadine treated groups. It would appear that by the time treatment was started and became effective, antibody stimulation had already begun and was not subsequently significantly altered. Jackson (5) has shown beginning antibody rise by 3 days post challenge in his volunteer studies. This was usually 48 hours after onset of illness. Assuming naturally acquired disease has a similar incubation period i.e. approximately 24 hours and amantadine therapy began to hasten recovery after another 24 hour period it appears plausible to assume antibody synthesis begins during these early days of infection and is not influenced by amantadine.

The antigenic stimulation for these antibodies is obviously the virus. One might guess that since no difference in antibody synthesis occurred between the 2 drug groups, the antigen i.e. virus persisted in both. Such indeed was the case. Table 4 illustrates this point. The data on this chart were obtained in the Walls and Wynne prisons in Texas. Virus recoveries were attempted during the first five days of participation in the study. It is apparent that amantadine treatment did not eliminate A_2 influenza virus from the pharyngeal area. Secondly virus disappearance was not associated with recovery from the disease since controls as well as amantadine treated patients were well by day 4 and most by day 3. The duration of viral carriage was not determined beyond 5 days but no evidence of lessening incidence of virus isolation by this day was evident suggesting prolonged persistence.

The results summarized here demonstrate a therapeutic efficacy of amantadine. Previous studies proved prophylactic effect. However, several questions remain to be answered. Of prime interest is the mechanism of therapeutic action. Amantadine does not have antipyretic or antihistamine activities. Perhaps the prevention of viral spread from the initial foci in