

CHEMOTHERAPY IN BUBONIC LYMPHOGRANULOMA VENEREUM

A Clinical and Serological Evaluation

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SYNOPSIS

The authors present the results of a study designed to measure the relative effectiveness of chloramphenicol, chlortetracycline (Aureomycin), oxytetracycline (Terramycin), and sulfadiazine in the treatment of lymphogranuloma venereum. The responses of the drug-treated patients are compared with those of a control group of patients who received symptomatic treatment only. The effectiveness of each method of treatment is evaluated in terms of the time required for resolution of the bubo and the rate of decline in the amount of complement-fixing antibody.

The literature on the chemotherapy of lymphogranuloma venereum,^{1-3, 7, 13, 15-18} which has been reviewed by Robinson,¹² shows that there is a considerable difference of opinion as to the relative effectiveness of the

sulfonamides and of the various broad-spectrum antibiotics. This disagreement appears to have resulted largely from the failure to take into account the extreme variability of the normal course of the disease and from the fact that control groups of patients from whom specific chemotherapy had been withheld were not included in the evaluations.

The present report summarizes the results of a study designed to measure the relative effectiveness of sulfadiazine, chloramphenicol, chlortetracycline (Aureomycin) and oxytetracycline (Terramycin) in the therapy of lymphogranuloma venereum and to compare the response of drug-treated patients with that of a control group of individuals for whom non-specific supportive measures alone were used. All the patients selected for study had bubonic lymphogranuloma venereum in the early stage in which chemotherapy might be expected to be most efficacious. In the study, the effectiveness of each method of treatment was evaluated in terms of objective clinical signs and of serological response over a long period of time. The data were subjected to analysis of significance by statistical procedures.

Materials and Methods

Patients

The patients included in the study were adults of both sexes who were residents of Washington, D.C. and who sought medical attention for early bubonic lymphogranuloma venereum at the Polk Health Clinics. The group was composed primarily of labourers and domestic servants of low socio-economic standing.

Each of the 43 patients investigated presented distinct evidence of lymphogranuloma venereum during the course of the illness. All the patients gave positive complement-fixation tests for lymphogranuloma venereum: 41 had complement-fixation titres of 1:40 to 1:640, and two individuals developed titres of only 1:20. Both the latter persons were in drug-treated groups in which effective chemotherapy might be expected to have suppressed the usual complement-fixing antibody response. In 35 patients the maximum complement-fixing antibody level had been reached by the time the first blood sample was withdrawn, while in the remaining 8 individuals, a fourfold or even greater rise in the complement-fixation titre was demonstrated in the progress of the illness. All but 5 of the patients had positive Frei reactions.

Some of the patients had genital lesions. These were examined repeatedly by darkfield examination but there was no evidence of *Treponema pallidum*. Smears or biopsy samples of all lesions were free of *Hemophilus ducreyi* and of Donovan bodies and none of the patients gave a positive Ducrey skin test. Urethral smears and cultures taken from all of the patients were free of detectable *Neisseria gonorrhoeae*. Routine complement-fixation and flocculation tests for syphilis, performed on serum specimens

collected at the time of the initial observation and at about 3-month intervals thereafter, were negative in all but 5 individuals. These latter persons were all latent syphilitics who had received adequate chemotherapy for syphilis previously; the flocculation and complement-fixation titres of these individuals ranged between 1:2 and 1:16, and the lymphogranuloma-venereum titres were from 1:40 to 1:640.

Method of study

The patients were seen on the average 15 days (the range was from 1 to 37 days) after the onset of the inguinal bubo, at which time the initial physical examination was made and specific or non-specific therapy was begun. The physical examination consisted of a thorough inspection of the genitalia, anorectum, and inguinal area; skin or eye manifestations and generalized lymphadenopathy were searched for. Examinations were made at bi-weekly intervals until the lesions, buboes, or draining sinuses were healed and then at monthly or quarterly intervals for up to two years.

The patients who received specific therapy were divided into groups, which were given chloramphenicol, chlortetracycline, oxytetracycline or sulfadiazine by the oral route. Patients received 1 g of antibiotic initially, followed by 0.5 g 4 times daily for 14 days. The dosage of sulfadiazine was 2 g initially, followed by 4 g daily, given in divided doses, for 10 to 28 days. The 17 patients treated symptomatically were given aspirin (coloured, aromatic, squared) as required to relieve pain and tenderness in the groin and to reduce the temperature. The fluctuant buboes were aspirated whenever the pain became so intense as to require relief. This was done on one or two occasions for 5 of the 17 patients treated symptomatically. Fourteen of the patients initially started on specific drug therapy were lost from the study because they failed to return to the clinic or were dropped because they developed an intercurrent infection with some other venereal-disease agent.

Blood samples collected at the time of the initial observations and at periodic intervals thereafter were transmitted to the Walter Reed Army Institute of Research, where the sera were separated from the clots and stored frozen at -20°C until tested for lymphogranuloma-venereum antibody.

The relative effectiveness of the specific and non-specific therapeutic procedures was measured in terms of the duration of inguinal adenopathy and the occurrence of other manifestations of the disease—namely, bubonic relapse, sinus formation or skin lesions. The buboes in all patients attained a diameter of 3 cm to 10 cm and became fluctuant. A bubo was considered healed when it regressed to a diameter of 1 cm or less, which was considered normal even though the lymph-nodes were often indurated and “shotty” for long periods after healing. Treatment was also evaluated serologically in terms of the antibody pattern following initiation of therapy.

Frei test. Lygranum ST and control antigens prepared by E. R. Squibb & Sons were employed. The intradermal test dose was 0.1 ml and the tests were read two days after the injection. A positive test was considered to be one in which there was a papule of at least 6 mm in both principal diameters. It was determined that injection of the Frei antigen did not exert a measurable change in the complement-fixation titre for lymphogranuloma venereum. From tests carried out on 14 persons not included in the study, it was found that the complement-fixation titre of blood collected 2-4 weeks after the injection was identical with the sample taken prior to the skin test.

Complement-fixation test. The complement-fixation tests were performed using a modification of a standard technique employed in this laboratory.¹¹ By this method, the patient's serum was inactivated at 56°C for 30 minutes and 0.25-ml amounts of serial twofold dilutions of the serum, covering a range from 1:10 to 1:1280, were allowed to react with 2 full units of guinea-pig complement in 0.5 ml and 2 exact units of lymphogranuloma-venereum antigen or normal control antigen in 0.25 ml. Following incubation at 37°C for 75 minutes, 0.5 ml of the haemolytic system, which consisted of 0.25 ml of 3% sheep-cell suspension and 0.25 ml of physiological saline solution containing 3 units of sheep-cell amboceptor was added. The tests were read after final incubation at 37°C for 30 minutes and the titre of the serum was considered to be the highest initial dilution of serum causing complete or almost complete fixation of complement. The antigen employed in the tests was prepared from yolk sacs of embryonated eggs infected with lymphogranuloma-venereum virus which were treated with phenol to enhance the potency.⁸ The control antigen was prepared in a similar way from the yolk sacs of uninoculated eggs.

In order to obviate the differences in complement-fixation titre that may result from day to day variation in the tests, all the serum specimens from each patient were retested at the end of the study on a single day and these values are the ones reported in this paper.

Findings

The clinical evaluation of the effectiveness of therapy in lymphogranuloma venereum is rendered difficult because of the paucity of symptoms and signs which can be measured objectively. The illness is typically an ambulatory one in which there is involvement, principally, of non-vital tissues and it is only in the late stages of the infection that fibrotic changes may affect the function of vital organs.

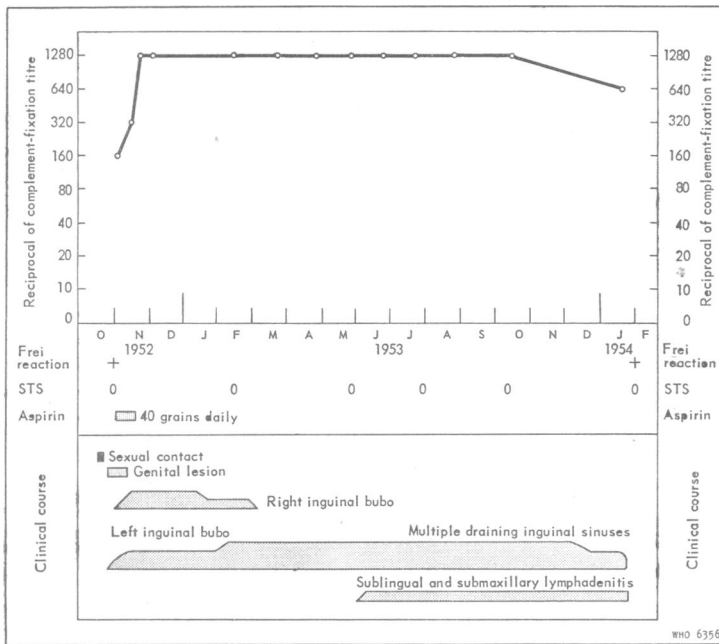
For the present investigation, a single clinical criterion only, i.e., duration of the bubo, was considered sufficiently objective to be generally indicative of the active viral process. The presence or absence of sinus formation, bubonic relapse or rash provided ancillary information. Measurement of

the complement-fixing antibody in the patient's serum provided indirect evidence of the duration of viral activity, and the probable significance of such findings is considered later.

Individual patients

In attempting to evaluate drug effectiveness by the above criteria, one is faced with the extreme variability of the natural course of the disease among patients who receive no specific treatment. This variation in clinical

FIG. 1. HISTORY OF PATIENT 1 (SYMPTOMATIC TREATMENT ONLY)



and serological pattern among three patients who were given symptomatic therapy only is exemplified in their case-histories, which are summarized in Fig. 1-3. Patient 1 (Fig. 1) was treated with aspirin only and presented a clinical course in which inguinal adenopathy persisted for at least 15 months. There was no significant diminution in the complement-fixation titre during the observation period. Patient 2 (Fig. 2), by contrast, had a rapid and spontaneous clinical recovery which was accompanied by a rapid decrease in antibody to the 1:10 level within 6 months after healing of the bubo. Residual viral activity, however, was suggested by the presence of complement-fixing antibody at a low level (1:10) for at least 7 months.

FIG. 2. HISTORY OF PATIENT 2 (SYMPTOMATIC TREATMENT ONLY)

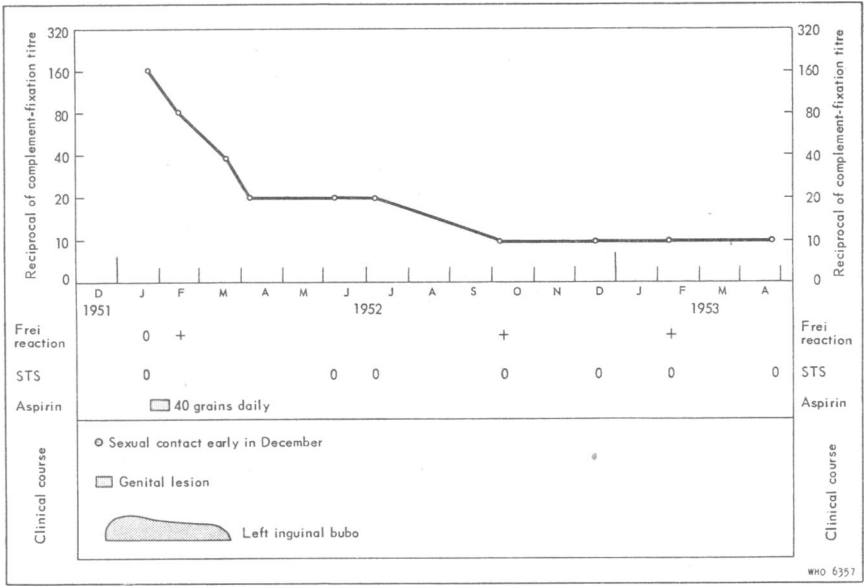


FIG. 3. HISTORY OF PATIENT 3 (SYMPTOMATIC TREATMENT ONLY)

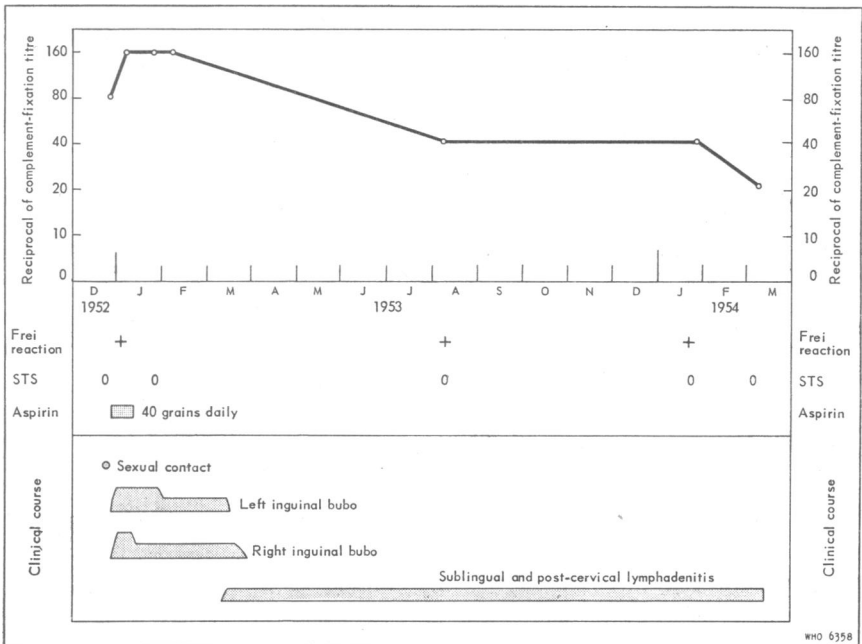


FIG. 4. HISTORY OF PATIENT 4 (CHLORTETRACYCLINE THERAPY)

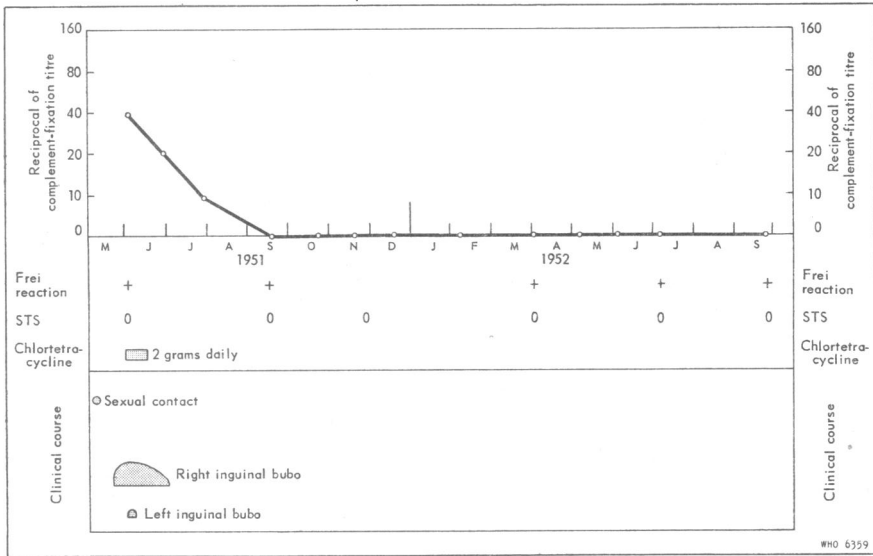
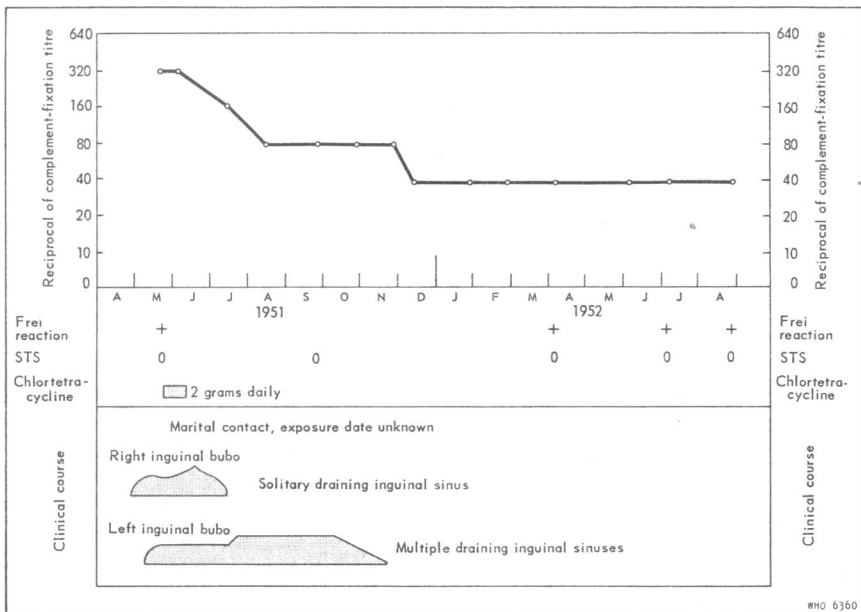


FIG. 5. HISTORY OF PATIENT 5 (CHLORTETRACYCLINE THERAPY)



Patient 3 (Fig. 3) presented a delayed but spontaneous remission of the inguinal adenopathy while lymphadenitis involving the head and neck persisted. The decrease in antibody was more gradual than that of patient 2, who did not exhibit signs of involvement of the head and neck.

Patients 4 and 5 (Fig. 4 and 5), who received chlortetracycline, represented the extremes of the clinical and serological pattern in the drug-treated cases. Patient 4 presented a course in which there was a prompt clinical remission and a rapid drop in the antibody titre following the administration of the drug. Patient 5, by contrast, had a relatively severe illness in which there was rupture of the bubo with formation of draining sinuses and inguinal adenopathy for at least 6 months after treatment was begun. There was a very slow diminution in the titre and the level did not fall below 1:40 during the period of observation.

Total group of patients

. Because of the variability in clinical and serological patterns in individual patients, it was considered expedient for the purpose of comparison to employ relatively large numbers of individuals and to consider the average response in the various groups when drawing conclusions. The following sections present the clinical and serological picture among the 43 patients given either specific therapy or symptomatic treatment.

Clinical evaluation. Table I summarizes the findings with respect to the duration of the bubo in drug-treated cases and in patients given symptomatic therapy. In the analysis, the stage of the disease when therapy was begun was measured by the duration of the bubo prior to starting treatment and the therapeutic effect by the persistence of the bubo after therapy had been initiated. There were wide individual variations among the cases. Thus, the duration of the bubo before treatment varied from 1 to 37 days and its persistence after treatment from 8 to 436 days. There was also marked overlap in the persistence of inguinal adenopathy among the different groups. Hence, for comparative purposes, the mean duration of the bubo before and after initiating treatment was used as the index for the groups, and the differences were compared statistically, using the standard error of the difference between two means.

It is seen in Table I that the duration of the bubo after treatment was about the same in all the drug-treated groups (23-51 days), indicating that the four drugs were essentially equally effective for treating the bubonic stage of the disease. The minor differences in the mean duration after therapy was begun were well within the sampling variation expected in groups of this size with a wide range of individual variation. On the basis of this particular type of observation it would appear that sulfadiazine was equally as efficacious as any of the three antibiotics tested and, moreover, that none of the antibiotics was superior to the others.

TABLE I. CLINICAL EVALUATION OF THERAPY

Treatment			Number of patients in group	Duration of bubo in days				
type	drug dosage (grams)	duration of treatment (days)		before treatment		after treatment		
				individual patients	mean	individual patients *	mean	median
Chloramphenicol	28.5	14	7	1, 5, 6, 14, 14, 15, 21	11	22, 36, 21, 61, 30, 8, 35	30	
Chlortetracycline	28.5	14	6	7, 14, 14, 23, 29, 30	20	34, 17, 27, 181, 31, 16	51	
Oxytetracycline	28.5	14	5	5, 7, 7, 10, 12	8	45, 14, 23, 16, 18	23	
Sulfadiazine	72	18	8	5, 5, 5, 8, 14, 21, 25, 28	14	13, 18, 25, 14, 13, 50, 14, 33	23	
Specific (all drug-treated cases combined)	—	—	26		13		31	23
Symptomatic (control group)	—	—	17	** 7, 8, 8, 9, 10, 11, 12, 13, 15, 19, 19, 21, 26, 26, 33, 35, 37	18	20, 27, 35, 21, 79, 31, 436, 8, 75, 171, 34, 37, 20, 44, 65, 23, 51	69	35

* Numbers correspond in sequence to same patients before treatment.
 ** Based on theoretical periods of time comparable to those of drug-treated cases.

Because of the similarity of response to the four specific agents, these groups were combined for comparison with the control group of patients treated symptomatically (see Table I). There was no significant difference between the mean duration of the bubo before therapy in the composite drug-treated group as compared with those given symptomatic therapy. After treatment was initiated, the bubo persisted on the average for 31 days in the composite group of drug-treated cases and for 69 days in the controls. This difference appears impressive but is influenced by some exceptionally high values in both groups. It is possible to test these values for significance by the use of a log-transformation. When this is done, a *t* value of 2.1 is obtained, indicating that the probability of this being a chance occurrence is 4.2 in 100. This indicates that there was perhaps some slight advantage in using the drugs but that it was not great. This is further borne out by a consideration of the median values for the duration of the bubo after treatment. These were 23 and 35 days, respectively, for the drug-treated and control groups. It was concluded from these findings that the clinical response to chemotherapy, as measured by the duration of the bubo after initiation of therapy, was not significant and did not appear to be greatly superior to the response to symptomatic therapy alone.

TABLE II. CLINICAL COURSE IN PATIENTS RECEIVING DRUG OR SYMPTOMATIC TREATMENT

Type of treatment	Number of patients treated	Number of patients showing clinical features of		
		bubonic relapse	sinus formation	skin lesions
Chloramphenicol	7	0	0	0
Chlortetracycline	6	0	1	0
Oxytetracycline	5	0	0	0
Sulfadiazine	8	1	1	0
Specific (all drug-treated cases combined)	26	1	2	0
Symptomatic (control group)	17	3	4	2

Other indices of clinical progress used to test the value of therapeutic agents were bubonic relapse following initial healing, sinus formation, and the occurrence of skin manifestations of the disease (see Table II). The number of persons who developed these complications was too small to show any significant differences but, in each instance, undesirable manifestations were more common among those treated symptomatically.

Serological evaluation. A serological evaluation of the effect of the four chemotherapeutic agents and of symptomatic therapy alone is presented in Table III. This table shows the geometric mean of the complement-fixation titres at the time treatment began and at regular intervals throughout the following 12 months. It can be seen that the initial mean titres for the four drug-treated groups varied considerably, ranging from 1:32 to 1:139. Nevertheless, the four drug-treated groups thereafter conformed to the same general pattern. The mean titre reached its maximum level before therapy began or shortly afterwards and declined consistently to a low level, ranging from 1:5 to 1:9, by the twelfth month. At the end of the year, all but one of the 26 patients receiving specific therapy showed a fourfold or greater decrease in titre and almost half gave negative results. This contrasted sharply with the response in the control group, in which the high levels of antibody were maintained for a long period and declined slowly. At the end of a year, the mean titre, which began at 1:83, had only been reduced to 1:34. Moreover, only 7 of the 17 patients in the control group showed a fourfold or greater

TABLE III. SEROLOGICAL EVALUATION OF THERAPY

Type of treatment	Number of patients	Mean complement-fixation titre for all patients in each group at indicated intervals (months) after initiating treatment						Number of patients showing after 1 year	
		0	1	3	6	9	12	a fourfold or greater decrease in titre	a zero titre *
Chloramphenicol	7	32**	24	14	10	10	6	6	3
Chlortetracycline	6	71	80	27	17	10	8	6	2
Oxytetracycline	5	139	80	40	19	15	9	5	2
Sulfadiazine	8	67	73	25	11	7	5	8	4
Specific (all drug-treated cases combined)	26	65	56	24	13	10	6	25	11
Symptomatic (control group)	17	83†	87	68	45	36	34	7	1

* A zero titre is one less than 1:10—the lowest serum dilution tested.

** Numbers are reciprocals of mean complement-fixation titres.

† Based on theoretical periods of time comparable to those of drug-treated cases.

decrease in antibody level and only one had a negative test. The differences in serological response of those receiving specific as compared with symptomatic treatment were statistically significant and indicated that therapy with any of the four agents resulted in more rapid loss of antibody than was afforded by symptomatic treatment.

Discussion

The present investigation, in attempting an objective evaluation of the effectiveness of chemotherapy in lymphogranuloma venereum, has clearly demonstrated the need for studying sufficient numbers of individuals in each of the groups to permit statistical comparisons and for including an adequate control sample. It has further stressed the necessity for applying serological as well as clinical criteria to the evaluation of the total response to treatment. The extreme diversity of the clinical patterns that follow specific or non-specific therapy has emphasized the difficulty of obtaining definitive results in small and uncontrolled studies and has offered an explanation for the widely divergent conclusions and opinions of various workers^{1-3, 7, 10, 13, 15-18} concerning the relative effectiveness of the various drugs.

The lack of significant difference in the persistence of the bubo among the groups of patients given chloramphenicol, chlortetracycline, oxytetracycline, or sulfadiazine indicated that none of the drugs was markedly superior to the others for treating the early disease. Further, specific chemotherapy had only a minimal effect in shortening the duration of the bubonic lesion. The slightly less frequent occurrence of complications such, as bubonic relapse, sinus formation or skin lesions among persons given specific treatment suggested that the drug was of some value in retarding or stopping viral activity and in preventing the further progress of the disease. This beneficial effect was more clearly shown in the marked increase in the rate of decline in antibody among the drug-treated persons and in the relative proportion of individuals whose complement-fixation titre fell below the detectable level.

The exact relationship between the persistence of antibody and the state of viral activity in patients with lymphogranuloma venereum has not yet been defined clearly. It has been shown,^{4-6, 8-10, 14} however, that the virus of lymphogranuloma venereum, like other agents of the psittacosis-lymphogranuloma-venereum family, has a marked tendency to establish latent or low-grade infections which persist for many years, thereby accounting for the continued synthesis of antibody over long periods of time, and for the gradual progress of the disease to late sequelae such as rectal stenosis, rectovaginal fistulae or elephantiasis of the genitalia. A reduction in or total loss of antibody titre may be categorically assumed, therefore, to be indicative of diminution in amount or elimination of the virus from the body.

It follows then that the marked reduction in antibody in the drug-treated cases in the present study should be regarded as a propitious event, and that specific chemotherapy should be routinely used in treating patients with lymphogranuloma venereum. Since the antibiotics were not superior to sulfadiazine, either in the clinical or in the serological response, the latter appears to be the drug of choice because of its greater availability and lower cost.

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RÉSUMÉ

La chimiothérapie de la lymphogranulomatose vénérienne a fait l'objet de fréquentes controverses entre les partisans des antibiotiques et ceux des sulfamidés. Ce différend peut s'expliquer par l'extrême variabilité du cours de la maladie et l'absence de groupes témoins dans les études effectuées jusqu'à maintenant.

Les expériences décrites par les auteurs de cet article ont porté sur cinq groupes de malades: un groupe témoin et quatre autres recevant respectivement du chloramphénicol, de la chlortétracycline, de l'oxytétracycline et de la sulfadiazine.

Les résultats ont montré la nécessité de recourir non seulement à des critères cliniques mais à des données sérologiques pour évaluer la réponse au traitement. L'absence de différences significatives dans la persistance du bubon entre les divers groupes traités par les divers médicaments, au cours de l'expérience considérée, ne permet d'attribuer la supériorité à aucun de ces derniers, dans le traitement des premiers stades de la maladie. La fréquence moindre des complications indique cependant que le traitement a freiné ou arrêté la progression de la maladie. Cet effet a été confirmé, du reste, par les réactions sérologiques, notamment par la baisse du taux des anticorps fixateurs du complément — même au-delà du niveau décelable — chez une plus forte proportion de malades.

D'après ce que l'on sait par ailleurs des réactions sérologiques produites par les virus du groupe psittacose-lymphogranulomatose vénérienne, on peut affirmer que cette baisse du taux d'anticorps est bien l'indication d'une régression du virus et même de son élimination. Ce fait permet d'encourager l'application de la chimiothérapie pour combattre la lymphogranulomatose vénérienne. La sulfadiazine, qu'il est facile de se procurer et qui est peu coûteuse, semble être le médicament de choix, puisque aucun des antibiotiques utilisés n'a donné de résultats nettement meilleurs.

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