Effect of Nitrogen Mustard on Serum Complement *in vitro* and in Patients with Neoplastic Disease. (18513)

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The object of the present study was threefold. First, to determine whether serum complement titers were normal in patients with neoplastic disease, especially of the lymphomatous types.† This seemed of interest since complement titers have been reported to be abnormal in certain infectious diseases(5,6). Second, to ascertain whether nitrogen mustard inactivates human complement *in vitro,* as it does guinea pig complement(1). Third, to determine whether the clinical administration of nitrogen mustard causes depression of complement titer. Because of clinical availability, this third part of the investigation was carried out with patients who received x-radiation in addition to nitrogen mustard, and hence the effect of x-radiation on complement titer has also been studied.

Methods. All sera were separated from the clot, frozen at −76°C within 2 hours of venipuncture, and were kept in deep freeze until titrated. Complement was measured by the technic of Kent, Bukantz, and Rein(4) in terms of the 50% hemolysis unit. Results are recorded as the fraction of a milliliter containing one unit of complement.

Complement titers in patients with neoplastic disease. The serum complement titers of 42 patients with neoplastic diseases, mostly lymphomas and leukemias, are presented in Table I, together with complement titers of 12 healthy adult controls. There was a greater range of variation among the complement titers of the patients than in the controls, but there was no significant difference between the mean titers of the several groups. Several sera were retitered after an interval of several days (at −76°C) with close reproduction of results.

TABLE I. Complement Titers of Patients with Neoplastic Diseases.

Diagnosis	No. of cases	50% hemolysis unit (ml) Range	Mean	S.D.
Ac. leukemia	6	.0015-.0065	.0030	.0012
Chr. myel. leukemia	7	.0023-.0072	.0047	.0017
Chr. lymph. leukemia	5	.0023-.0065	.0040	.0013
Lymphosarcoma	7	.0021-.0045	.0032	.0007
Hodgkin's disease	7	.0021-.0045	.0035	.0009
Bronchogenic Ca.	6	.0034-.0075	.0055	.0013
Breast Ca.	2	.0049-.0050	.0050	—
Nephroma	1	—	.0048	—
Osteogenic Sa.	1	—	.0058	—
All neoplasms	42	.0015-.0075	.0041	.0009
Normals	12	.0027-.0057	.0041	.0009

Effect of nitrogen mustard on serum complement in vitro. Freshly drawn serum from a normal male was divided into 6 portions and diluted with equal volumes of 0.85% saline or various concentrations of methyl-bis-beta-chloroethyl-amine hydrochloride dissolved in 0.85% saline. One sample of saline-diluted serum was then placed in a refrigerator at 3°C for 2 hours, while another saline control and all of the nitrogen mustard treated sera were kept at 37°C for two hours. Complement was then titered. This experiment was performed twice. Data are recorded in Table III. The nitrogen mustard in a concentration of 1.0 or 10.0 mg per ml of serum completely inactivated complement, but no significant change in complement titer was produced by the lower concentrations, as compared to the control subjected to the same conditions of incubation. The control incubated for 2 hours at 37°C showed a 25% to 50% loss of complement activity as compared with the control maintained at 3°C.

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† This phase of the study was done in cooperation with Dr. Anna Dean Dulaney formerly of this institute.

1. Watkins, W. M., and Wormall, A., *Nature,* 1948, v162, 535.

4. Kent, J. F., Bukantz, S. C., and Rein, C. R., *J. Immunol.,* 1946, v53, 37.

5. Dulaney, A. D., *J. Clin. Invest.,* 1948, v27, 320.

6. Ecker, E. E., Seifter, S., Dozois, T. F., and Barr, L., *J. Clin. Invest.,* 1946, v25, 800.

TABLE II. Serial Complement Titers in Cancer Patients Before and After Treatment with 0.4 mg/kg Nitrogen Mustard Plus Radiotherapy, and in Untreated Controls.

			50% hemolytic unit (ml)						
				Days post Rx					
Age	Sex	Dx	pre Rx	1	2	3	4	5	11
73	M	Broncho	.0075	.0067	.0088				
62	F	Nephroma	.0048	.0054					
56	M	Broncho	.0055	.0055	.0058				
60	M	"	.0064		.0063	.0042			
31	F	Breast	.0049	.0057	.0048	.0056	.0066		
65	M	Broncho	.0050	.0050					
52	M	"	.0049		.0048			.0046	
60	M	"	.0034	.0033			.0035		
34	M	Osteo.	.0058	.0062		.0062			.0056
33	F	Untreated breast	.0050	.0053	.0057	.0054	.0046	.0051	
30	M	Untreated normal	.0079	.0081	.0078	.0058		.0059	

TABLE III. Effect of Nitrogen Mustard on Human Complement Activity *in Vitro*.

N mustard conc. mg/ml of serum	2 hr incubation, °C	ml of serum containing 1 unit complement	
		Exp. 1	Exp. 2
0	3	.0081	.0067
0	37	.015	.0085
.01	37	.012	.0090
.10	37	.013	.0089
1.00	37	>.100	>.1000
10.00	37	>.100	>.1000

Serial complement titers after nitrogen mustard and x-ray therapy. Serial specimens of blood were obtained from 9 patients before, and for one to 11 days after, injection of nitrogen mustard. Each patient received a single dose of 0.4 mg/kg of methyl-bis-beta-chloroethylamine hydrochloride, given intravenously while circulation of both legs and one arm was completely occluded by tourniquets. Tourniquets were left in place for 5 to 10 minutes after the injection in order to concentrate the nitrogen mustard in the trunk during its short period of cytotoxic activity(2). The maximum theoretical concentration of nitrogen mustard that might occur by use of this method is approximately 0.01 mg/cc of serum (assuming a plasma volume of 5% of body weight, complete intravascular mixing, 50% of blood volume isolated by tourniquets, and no immediate intracellular or extra-vascular diffusion). Immediately after the nitrogen mustard injection, intensive fractionated-dose radio-therapy was given to the diseased areas from a million volt machine, and was repeated daily during or beyond the period of the complement studies.‡ Most of the patients had x-ray directed to the thoracic area. Dosage was in the range of 2000 r tumor dose in a period of 7 to 14 days. The patients' diagnoses are indicated in Table II. Two untreated controls, one normal and one cancer patient, were included in the study in order to determine the normal fluctuation in day to day complement titers. It is apparent from the data (Table II) that no significant variations in complement titer occurred. Daily fluctuations in the controls are as great as the changes in the treated patients.

Because it seemed possible that the nitrogen mustard (or x-ray) treatment might have caused an immediate drop in complement which was obscured in the above studies by rapid regeneration of complement in the first 24 hours after treatment, blood was drawn

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‡ The clinical efficacy of this combined method of therapy for inoperable neoplasms is being evaluated at Memorial Hospital by Drs. Ralph Phillips and D. A. Karnofsky, and is based on the additive effects of nitrogen mustard and x-rays in certain sequences in animals(3).

3. Karnofsky, D. A., Burchenal, J. H., Ormsbee, R. A., Corman, I., and Rhoads, C. P., Approaches to tumor chemotherapy, pp. 11-23, A.A.A.S., 1947.

from one patient immediately before injection of the nitrogen mustard, 5 minutes after injection but before tourniquets were released, 2 minutes after tourniquets were released, and 3 hours after treatment was completed. No significant change occurred. The titers, in the order in which the specimens were taken, were 0.0058 ml, 0.0061 ml, 0.0060 ml, and 0.0062 ml per 50% hemolysis unit.

Discussion. This study indicates that in the neoplastic diseases studied there is no consistent deviation of complement activity from normal, although greater variability may occur than in normals. In a series of patients with various infectious diseases, Ecker *et al.*(6) had similar findings. These workers, however, found frequent high complement titers in streptococcus and pneumococcus infections, and frequent low titers in meningococcus meningitis, which differences they felt to be significant. Dulaney(5) has reported frequent low complement titers in malaria. Ecker *et al.*(6) reported a possible relationship of complement to severity of illness and serum protein concentration, but no relation to fever or leukocytosis. In the present work serum proteins were not studied, but there was no apparent relation between complement titer and extensiveness of disease, fever, degree of debility, leukocyte count, or transfusions.

The results of the present *in vitro* experiments with nitrogen mustard and human complement are in general agreement with the results reported with guinea pig complement by Watkins and Wormall(1). The absence of any change in complement titer following clinical use of nitrogen mustard is what would be expected from a consideration of the *in vitro* studies, since the concentrations of mustard attained in patients are far below those which destroyed complement *in vitro*.

Conclusions. The mean serum complement titers of patients with various neoplastic diseases did not differ significantly from that for normal adults. The range of complement values was greater among cancer patients than among normals, but the erratic titers did not appear to be related to clinical status. No depression of human complement titer was observed following the clinical use of nitrogen mustard and x-ray in maximal dosage. Human complement is inactivated by high concentrations of nitrogen mustard *in vitro*, but not by concentrations in the range which is achieved in clinical usage.

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Influence of Vitamin B_{12} and Liver Extract on Nitrogen Balance of Normal and Hyperthyroid Rats.* (18514)

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The retardation of growth observed upon feeding thyroid hormone can be prevented by simultaneous administration of liver preparations in growing mice(1), chicks(2), rats(3-7), and rabbits(8). Similar effects have been obtained in rats with vitamin B_{12} (8,9), and with various liver preparations high in antipernicious anemia activity(8). Other

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