Serologic Analysis of Ehrlich Tumor-Cell Induced Ascites Fluid.* (25257)

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It has been reported that viral antibodies can be found in ascites fluid induced in mice by Sarcoma 180(1) or Ehrlich tumor cells (2). Munoz(3) was able to induce large quantities of peritoneal fluid in mice by injection of Freund's adjuvant. The fluid was shown to contain antibodies against certain specific albumins used as immunizing agents; however, Munoz reported that only 50 per cent of the mice responded with an ascites fluid when injected with Freund's adjuvant. Recently Lieberman *et al.* reported(4) that intraperitoneal injection into mice of *S. aureus* mixed with Freund's adjuvant resulted in all of the mice producing an ascites fluid. Since large quantities of peritoneal fluid can be obtained regularly in mice injected intraperitoneally with Ehrlich tumor cells, it was of interest to serologically analyze the fluid to learn if the ascites could be employed as source for large quantities of various humoral components of mouse origin.

Materials and methods. In all experiments adult albino mice (*Mus musculus*) of mixed gender weighing 25-35 g each were employed. Mice were immunized by intraperitoneal injection of heat-killed *S. typhimurium*. The injection schedule was 0.1 ml of vaccine administered at intervals of one week until 3 injections were completed. Immediately following last injection the mice were intraperitoneally injected with 0.25 ml of a heavy suspension of ascites cells taken from peritoneal cavities of infected mice. Another group of mice were injected only with the tumor cell suspension. The Ehrlich tumor cell challenged mice were sacrificed after 10 days at which time all animals used were markedly distended with ascites. The blood as well as the ascites fluid was collected and pooled. The average yield of fluid/mouse at this time was 20-30 ml. The average yield of blood following severance of neck vessels was 2-3 ml/mouse. The blood was allowed to clot 2 hours at room temperature; the clot was rimmed and removed by centrifugation at high speed in a refrigerated centrifuge. Cells in the ascites were removed by mild centrifugation in the cold. Aliquots of both serum and cell-free ascites fluid were then recentrifuged at 25,000 g for 15 minutes. The serum and ascites fluid were assayed for whole complement (C′) activity employing the micro-Kolmer method(5). Properdin assays were performed using the zymosan technic of Pillemer *et al.*(6). Other serologic tests were performed using standard technics(7). Electrophoretic patterns were obtained by paper strip technic employing a Spinco Model R apparatus. Total protein concentrations were determined by micro-Kjeldahl procedures.

Results. The data in Table I indicate that neither mouse ascites nor serum contain normal agglutinins for human or sheep red blood cells(8). The ascites from mice immunized against *S. typhimurium* contained agglutinating antibodies to the immunizing agent although the titer was lower than the serum titer. There was no detectable properdin in the ascites from immunized or nonimmunized mice, but serum properdin levels were within limits reported by others(9). The serum and ascites were tested for whole C′ titer and contained less than 3 units/ml; both fluids had similar anticomplementary properties which could be removed by 1:4 dilution in buffer of serum or ascites fluid.

The data in Table II show total protein concentrations of serum and ascites. In addition the concentration of the various protein components of the 2 fluids is shown. Gamma component of globulin in the ascites from immunized mice constitutes 37% of total protein while the same component in the ascites from the nonimmunized mice comprises 29%. The major difference between the ascites from immunized and nonimmunized mice appears

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TABLE I. Serological Analysis of Mouse Serum and Ascites Fluid from Ehrlich Tumors.*

	Mouse serum		Mice with Ehrlich ascites tumor			
			Nonimmunized mice		Immunized mice	
	Normal	Immunized	Serum	Ascites	Serum	Ascites
Human RBC agglutinin titer	neg.		neg.	neg.		
Sheep " " "	"		"	"		
Properdin titer (units/ml)	10	10	10–20	<4	10–20	<4
Whole C′ titer (units/ml)	<3	<3	<3	<3	<3	<3
Anti C′ property removed at	1:4	1:4	1:4	1:4	1:4	1:4
S. typhimurium agglutinin titer	neg.	1:8192	neg.	neg.	1:8192	1:2048

* All results similar with specimens centrifuged at 25,000 g or low speed.

to be in the beta globulin component. It is apparent from the results that the protein synthesizing capacity is not impaired as a result of tumor cell growth. To the contrary, it appears that compensatory synthesis occurred to make up the protein content of the ascites.

Discussion. Intraperitoneal injection of Ehrlich tumor cells into mice regularly induced an ascites which amounted to 20-30 ml/mouse when the fluid was removed from animals 10 days post-challenge. The ascites and serum did not contain any normally occurring agglutinins for human or sheep erythrocytes or for *S. typhimurium*. The ascites contained specific agglutinating antibody to *S. typhimurium* when the mice were immunized against this agent. The agglutinin titer of the ascites was lower than that present in serum of immunized mice but of sufficiently high titer to be employed for study of antibody reactions.

The ascites did not contain any detectable properdin activity at the dilution of fluid (1:4) employed in the assay. The whole C′ titers of serum and ascites were of the same magnitude, that is, less than 3 units/ml in each case. Both fluids were anticomplementary, however this effect could be overcome by 1:4 dilution in both cases.

Values for concentration of serum and ascites protein constituents were obtained from pooled fluid samples. This design was adopted to decrease the error involved in an experiment of this type.

Concentrations of protein components in serum from normal or specifically immunized mice are very similar to those reported by Clausen *et al.*(10). Serum obtained from mice free of the neoplasm exhibited a high concentration of beta globulin as compared to alpha and gamma fractions. Clausen *et al.* (10) reported that serum from (CBA × DBA/2) F_1 hybrid mice, infected with plasma cell leukemia, showed increased amounts of beta globulin components and decreased amounts of gamma components while no significant change occurred in the alpha components. In the present investigation a loss of beta globulin from the serum appeared to occur as a result of Ehrlich tumor cell infection.

TABLE II. Paper Strip Electrophoretic Analysis of Mouse Serum and Ascites from Ehrlich Tumors.

Material	No. of specimens pooled	Total protein	Albumin	Globulin α	Globulin β	Globulin γ
Normal mice serum‡	12	6.78*	2.95	1.22	1.81	.8
Immunized mice serum†	12	5.7	2.6	1.0	1.4	.7
Mice with Ehrlich ascites tumor						
Nonimmunized mice serum	15	4.9	2.11	.7	.6	1.5
Nonimmunized mice ascites§	15	3.1	.9	.2	1.1	.9
Immunized mice serum	14	6.45	3.5	.7	1.5	.75
Immunized mice ascites	14	3.5	1.36	.39	.44	1.31

* g %.
† Immunized by 3 intraper. injections of heat-killed *S. typhimurium* given one week apart.
‡ Avg yield of serum/mouse .5-1.5 ml.
§ " " " ascites/mouse 20-30 ml.

It is of interest to note that serum of non-immunized mice infected with tumor cells showed less beta and more gamma globulin protein than the ascites fluid obtained from the same group of mice. In contrast, the serum of immunized mice infected with tumor cells showed more beta and less gamma globulin protein than the ascites fluid obtained from the same pool of mice.

It is apparent that by suitable fractionation of ascites fluid large amounts of purified antibody, if not properdin, can be obtained for study of mouse serologic reactions.

Summary. Ascites fluid induced in mice by intraperitoneal injection of Ehrlich tumor cells contained specific antibody to *S. typhimurium* when mice were immunized against this organism. The serum and ascites were identical in their anticomplementary properties and neither contained detectable complement. The serum had normal properdin levels, but the ascites did not contain detectable properdin under the assay conditions employed.

1. Herrmann, E. C., Jr., Engle, C., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 257.
2. Wagner, R. R., *J. Immunol.,* 1955, v74, 439.
3. Munoz, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 757.
4. Lieberman, R., Douglas, J. O. A., Humphrey, W., Jr., *Science,* 1959, v129, 775.
5. Kolmer, J. H., Spaulding, E. H., Robinson, H. W., *Approved Laboratory Technic,* Appleton-Century-Crofts, 1951.
6. Pillemer, L., Blum, L., Lepow, I. H., Wurz, L., Todd, E. W., *J. Exp. Med.,* 1956, v103, 1.
7. Kabat, E. A., Mayer, M. M., *Experimental Immunochemistry,* Charles C. Thomas, 1948.
8. Marcus, S., Esplin, D., Donaldson, D. M., *Science,* 1954, v119, 877.
9. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., Wardlaw, A. C., *ibid.,* 1943, v120, 279.
10. Clausen, J., Hermans, J., Heremans, M. T., Rusk-Nielsen, R., *J. Nat. Cancer Inst.,* 1959, v22, 57.

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Effects of Nitrogen Mustard N-Oxide, X Rays, and Cortisone on Yoshida Sarcoma Metastasis Development. (25258)

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At present, the most effective treatment for cancer is complete removal of malignant tissue by means of surgery. Despite the great value of this method, however, its usefulness is unfortunately limited by the difficulties involved in locating metastases. This particular limitation does not apply to carcinostatic agents, since their effects are systemic. Nevertheless, the mode of action of such therapeutic agents is but poorly understood, and many problems remain to be solved. For example, we have observed that the use of carcinostatic agents has sometimes been followed by an increase in tumor size or by the production of metastases, thus multiplying the problems connected with surgery.

Materials. An outbred strain of Gifu male rats weighing about 100 g was used in this investigation. Eight million cells of a 7-day-old Yoshida ascites tumor were transplanted subcutaneously into the right axillary area. Three weeks after tumor implantation, the animals were autopsied and examined for metastases. Certain batches of injected cells were taken from a tumor which had been made resistant to the carcinostatic effects of nitrogen mustard N-oxide ("NMO").† This tumor, which was kindly supplied by Dr. I. Hirono(1), had been made resistant to NMO

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† The NMO used was supplied under trade-name NITROMIN by Yoshitomi Pharmaceutical Industries, Osaka, Japan. The chemical name for this brand of NMO is methylbis(2-chlorethyl)amine N-oxide hydrochloride.