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A Modified Seroflocculation Reaction in Relationship to Invasive Neoplasms.* (23106)

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In a previous communication(1) a seroflocculation reaction was described as a laboratory aid in diagnosis of invasive cancer in humans. This reaction consisted of mixing serum with ethyl choladienate under controlled conditions and analyzing the resulting flocculation reaction. While a preponderance of positive reactions was obtained with sera from histologically proven malignancies, a considerable number of positive reactions was given by sera from normal individuals and from some patients with non-malignant pathological conditions. This is to report on a modification in the procedure whereby the incidence of "non-specific" positives can be significantly reduced. As a result of an investigation by Kidd(2), who noted that non-specific reactions to the Brown-Pearce tumor antigen by normal rabbit sera could be eliminated through heating the sera at 65°C for 30 minutes, the following study was made to determine whether a similar treatment of human sera would reduce the incidence of non-specific positives in the flocculation test for malignancies. Preliminary studies, in which sera were heated at various temperatures ranging from 56°C to 65°C, showed that 64°C was optimum. Higher temperatures tended to coagulate certain sera. Sera from 2,747 individuals were tested (Table I); 220 of these individuals had biopsy proven cancer (Table II), 1,822 were normal blood donors, and 705 had non-malignant diseases (Table III). The results clearly indicate that this modified procedure reduces the number of false positives.

Materials and methods. Blood was obtained by venipuncture under aseptic precautions and stored overnight at temperatures between 4°-6°C. Serum was separated from the clot by decantation and centrifugation. Each serum was then divided into approximately 2 equal volumes. One was heated at 56°C and the other at 64°C for 30 minutes simultaneously. *For testing, all sera and solutions were maintained at 15°C (iced water bath).* The following solutions were required: (A) Buffered saline solution(3) consisting of 6.5 ml of 0.1M citric acid, 43.5 ml of 0.2M anhydrous disodium phosphate, 42.5 ml of 5% sodium chloride and then diluted to 250 ml with water; (B) 0.1 mg percent of bovine albumin solution (Fraction V Armour Laboratories) in buffered solutions; (C) 1% cholesterol in absolute ethanol; (D) Ethyl choladienate 15 mg per ml in 95% ethanol. The *antigen suspension* was prepared by depositing at the bottom of a tube 0.013 ml of the cholesterol solution (C), plus 0.1 ml of the antigen solution (ethyl choladienate solution D) and mixed by rapid rotation of the tube. To the alcoholic solutions C and D was added 0.4 ml of the citrate phosphate buffered nor-

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TABLE I. Analysis of Cases Studied.

Category	Total No. of cases	Positive reactions with heated serums 56°C	64°C
	2747		
		(%)	(%)
Carcinoma	220	204 (93)	202 (92)
"Normal" individuals and blood bank sera	1822	238 (13)	11 (0.6)
Non-malignant hospitalized cases	705	275 (38)	71 (10)

mal saline containing bovine albumin (B). These were thoroughly mixed by gently inverting the tube several times. (Too rigorous agitation may precipitate suspension.) The suspension was used within 15 minutes after preparation. The test was carried out as follows: All glassware was thoroughly cleansed, rinsed in distilled water and oven-dried. Two tubes (13 x 100 mm) were used. To one was added 0.1 ml of serum and 0.15 ml of antigen suspension; to the other 0.07 ml of serum and 0.15 ml of antigen suspension. Rack was shaken in Kahn shaker for 3 minutes. To each tube was then added 0.8 ml of buffered normal saline (A), and centrifuged at 3,000 rpm for 10 minutes. Readings were made in diffuse standardized light after shaking tubes to disperse precipitate at the bottom. Positive reactions gave particles in a crystal clear medium in one or both tubes. Negative reactions gave various degrees of turbidity with or without particles. Occasionally there occurred a very slight turbidity in the test serum as compared with the sparkling clarity of the positive control. These "doubtfuls" were considered negatives. Sera were tested in units of thirty. In each unit was included a known positive and a known negative control.

Results. Comparison of the earlier and the present modified methods (Table I) shows a striking reduction in the incidence of "false positives." Tests on the normal group ranging from 20 to 70 years of age, clearly demonstrated the reduction of "false positives" (13% to 0.6%) by heating the serum at 64°C. The results also show that heating at 64°C did not diminish sensitivity of the reaction with respect to detection of malignancies. It should be noted that this group of malignant tumors does not contain carcinomas *in situ*. The seroflocculation reaction has been concerned primarily with invasive malignancies. The limited number of neoplasms of female sex organs and none in children is due to the fact that the sera were obtained almost exclusively from the male hospital wards.

Occurrence of positive reactions in a wide

TABLE II. Cancer Sera.*

Site of malignancy	Total cases	Reactions with heated serums 56°C No. positive	56°C No. negative	64°C No. positive	64°C No. negative
	220	204	16	202	18
Head and neck	41	40	1	40	1
Chest	28	28	0	28	0
Gastro-intestinal	34	33	1	33	1
Genito-urinary	45	43	2	42	3
Bone	10	9	1	9	1
Female breast	7	5	2	4	3
" pelvic	3	2	1	2	1
Metastasis, primary unknown	3	3	0	3	0
Lymphoma	9	9	0	9	0
Squamous-cell epithelioma	21	17	4	18	3
Basal-cell epithelioma	14	11	3	10	4
Melanoma	5	4	1	4	1
% positive		93%		92%	

* All diagnoses based on histopathological findings exclusively.

TABLE III. Non-Cancer Serum.

Diagnosis	Total cases	Positive reactions with heated serums 56°C	64°C
	705	275	71
Rheumatoid arthritis, active	96	38	6
Endocrine disturbances + medication	143	59	16
Allergy disease + medication	113	32	12
Cirrhosis	14	10	4
Gastric ulcer	30	11	3
Duodenal "	13	3	0
Active tuberculosis	25	16	4
Bronchiectasis	7	4	0
Diabetes *on* insulin	66	33	7
" *no* "	46	10	2
Pregnancy	14	6	1
Chronic cystic mastitis	7	2	1
Menorrhagia	31	19	2
Infection with temperature	20	12	10
Keratosis	26	4	0
Bone cyst	4	0	0
Lipogranuloma	2	1	0
Benign prostatic hypertrophy	48	15	3
% positive		39%	10%

variety of other pathological conditions (non-malignant group) was also considerably diminished (38% to 10%). The persistence of positive reactions in this group might suggest the presence of some change in plasma which is similar to that occurring in malignancies.

Summary. A method is described for an improved laboratory aid in mass screening for invasive neoplasms. The modification of the described serofloccculation test consisted essentially in heating sera at 64°C for 30 minutes.

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Transfer of Lymph Node Cells to Recipient Rabbits Pre-Injected with Blood Leucocytes of Donors.*† (23107)

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Studies of antibody formation in this laboratory have recently involved the technic of transfer of lymph node cells from donor to recipient animals, as described by Chase (1,2). In earlier studies, cells to be transferred were obtained from lymph nodes draining sites of injection of antigen into donor rabbits(3,4); more recently lymph node cells obtained from uninjected donors (or from donors injected with heterologous antigens) were incubated *in vitro* with the antigenic substance and then transferred to X-irradiated recipient rabbits(5,6). In each case antibody to the antigen referred to appeared subsequently in sera of recipients. In both systems it was found that living cells were necessary, and that antibody did not appear in recipients of injured cells. In studies with other systems involving humoral antibody, or resistance to tumor homografts, various workers also found that injury to lymph node cells prior to transfer results in failure of antibody to appear in the recipient(1,2,7-9). However, the role of transferred cells in the formation of the antibody subsequently found in the recipient has not been clarified. In the present study the relation of transferred cells to such antibody was approached by attempts to alter the host-tissue environment of the transferred cells, rather than by manipulation of the cells themselves. This approach was suggested by

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