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Effects of Blood Taken from Patients with Neoplastic Disease on the Chick Embryo.* (28946)

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Handler *et al*(1) reported that whole blood or blood fractions, obtained from patients with various forms of neoplastic disease, were toxic to chick embryos when inoculated onto the chorioallantoic membrane (CAM) of the chick embryo at the 6th to 9th day of incubation. They state that a comparison of the toxicity in chick embryos of blood from patients with and without neoplastic disease revealed that blood from 186 of 187 patients with neoplastic disease tested was toxic in eggs. Of 35 samples of normal blood, 5 were toxic; 3 of these patients had hairy or pigmented nevi, one had an osteochondroma with fever, and one a laceration, presumably under treatment. The toxic factor was present in whole blood, plasma and plasma suspensions of leukocytes. Handler(1) suggests that a subcellular factor exists in the blood of patients with neoplastic disease that is involved in the high rate of chick mortality, and furthermore, that the utilization of these techniques may be a possible tool for clinical diagnosis. Because of the important implications of this report, their technique has been used in an attempt to confirm the presence of a factor in the blood of patients with neoplastic disease toxic to the 8-day chick embryo.

Materials and methods. Fertile chicken eggs were obtained from a commercial source and incubated at 38°C. The embryos were checked for viability on the 8th day. A window was made over a major blood vessel and the CAM was dropped. Blood was obtained

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TABLE I. Summary of Results Obtained with Blood from Normal Individuals and Patients with Neoplastic Disease Inoculated onto the CAM of the 8-Day Embryo.

Diagnosis	No. patients	No. tests	Embryo deaths/test (number out of 10 embryos) "Non-toxic" 0	1	2	3	"Toxic" 4	5	6	7	No. tests "toxic" (%)
Controls	35	38	23	7	3	5					0
Acute leukemia	28	31	12	9	6	3	3	1			13
Chronic myelocytic leukemia	14	19	4	6	3		2		3[1]		26
Chronic lymphocytic leukemia	6	10	3	2	1	2	1[2]				10
Lymphomas	13	13	5	3	3	1	2				15
Carcinomas	20	31	8	10	6	3	2[3]		1[4]	1[5]	13
Sarcomas	8	10	3	2	3	1		1			10
Total	124	152									

[1] Test repeated 3 more times, "non-toxic." [2] Test repeated once, "non-toxic." [3] Test repeated twice, "non-toxic." [4] Test repeated 3 additional times, 2 "toxic," 2 "non-toxic." [5] Test repeated once, "non-toxic."

from patients and apparently normal individuals in sterile heparinized tubes (B-D Vacutainer). The tube was immediately sent to the laboratory, and in each test 10 embryos were each inoculated with 0.1 ml onto the CAM. No more than 30 minutes elapsed, in the great majority of cases, from collection of the blood to its introduction onto the CAM. The opening in the shell was sealed with scotch tape. The eggs were candled daily and the dead ones examined for abnormalities. Dead embryos were also cultured and in the rare instances where bacterial contaminants were found, the tests were discarded. The surviving embryos were sacrificed on the 16th to 18th day of inoculation, the embryos were examined for gross abnormalities and their spleens weighed.

Results. One hundred and fifty-two tests were performed on a total of 124 patients; their diagnoses and the results are summarized in Table I.

Twenty-eight tests, using fresh specimens, were repeated to determine the reproducibility of the method.

Some embryo deaths may be expected due to handling the eggs, and we have followed Handler *et al*(1) in classifying up to 3/10 deaths in a test as "non-toxic," whereas 4 or more deaths in 10 injected eggs represents "toxicity."

The 35 control patients are shown in detail in Table II. The tests were "non-toxic" by Handler's criterion, and there was a 7% mortality in the overall group. Table III lists results obtained on the bloods of the patients with neoplastic disease. Of 114 tests, 15 tests were positive; 6 of the "toxic" specimens were "non-toxic" where retested on fresh blood drawn from the patient. There was a 16% mortality among all the embryos treated with blood from patients with neoplastic diseases, and the embryos sacrificed at 16 to 18 days, showed no significant gross abnormalities or change in spleen weight. Two embryos, at time of sacrifice, one control and one inoculated with acute leukemia blood, had developmental defects.

Discussion. Using Handler's(1) technique, we did not observe an impressive increase in the toxicity, to the 8-day chick embryo, of blood obtained from patients with neoplastic disease as compared with blood from control individuals. The over-all mortality among embryos in the neoplastic series was 16% as compared with 7% in the controls, and in 6 instances where the blood from cancer patients was "toxic," they were "non-toxic" on repeat tests. Our results, therefore, do not indicate the regular presence of a toxic factor for the 8-day embryo in the blood of patients with neoplastic disease, and the procedure gave no suggestion that it was of diagnostic significance. It seems relevant to note, also, that a number of attempts have been made to grow human cancer cells in the chick embryo, by implanting the cells on the CAM or by intravenous injection, and there was no specific evidence of enhanced toxicity(2-9). Embryos receiving cancer cells

often died at a later date, and this was associated with the proliferation of neoplastic cells in the embryo.

The reasons for the differences between Handler's(1) and our findings are not apparent. While our data suggest a trend toward an increased toxicity of cancer blood for the chick embryo (Table I), qualitative differences in toxicity were not found between the normal and cancer bloods in this study. Furthermore, the result on individual blood samples may not be consistent, in that when

TABLE II. Effects of 0.1 cc of Whole Blood from Apparently Normal Individuals Inoculated onto the CAM of the 8-Day Chick Embryo; 10 Eggs in Each Group.

Subjects		Embryos				
		Deaths			Avg	Avg
		Days of incubation		No. of	body	spleen
No.	Age* & sex†	9-11	12-sac.	survivors	wt % controls	wt
1	AM			10	99	103
2	"			10	105	92
3	"	2	1	7	81	77
4	AF			10	99	107
Repeat		3		7	94	115
"				10	102	108
5	AM	2	1	7	80	85
6	AF		1	9	112	101
7	"			10	88	76
8	"			10	98	93
Repeat				10	103	97
9	AM		1	9	103	102
10	"			10	91	90
11	"			10	115	111
12	"	3		7	85	98
13	"			10	98	105
14	AF			10	103	100
15	AM			10	100	101
16	AF			10	103	116
17	"			10	113	89
18	"			10	110	111
19	"			10	101	95
20	"	2		8	94	100
21	"	1	1	8	99	98
22	"	1		9	118	98
23	"		1	9	93	95
24	"			10	101	102
25	"			10	105	104
26	"			10	106	105
27	AM	1		9	102	99
28	AF			10	107	100
29	"			10	103	106
30	AM	1		9	94	108
31	"	1		9	102	112
32	AF	3		7	89	88
33	"			10	103	96
34	"			10	92	79
35	AM			8	97	103

* A, adult; C, child.
† M, male; F, female.

TABLE III. Effects of 0.1 cc of Whole Blood from Patients with Neoplastic Disease Injected onto the CAM of the 8-Day Chick Embryo; 10 Eggs in Each Group.

Subjects		Embryos				
		Deaths			Avg	Avg
		Days of incubation		No. of	body	spleen
No.	Age* & sex†	9-11	12-sac.	survivors	wt % controls	wt
		Acute leukemia				
36	AF			10	116	113
37	"	2		8	117	111
38	CF	1		9	86	92
Repeat				10	100	120
39	CM	3		7	82	90
40	CF		1	9	84	83
41	CM	3		7	83	76
42	CF	2	1	7	75	70
43	CM	2		8	87	69
44	CF	2		8	82	83
45	CM			10	100	94
46	"			10	109	97
47	CF			10	105	91
48	CM			10	102	83
49	CF	1		9	102	86
50	CM			10	104	91
51	AF	2		8	106	134
52	CF			10	108	88
53	"	1		9	103	96
Repeat		2		8	94	103
54	CF	1		9	99	96
55	"	4	1	5	71	76
56	"	1		9	102	80
57	"			10	106	93
58	CM			10	90	77
59	CF	1		9	98	95
60	CM			10	97	90
61	CF			10	110	93
62	CM	1		9	107	101
63	AF	1	1	8	114	120
Repeat		1		9	86	105
		Chronic myelocytic leukemia				
64	AM			10	102	97
65	"			10	105	97
Repeat		1		9	89	80
66	AF		1	9	90	95
Repeat		1		9	92	110
67	AF	6		4	81	79
68	AM	6		4	87	83
69	"	4		6	76	83
70	"	2		8	87	90
71	"	2		8	111	99
72	AF	3	1	6	92	93
73	"	1		9	90	89
74	AM	4		6	82	70
75	AF	5	1	4	85	88
Repeat				10	102	93
"		1		9	107	91
"		1		9	95	105
76	AF			10	98	113
77	AM	3		7	82	82
		Chronic lymphocytic leukemia				
78	AF	2		8	84	80
79	AM	3	1	6	85	83
Repeat				10	97	95
80	AM			10	109	118

TABLE III (continued)

Subjects		Embryos				
		Deaths			Avg	Avg
		Days of incubation		No. of	body	spleen
No.	Age* & sex†	9-11	12-sac.	survivors	wt	wt
					% controls	
Chronic lymphocytic leukemia (continued)						
Repeat		2	1	7	89	89
"				10	93	110
"		1		9	102	110
81	AF	2	1	7	101	88
82	AM	4		6	87	95
83	AF		1	9	94	105
Lymphomas						
84	AM	1		9	88	89
85	AF			10	102	93
86	AM			10	94	85
87	CF	1		9	107	93
88	AF	2		8	86	100
89	"	3		7	93	105
90	AM	3	1	6	89	80
91	"	2		8	88	110
92	"			10	89	94
93	AF			10	97	103
94	AM			10	108	114
95	"	1		9	90	105
96	AF	2		8	89	103
Carcinomas						
97	AF	2		8	97	77
Repeat			3	7	73	82
"		1		9	100	114
98	AF	1		9	97	88
Repeat		3		7	94	84
99	AF			10	100	104
100	"	7		3	80	85
Repeat				10	106	113
101	AF		1	9	116	114
Repeat				10	105	102
102	AF			10	110	116
103	"	1		9	87	89
Repeat		3	1	6	84	76
"		1		9	95	91
"		6		4	82	89
104	AM	2		8	87	89
105	AF			10	99	98
106	"	2		8	100	99
107	"			10	110	115
108	"		1	9	104	108
109	AM	4		6	85	66
Repeat		1	1	8	97	82
"			1	9	99	125
110	AF	1		9	99	103
Repeat				10	115	105
111	AM	1		9	84	102
112	"	1	1	8	79	97
113	AF	1		9	90	86
114	"	2	1	7	88	88
115	"			10	97	81
116	AM	2		8	95	120
Sarcomas and miscellaneous tumors						
117	CM	1	2	7	94	70
Repeat		1		9	97	98
118	AM	2		8	96	92
119	AF	2		8	88	93
120	"	1	1	8	117	108

TABLE III (continued)

Subjects		Embryos				
		Deaths			Avg	Avg
		Days of incubation		No. of	body	spleen
No.	Age* & sex†	9-11	12-sac.	survivors	wt	wt
					% controls	
Sarcomas and misc. tumors (continued)						
Repeat			5	5	87	92
121	AM			10	103	97
122	"		1	9	105	105
123	CM			10	114	108
124	"			10	111	91

* A, adult; C, child.
† M, male; F, female.

some tests were repeated, "toxic" bloods were often "non-toxic." A quantitative comparison of the Handler phenomenon in normal and cancer bloods would require separate surveys of the toxicity of blood from different forms of cancer, the inoculation of varying amounts of blood from each subject in an adequate number of eggs to determine the minimum lethal dose (MLD) for each blood sample, repeated studies on each patient to determine the consistency of the MLD, and also, it might be desirable to determine the MLD for each blood sample in embryos at different stages of development. From our data such an extensive undertaking did not seem to be mandatory.

Summary and conclusion. 1. Whole blood from 89 patients with neoplastic disease and 35 apparently normal control individuals was inoculated onto the chorioallantoic membrane of the 8-day chick embryo; 10 eggs were used for each sample. 2. In 35 controls the embryo mortality was 7% and the mortality did not exceed 3/10 eggs from any blood specimen. Of the 89 patients with neoplastic disease tested, the over-all embryo mortality was 16%, and in 14 instances more than 3/10 embryos died; 6 "toxic" bloods, however, were "non-toxic" on repeat examination. 3. This study failed to demonstrate the presence of a reproducible or a consistent factor in the blood of patients with neoplastic disease toxic to the 8-day chick embryo.

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A Simplified Tissue Culture Tube Neutralization Test for Rous Sarcoma Virus Antibodies. (28947)

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Various methods have been described for the assay of neutralizing antibodies to Rous sarcoma virus. Most commonly used are those based on prevention of tumors in young chickens or in chicken embryonic tissues (1-17) and prevention of a hemorrhagic disease in chicken embryos(18,19). Recently, Rubin(20) described an *in vitro* petri dish tissue culture test based on the neutralization of a discrete foci of altered cells in monolayer cultures of chicken embryo fibroblasts.

Recently we found that antibodies to Rous sarcoma virus could be assayed efficiently by means of a simple tissue culture tube neutralization test using the constant virus-serum dilution technique. This paper describes the test, its relative sensitivity and specificity, its value in distinguishing between different serotypes of Rous sarcoma virus, and preliminary observations on its application to surveys for antibody in various populations.

Materials and methods. Virus. Two strains of Rous sarcoma virus, the Bryan strain and the Schmidt-Ruppin strain(21) were used. The Bryan strain was kindly provided by Dr. W. Ray Bryan, Nat. Cancer Inst., as partially purified virus suspension CT 929(22) which was stored at —60°C and used directly in the experiments to be reported. The Schmidt-Ruppin strain (hereafter referred to as S-R strain) was kindly provided by Dr. C. G. Ahlstrom, Univ. of Lund, Sweden. The stock virus for this strain was made as follows: 11-day-old chick embryos were inoculated on the chorioallantoic membranes with a 20% clarified extract of wing web tumors induced by this virus. Membranes with confluent lesions 6 days later were made into a 20% suspension in Eagle's medium(23) and the suspension was clarified at 2500 rpm for 10 minutes in the International refrigerated centrifuge, distributed in screw-capped vials, and stored at —60°C until used.

Solutions and media. Chicken embryo fibroblasts were grown in a medium (EGM) consisting of 90 parts of modified Eagle's medium(23), 5 parts calf serum‡ (inactivated at 56°C for 30 minutes), 5 parts Bacto tryptose phosphate broth and one part stock antibiotic solution to give a final concentration of penicillin 250 units per ml and streptomycin 250 μg per ml. Tris buffer (0.05 M) in isotonic sodium and potassium chloride solution, pH 7.4 was used as wash fluid and as diluent for trypsin in preparation of tissue cultures. The trypsin solution used con-

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