sorbed by the renal tubule. Ampuled inulin suspected to contain it should not be used for measuring filtration rate unless all samples are yeasted before analysis.

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Inhibition of Serous Exudation and Tumor Cell Proliferation in Peritoneal Cavities of Mice Given Cortisone.* (20957)

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Previous tests for effects of cortisone on various mouse tumors (including Sarcoma 180) have revealed as a rule no inhibition of their growth(1-3). Moreover, several reports have stated that cortisone treatment facilitated the growth of transplanted tumor cells (4) by modifying host environment, *i.e.*, by "diminishing the awareness of the host"(5) or by inhibiting antibody formation(6). On the other hand, some authors working with different tumors and under different experimental conditions recorded complete(7) or moderate(8-10) inhibition of tumor growth. However, no investigation on this problem has thus far been carried out with mouse tumors growing in a peritoneal exudate and on the serosa. Sarcoma 180 cells can be cultured *in vivo* in this way(11,12), and it occurred to us that these experimental conditions might provide a suitable tool for measuring quantitatively the effect of cortisone both on tumor cells and on their environment. Accordingly, we have inoculated mice i.p. with requisite numbers of Sarcoma 180 cells and treated some of them with cortisone while keeping others as controls. Later, the amount and cellular composition of the exudate in the peritoneal cavities of the experimental and control mice were determined, as was also the amount of tumor implanted on the serosa. The results are reported below.

Material and methods. 1. *Tumor and animal strains.* We have previously described (12) the ability of S-180 to grow as free cells in serial transfers in the peritoneal or pleural exudate of CFW mice. 2. *Inoculation and exploratory abdominal puncture.* Doses of inoculated tumor cells were high enough (10 to 15 millions) to insure takes and to reduce individual variations. Technics of inoculation, of counting tumor cells and of measuring the amount of peritoneal exudate by means of glass capillaries have been described elsewhere(11). 3. *Treatment.* Cortisone and hydrocortisone, both alcohol free and acetate preparations† were used, but since they produced similar effects the results were pooled. All mice received the same total dose of cortisone (5 mg); this was started on the day of implantation and given in one or 2 daily doses either of one mg (concentrated treatment) or of 0.5 mg (fractionated treatment). Control series of treatments were given to mice with peritoneal exudates induced by the i.p. injection of normal tissue cells (ground liver, spleen, lung, and brain) in order to study the effect of cortisone on exudate devoid

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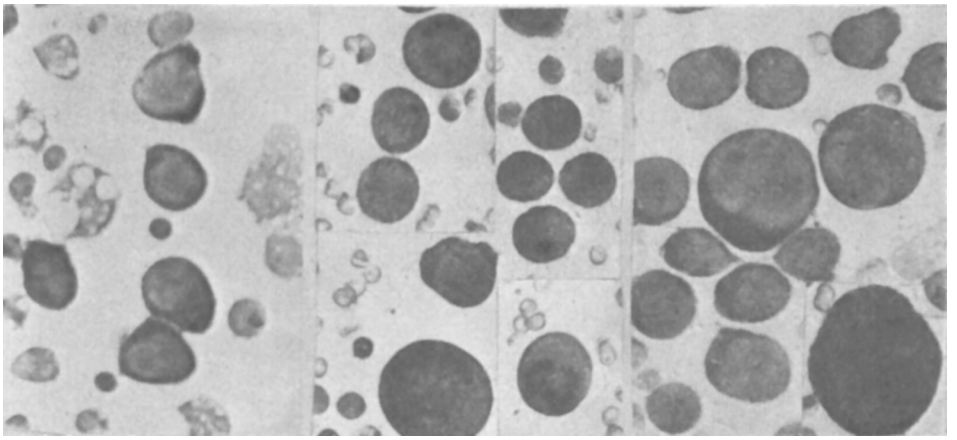

FIG. 1. S-180 cells and leukocytes in peritoneal fluid of CFW mice after treatment with cortisone or untreated. Left: untreated; center: treated with 5 × 1 mg cortisone i.p.; right: treated with 5 × 1 mg cortisone s.c. Wright's stain × 450. Note: (a) abundance of macrophages in the exudate of untreated mice; (b) "swollen" cells and abnormal mitoses in treated mice; (c) polymorphonuclears in i.p. treated, scarcity of leukocytes in s.c. treated.

of tumor cells.‡

Results. Implanted but untreated control mice, and those treated by the fractionated method, died consistently after 8 to 10 days. About 15 to 20% of mice treated by the concentrated method died on the 7th day. All control animals injected i.p. with normal tissue cells survived the concentrated treatment, even when 3 additional daily doses of one mg were given.

Specimens of exudate withdrawn on the third day from mice given the "concentrated" treatment showed consistently a considerable number of "swollen" tumor cells, some with abnormal mitoses in the prophase stage of division (Fig. 1). Transfer of these specimens into new hosts resulted in tumor cell growths quite similar to those resulting from peritoneal exudates of untreated mice.

Further results of the experiments are given in detail in Table I. From the findings it appears that the "concentrated" treatment, and in a lesser degree the "fractionated" treatment also, decreased the amount of the peritoneal exudate, sometimes below the level of detection by exploratory puncture. This effect was not specific for exudate induced by tumor cells since it was observed also in mice with exudate provoked by normal tissue cells. The decrease in the amount of exudate was reflected in several mice by higher concentration of its cells, *i.e.*, higher cell counts per cmm, but in most of the cortisone-treated mice these counts were lower than in controls. In third-day counts, for example, the percentage of tumor cells was higher in treated animals than in the controls, owing to a striking depression of lymphocytes (which was observed also in the control group 2 with exudate induced by normal cells). However, on the 7th day the proportions were reversed and the decrease both in total number of cells and in number of tumor cells indicated inhibition of growth of the tumor cells. Furthermore, gross implantation on the serosa was completely inhibited by the "concentrated" treatment (group 1, series 1, Table I) in a large proportion of the cases and more or less inhibited in the remainder (Fig. 2), and partial inhibition was effected by the "fractionated" treatment also, as was revealed by small size and early necrosis of the implants in this group. Our results indicate that cortisone suppresses not only defensive tissue reactions (antibody formation, leukocytosis(5,6)) but also those which are unfavorable for the host, in our experiments, the exudate formation promoting tumor cell growth and spread. Our experiments deal with a special type of growth in a fluid medium enclosed in a serous cavity,

‡ Mr. Wendell Gillett, medical photographer at Vanderbilt Medical School, prepared the photographs.

TABLE I. Effects of Cortisone on Induction of Peritoneal Exudate by S-180 Cells and on Growth and Implantation of These Cells in the Peritoneum of the Mouse.

	Date of examination	Implanted but untreated (controls)	Implanted and treated with cortisone Intraperitoneally	Subcutaneously
Group 1—Mice implanted i.p. with S-180 cells				
			First series "conc. treatment" 5 mg in 48 hr	
Amt peritoneal exudate	A	100% +++	8% ++, 58% +, 34% 0	54% +, 46% 0
	B	100% +++	32% ++, 56% +, 12% 0	18% ++, 64% +, 18% 0
Total cell No. (thousands/mm³)	A	86 (15– 55)	152 (55–199)	210 (85–345)
	B	75 (34–106)	85 (53–119)	64 (24– 84)
% tumor cells	A	58 (35– 64)	72 (45– 83)	78 (51– 88)
% lymphocytes		27 (21– 35)	3 (0– 12)	9 (0– 14)
% monocytes and macrophages		15 (8– 39)	8 (2– 14)	12 (4– 25)
% polymorphs		6 (3– 12)	21 (11– 33)	11 (4– 22)
% tumor cells	B	64 (49– 78)	35 (28– 49)	47 (22– 61)
% lymphocytes		26 (15– 52)	32 (15– 41)	28 (9– 37)
% monocytes and macrophages		20 (8– 33)	11 (8– 19)	19 (13– 27)
% polymorphs		6 (2– 14)	28 (8– 41)	10 (4– 19)
Amt peritoneal implant growth	B	76% +++, 24% ++	68% +, 32% 0	46% +, 54% 0
			Second series "fractionated treatment" 5 mg cortisone in 6 days	
Amt peritoneal exudate	B	100% +++	36% +++, 64% ++	44% +++, 56% ++
Total cell No. (thousands/mm³)	B	123 (75–276)	72 (34–178)	66 (24–184)
% tumor cells		41 (27– 56)	46 (18– 63)	62 (35– 70)
% lymphocytes		24 (14– 31)	12 (5– 20)	17 (13– 24)
% monocytes and macrophages		34 (10– 49)	16 (8– 22)	12 (8– 21)
% polymorphs		12 (7– 24)	34 (5– 57)	20 (7– 31)
Amt peritoneal implant growth	B	76% +++, 24% ++	22% ++, 78% +	28% ++, 72% +
Group 2—Controls: exudate induced by inj. of normal tissue cells; treated with cortisone				
Amt peritoneal exudate	A	22% +++, 78% ++	72% +, 28% 0	32% +, 68% 0
Total cell No. (thousands/mm³)	A	157 (107–191)	213 (188–272)	243 (185–292)
% lymphocytes		44 (15– 62)	28 (9– 47)	32 (17– 45)
% monocytes and macrophages		42 (18– 78)	36 (14– 60)	44 (10– 56)
% polymorphs		22 (14– 30)	53 (23– 75)	32 (11– 55)

Group 3—Controls: not implanted, untreated

Amt of peritoneal exudate	Total cell No. (thousands/mm³)	% of lymphocyte	% of monocytes and macrophages	% of polymorphs
8% ++, 92% +	93 (63–160)	72 (61–81)	32 (12–51)	.6 (0–1.5)

Fifty mice in each experiment of first 2 series (group 1), 30 in group 2 and 20 in group 3. For total numbers and differential counts averages and extremes are indicated. Amount of exudate estimated* as proportionate to volume of fluid obtained by insertion of glass capillary in peritoneal wall: +++ = 0.5 mm³ or more, ++ = <0.5 but >0.1 mm³, + = <0.1 mm³, 0 = no fluid. Condition of peritoneal implants graded as follows: +++ = lumps of healthy tumor tissue, >0.5 cm in each diameter; ++ = <that size or partly necrotic tumors; + = mostly necrotic nodules; 0 = no implant. Exudate withdrawn on 3rd (A) or 7th (B) day after inoculation. All autopsies performed on 7th or 8th days (B).

* The reliability of this estimate was shown by comparison with the amount of exudate measured at autopsy.

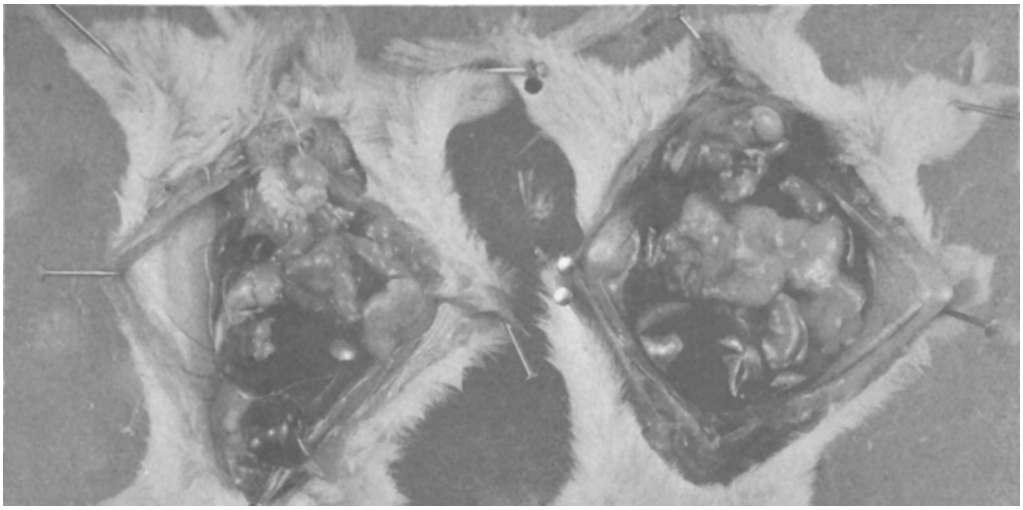

FIG. 2. Absence of large peritoneal S-180 implants in CFW mice treated with cortisone: Mouse 1 (left) = untreated, 2 (right) = i.p. treated with 5 × 1 mg cortisone.

but similar results may be demonstrated conceivably in other conditions of tumor growth.

Summary and comment. The results as a whole indicate that the treatment with cortisone impaired the growth of intraperitoneally implanted Sarcoma 180 cells, as was shown by morphological abnormalities in the tumor cells of treated animals, by a decrease in the number of tumor cells present in the peritoneal exudate, and by the lack or scarcity of serosal implants. This effect was not due to direct contact between cortisone and tumor cells, since i.p. treatment was not more efficient than s.c. injections. Furthermore, tumor cells of cortisone-treated mice grew freely when transferred into new hosts. Hence, it seems reasonable to suppose that the cortisone influenced the tumor cells only indirectly by changing their environment unfavorably. The essential change appeared to consist in a suppression of the serous exudate which is induced by the presence of tumor cells in the peritoneal cavity and constitutes a necessary condition for their growth and implantation. The scarcity or lack of serous fluid in spite of abundant growth of free tumor cells in the peritoneal cavity may be attributed to the well known suppressing action of cortisone on capillary permeability (13,14).

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