4. Saxton, J. A., Boon, M. C., Furth, J., *Cancer Research,* 1944, v4, 401.
5. Tannenbaum, A., Silverstone, H., *Advances Cancer Research,* 1953, v1, 451.
6. Andervont, H. B., *J. Nat. Cancer Inst.,* 1944, v4, 579.

Received June 22, 1959. P.S.E.B.M., 1959, v102.

Prevention of Peritoneal Free (Ascites) Tumor Cell Growth and Implantation by Pretreatment with Radioactive Yttrium.* (25225)

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It is well known(1,2) that thoracic and abdominal malignant tumors extending into pleural or peritoneal cavity may induce there accumulation of an exudate containing numerous malignant, frequently mitotic cells released from the tissue. Some of these cells eventually implant into the cavity wall and give rise to secondary growth of "coelomic metastases"(3). Müller reported(4) "undoubtful therapeutic results" of intraperitoneally injected colloidal Au^{198} in 8 female patients with "carcinomatous peritonitis." We showed(5) that peritoneal carcinosis (later designated as "ascites tumor") of the mouse(6) regressed completely after a single injection of colloidal Au^{198}; we obtained analogous results by similar treatment of free tumor cell growth in the pleural exudate(7). Later experiments(8,9,10) suggested the use of radioisotopes for inactivation of tumor cells remaining in healthy tissues or spilled there after tumor removal. We recently investigated the possibility of preventing growth and implantation of cells separated spontaneously or by trauma from abdominal tumors and entering peritoneal cavity. Accordingly, we reversed the procedure of our earlier experiments by injecting a radioisotope intraperitoneally into mice before and not after tumor inoculation by the same route. Eventual effect on tumor cells was checked by comparison of the amount of effusion, its content of free tumor cells and the extent of implantation in experimental and control animals. The results are recorded below.

Material and methods. a) *Mice and tumor strain.* Swiss Albino mice (Albino Farms, Red Bank, N. J.) females of 24 to 27 g weight were used. Krebs-2 carcinoma was maintained as "ascites tumor" by several intraperitoneal transfers. b) *Tumor cell count in ascitic fluid.* Methods of isotope injection, of inoculation with requisite numbers of tumor cells, of exudate withdrawal from the cavity and of staining were described previously (5,7). Standard pattern of implantation in female mice (periuterine localization) was obtained(6). c) *Isotopes.* In preliminary experiments we have tested on parallel lines preparations of short-living and pure beta emitting isotopes: $CrP^{32}O_4$, $Y^{90}PO_4$, and $ZrP^{32}O_4$ obtained from Abbott Laboratories, Oak Ridge, Tenn. Since most consistent results were produced with $Y^{90}PO_4$, this isotope was selected for experiments presented below. Decayed (no radioactivity detected by standard counters) isotope was given to control mice for assaying any chemical effect of the compound independent from its radioactivity. d) *Amount of ascitic fluid.* Amount of exudate was estimated as proportionate to volume of fluid obtained by insertion of a glass capillary in peritoneal wall: +++ = 0.5 ml or more; ++ = <0.5 but >0.1 ml; + = <0.1 ml; 0 = no fluid. The reliability of this estimate was shown by comparison with amount of exudate measured at autopsy. e) *Recording spontaneous implantation.* Extent of implant growth of periuterine localization (6) was graded as follows: 0 = no macroscopic implants; + = small nodules (less than 2 mm in diameter); ++ = tumor (2-5 mm in longer diameter); +++ = growth

* This investigation was supported by grants from U. S. Atomic Energy Comm. and Damon Runyon Fn.

TABLE I. Effect of Intraperitoneal Pretreatment with $Y^{90}PO_4$ on Accumulation of Ascitic Fluid and on Growth and Implantation of Its Free Tumor Cells.

Schedule of pretreatment			Findings in peritoneal cavity		
Series No.	Dose of inj. isotope (mc)	Days between isotope inj. and tumor inoc.	Amount of ascitic fluid	Conc. of tumor cells (thous./ml) in ascitic fluid	Extent of implantation from fluid into peritoneum
1	.25	1	++ ++ - +++	38.9 14.3-40.1	+ + - +++
2	.25	3	+ 0 - ++	16.9 4.7-22.2	+ + - ++
3	.25	6	+++ ++ - +++	43.7 34.8-59.1	++ ++ - +++
4	.25	Controls	+++ ++ - +++	61.4 51.4-71.2	+++ ++ - +++
5	.15	1	++ ++ - +++	40.8 33.1-59.8	++ ++ - +++
6	.15	3	++ + - +++	29.3 12.5-47.4	++ + - +++
7	.15	6	++ ++ - +++	61.6 39.5-71.0	++ ++ - +++
8	.15	Controls	+++ ++ - +++	59.9 51.4-67.9	+++ ++ - +++

Avg and variation extremes are given for each group (20 mice). Technics of cell counts, of fluid volume estimates and of grading the extent of implantation were recorded in *Material and Methods*. Each of two groups of controls included 20 untreated mice and 5 treated with completely decayed isotope (no activity detectable by routine counters).

larger than ++. f) *Pattern of experiments.* Graded doses (0.1, 0.2, 0.25, 0.3, 0.4 millicuries) of $Y^{90}PO_4$ were injected intraperitoneally into several series of mice. Three or 6 days later, these mice were inoculated with standard doses (10^5) of Krebs-2 tumor cells: 3 to 4 days later their ascitic fluid was withdrawn, its volume was estimated and the number of tumor cells per ml were determined; a day or 2 later, the animals were sacrificed and inspected for presence of implants in the peritoneum; sections of their peritoneal wall were examined for pathological changes and for presence of microscopic implant growth.† The results are recorded below.

Results. Lethal toxicity of the isotope. Preliminary experiments have shown that intraperitoneal injection of 2 ml of 0.85% NaCl solution immediately before injection by the same route of 0.3 mc of isotope in a volume of 0.2 ml was followed by death of less than 5% of mice within 10 days; the same dose of isotope alone was lethal for about 50% of animals. Dose of 0.25 mc injected into the cavity previously injected with 2 ml of saline was rarely lethal, but without saline it was lethal for 15% of mice. Intraperitoneal inoculation of tumor cells increased mortality in both groups pretreated with 0.3 mc but not in the group given saline solution and 0.25 mc. Accordingly, we have recorded in Table I only the results obtained with saline and the latter dose and, for comparison, with much lower isotope dose of 0.1 to 0.15 mc.

It appears that the optimal intraperitoneal dose of $Y^{90}PO_4$ was very close to 0.25 mc (series 1 and 2 of Table I) injected into saline filling the cavity. Lower doses (series 5 and 6) affected only slightly the results of inoculation; higher doses (0.3 mc) were not more efficient, but less safe.

The optimum interval between pretreatment with isotope and tumor inoculation was 3 days (Table I) in series 2 and 6, *i.e.*, maximum effect on tumor growth was obtained in these series as compared with series 1, 3, 5 and 7. Growth initiated after 6 days (series 3 and 7) was influenced by pretreatment, as compared with controls (series 4 and 8) only in few mice (higher minimum levels than in controls).

The most conspicuous effects of the optimal

† Sid Cox, Medical Photographer, Vanderbilt Univ. Med. School, prepared the photographs.

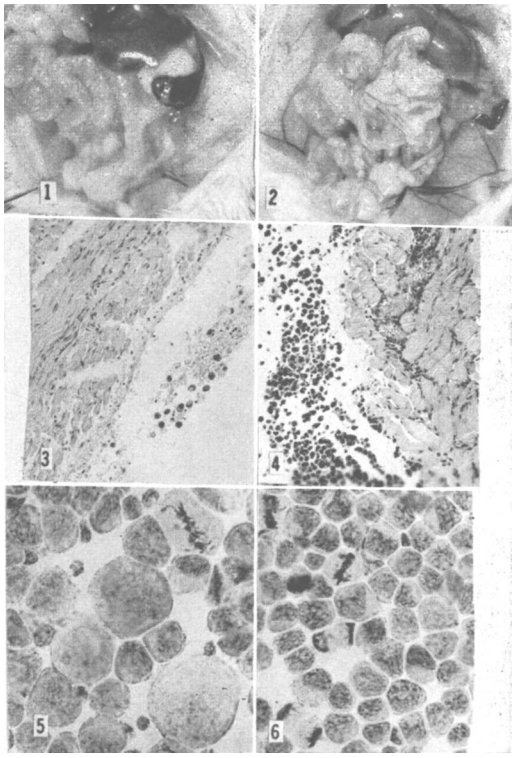

FIG. 1 and 2. Peritoneal implants 6 days after inoculation of 10^5 Krebs-2 tumor cells. Fig. 1, pretreated with 0.25 $Y^{90}PO_4$; Fig. 2, control.

FIG. 3 and 4. Peritoneal wall 4 days after inoculation of 10^5 Krebs-2 tumor cells. H + E; × 64. Fig. 3, pretreated with 0.25 $Y^{90}PO_4$; Fig. 4, controls.

FIG. 5 and 6. Free tumor cells in ascitic fluid 4 days after inoc. of 10^5 Krebs-2 tumor cells. Aceto-Orcein; × 128. Fig. 5, pretreated with 0.25 Y^{90}-PO_4; Fig. 6, controls.

pretreatment method (Table I, series 2) were the decrease in the amount of ascitic fluid and reduction of macroscopically (Fig. 1 and 2) and microscopically (Fig. 3 and 4) visible implantation. Free tumor cells in the fluid appeared (Fig. 5 and 6) swollen with highly distorted mitotic figures. Intraperitoneal transfer of a standard amount of this fluid (0.2 ml) into new mice (biological test of viability) induced sizable growth considerably later (not before the 15th day) than did the fluid from controls (not later than on the 4th day).

Sections from peritoneal wall (serosa, subserosa and muscle) of pretreated and inoculated mice showed edema of connective tissue (Fig. 3). Variable numbers of tumor cells were scattered or arranged in clumps on the serosa. No edema was noted in controls in the same stage of tumor growth (Fig. 4); tumor cells in their serosa not only were more numerous but they spread into subserosa and infiltrated interfibrillar spaces of the muscle.

In a group of mice, pretreatment with 0.25 mc was supplemented 2 days after inoculation with a low dose (0.1 mc) of the same isotope. The results (Table II) showed that additional injection did not improve the effect of pretreatment on fluid accumulation and implant growth, but it did achieve extensive or complete destruction of free tumor cells in a number of the animals. The implication of these findings is discussed below.

Discussion. Occurrence of maximum growth inhibition in tumors inoculated 3 days after pretreatment, *i.e.*, after substantial decay of the isotope *in vivo* (half life of Y^{90} = 65 hours) suggested an indirect mechanism of damage to peritoneal growth by radioactivity and some direct effect on tumor cells. Free tumor cells and, in particular, their mitotic forms were considerably altered (Fig. 5 and 6) but in no instance were all cells destroyed; on the other hand, accumulation of ascitic fluid and growth of implants were considerably reduced with only a few exceptions. The latter effect may be attributed to edema of irradiated tissue disrupting fluid circulation, but it is also conceivable that irradiated peritoneal elements offer resistance to tumor cell invasion. Andrews, *et al.*(11,12) suggested that "some unknown mechanism in action, possibly upon surfaces not involved with tumor may be important" for therapeutic action of Au^{198} in human peritoneal carcinosis. Whatever is the nature of tissue reaction induced by radioactivity and opposing invasion of peritoneum by tumor cells and accumulation of ascitic fluid, it develops gradually, reaches its maximum effect on the 4th day and appears to be almost without effect about the 7th day.

In animals pretreated with 0.25 mc of Y^{90} PO_4, a low dose (0.1 mc) of the same isotope given 1 or 2 days after inoculation destroyed free tumor cells in higher numbers than did post-treatment alone (Table II, Series 5). It may be presumed that tumor cells altered or damaged by pretreatment were an easily vul-

TABLE II. Effect of Low Dose of $Y^{90}PO_4$ on Krebs-2 Free Tumor Cells Growing in Ascitic Fluid of Mouse Pretreated with Higher Dose.

Schedule of treatment			Findings in peritoneal cavity		
Dose (mc) of pretreating inj.	Dose (mc) of treating (after inoc.) inj.	Days between inoc. and treating inj.	Amount of ascitic fluid	Conc. of tumor cells (thous./ml) in ascitic fluid	Extent of implantation from fluid into peritoneum
.25	.1	2	+ 0 - +	5.8 1.5-9.4	+ + - ++
.1	.1	1	++ + - ++	19.8 11.6-43.5	++ + - +++
.1			++ ++ - +++	49.2 42.1-55.8	++ ++ - +++
	.1		++ ++ - +++	37.1 28.4-44.6	++ ++ - +++
Untreated controls			+++ ++ - +++	52.6 41.1-58.1	+++ ++ - +++

Twenty mice in each group. For each group, avg and variation extremes were recorded. Doses of 10^5 Krebs-2 tumor cells were inoculated to all animals. For technic of cell counts, of fluid estimate and of implants grading, see *Material and Methods*. Fluid was withdrawn for tests 24 hr after last inj. of isotopes; autopsies were performed 2 days later.

nerable target for even a small additional irradiation dose.

The association of pretreatment and post-treatment combined advantages of both methods, *i.e.*, inhibition of fluid accumulation and cell implantation was combined with extensive destruction of free tumor cells. Perhaps the principle of combined pretreatment and post-treatment with an isotope may be developed, with suitable modifications, into a method for prevention of transcoelomic metastases in patients with operated abdominal tumors. At present we are testing a combination of pretreatment with Y^{90} and of post-treatment with chemotherapeutic agents.

Summary and conclusions. (1) Doses of 0.25 mc of radioactive yttrium phosphate ($Y^{90}PO_4$) were injected into peritoneal cavity of Albino mice immediately following administration of 2 ml of 0.85% NaCl solution; on 3rd to 4th day 10^5 cells of Krebs-2 Ascites Tumor were inoculated by same route into pretreated animals and normal controls; their accumulated fluid was withdrawn after 3 or 4 days for estimate of its volume, tumor cell counts and smears; they were sacrificed for autopsies 1 or 2 days later. (2) Amount of ascitic fluid and extent of implant growth were considerably reduced in pretreated animals as compared with controls; free tumor cells in the fluid appeared enlarged, altered (distorted mitoses) and less viable, after transfer into new mice, than in controls; decrease of their concentration in the fluid was not as rapid and extensive as in animals treated with an isotope after inoculation in previous experiments. (3) Cell destruction was increased by injection of 0.1 mc into pretreated mice. (4) It was concluded that pretreatment with radioactive yttrium increased resistance of the peritoneum to invasion with tumor cells, thus decreasing implantation of these cells and exudation of the fluid. (5) It was presumed that the principle of this method may be eventually applied for prevention of "transcoelomic metastases" from some abdominal tumors, before or after their removal.

1. Beneke, R., *Deutsch. Arch. f. Klin. Med.*, 1899, v64, 237.

2. Zemanski, A. P., *M. J. Med. Sc.*, 1928, v175, 489.

3. Willis, R. A., *The Spread of Tumours in the Human Body*, London, Mosby, 1952, Chap. 5, p49.

4. Muller, J. H., *Bull. Schweiz. Akad. d. Med. Wissensch.*, 1949, v5, 484.

5. Goldie, H., Hahn, P. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 634.

6. Goldie, H., *Ann. N. Y. Acad. Sci.*, 1956, v63, 711.

7. Goldie, H., West, H. D., Jeffries, B. J., Butler, C. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 327.

8. Goldie, H., West, H. D., *Cancer Res.*, 1956, v16, 484.

9. West, H. D., Goldie, H., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 116.
10. Goldie, H., Cooper, E., Chess, R., Freeman, R., *Proc. Cancer Res. Assn.*, 1958, v2, 301.
11. Andrews, G. A., Root, S. W., Kniseley, R. M., *Cancer*, 1953, v6, 294.
12. Kniseley, R. M., Andrews, G. A., *ibid.*, 1952, v6, 303.

Received June 25, 1959. P.S.E.B.M., 1959, v102.

Prevention of Salt Hypertension by Propylthiouracil Treatment in Rats.* (25226)

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The anti-thyroid drug, propylthiouracil, has been used successfully in rats to prevent development of renal hypertension(1,2); desoxycorticosterone-acetate induced hypertension(3) and adrenal-regeneration hypertension(4). Our studies were to determine whether this drug would also prevent a fourth type of experimental hypertension, namely that accompanying administration of hypertonic NaCl solution(5,6).

Methods. Holtzman rats were kept 2 or 3/cage in thermoregulated room at 25 ± 1°C, illuminated from 8 a.m. to 6 p.m. Blood pressures and body weights were measured during one-week control period and weekly thereafter for duration of experiment. Systolic blood pressure was measured by the microphonic manometer technic(7) but without anesthesia, as described previously(8). Immediately after the control period, 15 male rats (Exp. 1) were given 1.75% NaCl solution and 12 female rats (Exp. 2) 1.9% NaCl solution as sole drinking fluid. Eight rats in Exp. 1 and 6 rats in Exp. 2 received in addition ground Purina chow† containing 0.1% propylthiouracil (PTU)‡ The remainder of the rats received ground chow without the drug. After 11 weeks, all rats in Exp. 1 were killed by ether and heart, kidneys, testes, seminal vesicles, prostate, thyroid, thymus and adrenal glands were removed, trimmed, drained of excess fluid or blood, blotted on filter paper and weighed on torsion balance. Results are expressed as ratio of organ weight to body weight (mg/100 g B.W.). Since all rats in Exp. 2 lost nearly 50 g in 3 weeks, 1.75% NaCl solution was substituted for 1.9% NaCl solution. The substitution seemed to ameliorate weight loss. Only 4 rats in each group survived the total 14 weeks, then they were killed by ether and adrenals, thyroid, thymus and ovaries were removed and weighed as described above. Simultaneous means of control and experimental groups were compared using the "t" test for the 95% confidence limit (9).

Results. Fig. 1A illustrates that 1.75% NaCl solution given male rats produced a marked elevation in systolic blood pressure from 143 mm Hg to 165 mm Hg at end of Exp. 1. In contrast, rats receiving PTU in addition showed a decrease from 138 mm Hg to 128 mm Hg. Differences were significant ($P = .02$) by the second week and highly significant ($P < .01$) by 5th week. Mean body weight of rats receiving chow and salt fell during the experiment while that of PTU-treated group remained constant (Fig. 1B).

Systolic blood pressures of female rats in Exp. 2 showed results similar to, but more variable than, those of comparably treated groups of male rats in Exp. 1 (Fig. 2A). Control, salt-treated rats increased mean systolic blood pressure from 137 mm Hg to approxi-

* Supported by Grants from Nat. Heart Inst., Bethesda, Md., and The American Heart Assn.

† Analysis of Purina laboratory chow by wet ashing with concentrated nitric acid and subsequently determining sodium and potassium content by flame photometry indicated a sodium content of 127 mEq./Kg and potassium content of 218 mEq./Kg.

‡ 6-n-propyl-2-thiouracil, Nutritional Biochemicals Corp., Cleveland O.