by triparanol. Degree of growth depression due to triparanol was proportional to logarithm of dose.

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Prevention of Malignant Cell Growth and Spread by Tropical Pretreatment with Radioactive Chromic Phosphate.* (25949)

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We have reported(1) that intraperitoneal pretreatment with radioactive Yttrium (Y^{90}) compounds prevented growth and implantation of malignant cells not only by acting directly on tumor cells, but also by increasing indirectly the resistance of the cavitary wall to their invasion. This line of research was continued in our recent experiments, using as the radioactive agent an isotope with a longer halflife and, as targets, tumor inocula in different locations. The results are reported below.

Material and methods. (A) *Mice and tumor strain.* Swiss Albino mice (Albino Farms, Red Bank, N. J.) and CFW mice (Carworth Farms, New City, N. Y.) of either sex, 24 to 27 g weight, were used. Krebs-2 tumor was maintained in serial transfers as "ascites tumor." (B) *Tumor cell inoculation and pretreatment with isotope.* Methods of inoculation of requisite number of tumor cells, of ascitic fluid withdrawal and of the work with isotopes have been described(2,3,4,9,10). *Radioactive chromic phosphate* in colloidal suspension was obtained from Abbott Labs, Oak Ridge, Tenn. This compound of a pure beta emitting phosphorus P^{32} is widely used as a substitute for colloidal radiogold (Au^{198}) which is a beta and gamma emitter(5,6,7,8, 11,12). (C) *Technic of injections.* (aa) *Intrapleurally.* Puncture of most areas of the thoracic wall will produce hemorrhages from the surfaces of the lung. However, it is quite safe to puncture the phrenico-costal sinuses (which are not occupied by the lung), in the area of the right thoracic wall at intersection of posterior axillary line and lowest intercostal space. This procedure was done. (bb) *Subcutaneously into scalp.* We have reported(4) sharply localized growth of scalp tumors in proportion to size of inoculum and their suitability for estimation of normal tissue invasion by tumor outgrowth. For either tumor inoculation or isotope injection a gauge 23 needle was introduced through the external ear into subcutaneous tissue of neck, scalp, nasal region and supraorbital region (see be-

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low "Pattern of Experiments"). (cc) *Subcutaneously* into abdominal wall and (dd) *intramuscularly* into leg. Routine technics were used, but the isotope was injected into the selected area in 10 fractions of the requisite dose, diluted for this purpose to the volume of 2.0 or 3.0 ml. After skin (and muscle) puncture the direction of the needle was changed after each injection of 0.2 or 0.3 ml of diluted dose until 10 fractions of the dose had been infiltrated into the selected area at each of 10 different points. For intrapleural pretreatment dose of the isotope was injected only once, in a volume of 0.5 ml. Tumor inoculation after pretreatment was given in a single injection in all series. (D) *Safety of pretreatment method.* We have assayed by various routes increasing doses (.1 to .3 mc) of $CrP^{32}O_4$ for their systemic (lethal) and local (in normal tissue of injected area) toxicity in mice. (E) *Scale of quantitative estimate of tumor growth.* (aa) *Amount of pleural exudate* was estimated as proportionate to volume of fluid obtained by insertion of a glass capillary through the thoracic wall: + = 0.2 ml or less; ++ = 0.2 to 0.5 ml; +++ = more than 0.5 ml. (bb) *Invasion of pleural fluid by free tumor cells.* Technic of tumor cell counts in the fluid has been described(2,3,4). According to our experience, decrease in number of tumor cells per cu. mm (4,9,10) and/or in their percentage in the cellular exudate(9,10) reflected the effect of injected radioisotope. (cc) *Extent of intrapleural implantation*: 0 = no implant; + = implant either in superior or inferior mediastinal pleura; ++ = implants in both areas; +++ = spread of mediastinal pleura implant on diaphragmatic or costal pleura. (dd) *Extent of tumor spread in scalp*: 0 = no growth; + = circular elevation on the top of scalp reaching eventually orbits, but without involvement of eyelids; ++ = swelling covering the whole area between neck and orbits (including eyelids); +++ = growth extension on face and neck; ++++ = extension on the back. (ee) *Subcutaneous and intramuscular implants*: 0 = no implants; + = diffuse induration at site of inoculation; ++ = nodule 0.2 to 0.5 mm in diameter; +++ = tumor larger than 0.5 mm in diameter. (F) *Pattern of experiments.* A single dose of 0.1, 0.15, or 0.2 mc of $CrP^{32}O_4$ was given either in a single injection in a volume of 0.5 ml (intracavitary) or infiltrated in a volume of 2 to 3 ml in 10 fractions, each of 0.2 to 0.3 ml, (subcutaneously or intramuscularly) into a selected tissue area. One, 3, or 5 days later an inoculum of 10^6 to 10^7 tumor cells was introduced into pretreated cavity or tissue area. In intrapleural experiments, fluid was withdrawn from the cavity for tumor cell counts on 4th day after inoculation; 2 days later the mice were sacrificed for autopsies and recording of implants; in 5 pretreated animals of each series the withdrawn fluid was inoculated intraperitoneally into new mice for assay of tumor cell viability(3); smears were prepared from all fluid specimens (undiluted) and stained with Acetoorcein(2). In scalp experiments, size and extension of tumor were recorded on 10th day; in abdomen and leg experiments, on 7th day. Recorded data were tabulated and are presented below.

Results. A. *Safety controls.* All new mice survived doses of .2 mc intrapleurally and 0.25 mc subcutaneously and intramuscularly without any macroscopic damage to normal tissues. Higher doses induced occasionally local damage (pleural hemorrhages, crusting and loss of hair in the skin). Dose of 0.3 mc was lethal for all mice. Accordingly, doses of .15 mc and .2 mc injected for pretreatment into new mice were well tolerated. B. *Pleural cavity.* In mice inoculated intrapleurally 1 to 3 days after topical pretreatment, initial proliferation of free tumor cells was arrested, their mitoses disappeared, and their cytoplasm became swollen, thus enlarging their size and leading to their final disintegration (Fig. 2 as compared with controls in Fig. 1). The remaining cells were scanty and non-viable as it appeared from their inability to initiate intraperitoneal growth after transfer into new mice (bioassay of viability, see ref. 4). Their ability to implant into the pleura or to grow there after early implantation was considerably impaired (Fig. 4 as compared with Fig. 3). Table I shows the relationship between extent of these effects, on the one hand, and

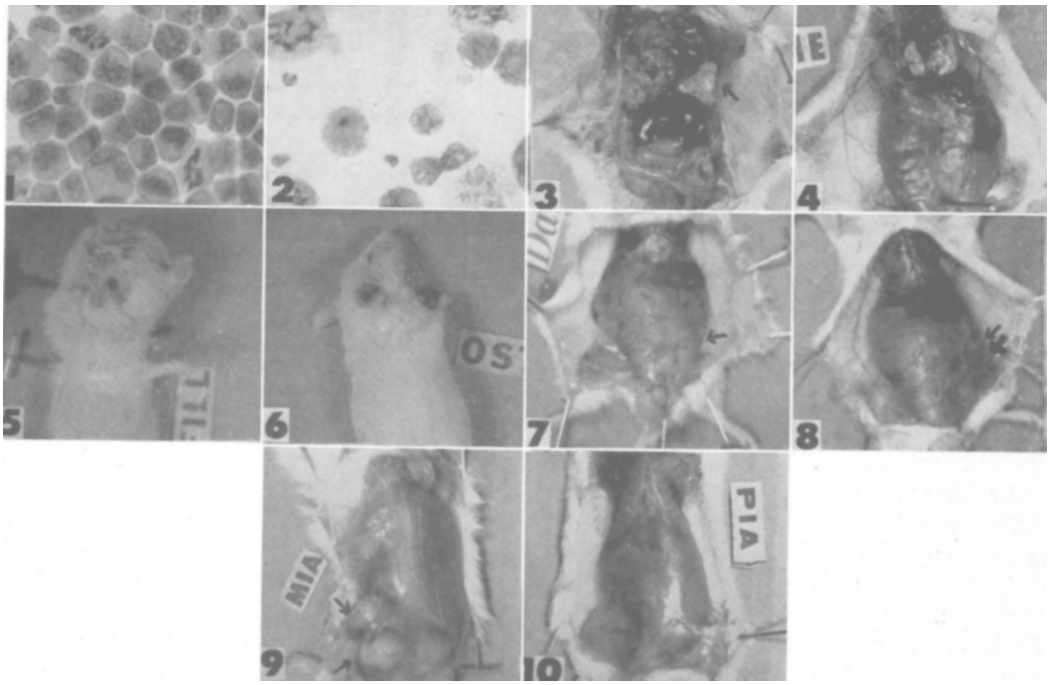

FIG. 1 and 2. Krebs-2 tumor cells in undiluted pleural exudate of Swiss Albino mice on 4th day after inoculation of 10^6 cells. Smears from 0.2 mm^3 of undiluted fluid. Fig. 1, control: numerous typical tumor cells and their mitoses. Fig. 2, pretreated intrapleurally with 0.15 mc $CrP^{32}O_4$: scarce, distorted tumor cells, macrophages and leukocytes.

FIG. 3 and 4. Pleural implants on the 5th day after inoculation (as in mice 1 and 2). Fig. 3, control: large tumor on diaphragm. Fig. 4, pretreated: no visible tumor growth.

FIG. 5 and 6. Krebs-2 implant in the scalp, 10 days after inoculation of 10^7 cells. Fig. 5, control: whole scalp thickened by tumor growth spreading on neck. Fig. 6, pretreated with 0.15 mc of $CrP^{32}O_4$ into the scalp: tiny necrotic nodule on top of normal looking scalp.

FIG. 7 and 8. Tumor in mouse 6 days after inoculation into skin of abdomen. Fig. 7, control: diffuse spread of tumor tissue covering peritoneum of low abdomen. Fig. 8, pretreated in the same skin area: few tiny necrotic nodules.

FIG. 9 and 10. Tumor 6 days after intramusc. inj. into thigh. Fig. 9, control: large round tumor masses infiltrating thigh and dorsal muscles. Fig. 10, pretreated in the same muscle: small necrotic nodule in right thigh muscle.

dose of radioisotope and interval between pretreatment and inoculation, on the other hand.

It appears from Table I that pretreatment with a higher dose (0.2 mc) shortly (1 to 3 days) before inoculation inhibited completely (or almost so) fluid accumulation and proliferation of free tumor cells and their implantation, while a lower dose (0.15 mc) or an earlier pretreatment (5 days before inoculation) achieved similar effects generally at a lower level and in smaller number of animals. Still, even in series 7 (0.15 mc, interval of 5 days) the results in some mice were close to findings in series 2 (0.2 mc, 3 days). High effect was obtained occasionally even with a still lower dose (0.1 mc). The significance of these variations will be discussed below.

B. *Subcutaneous and intramuscular growth.* Pretreatment with either 0.15 mc or 0.2 mc produced about the same level of tumor inhibition: effects of lower doses (0.1 mc) and of earlier pretreatment (5 days before inoculation) varied widely. Data of experiments in 3 different body areas are reviewed in Table II. According to Table II, standard procedures of pretreatment inhibited practically completely tumor growth in the scalp from standard inocula (Fig. 6). In pretreated areas of the abdominal subcutaneous tissue and of leg muscle, only a small amount of growth, mostly necrotic, was recorded (Fig.

TABLE I. Inhibition of Free Malignant Cell Growth in Pleural Cavity by Intracavitary Pretreatment with Radioactive Chromic Phosphate, in 8 Series.

Schedule of inoculation and treatment		Findings in pleural cavity (avg / variation extremes)			
Dose of inj. radioisotope (mc)	Days between pretreatment and inoc.	Approx. amt of pleural fluid	% of tumor cells in the fluid	No. (thousands) of tumor cells/mm³	Amt of implantation
.2	1	0 / 0 - +	3.5 / 1.7- 5.1	18.8 / 6.1-24.2	0
.2	3	" / "	10.4 / 6.5- 14.2	28.2 / 16.3-44.4	0
.2	5	+ / + - ++	12.2 / 7.1- 16.6	48.8 / 31.5-57.5	+ / 0 - +
Untreated control		++ / ++ - +++	97.4 / 91.0- 98.0	68.2 / 53.1-78.0	++ / ++ - +++
.15	1	0 / 0 - +	8.1 / 4.6- 17.1	21.6 / 7.7-29.2	0 / 0 - +
"	3	+ / + - ++	9.4 / 6.7- 19.9	34.4 / 16.6-41.3	+ / 0 - ++
"	5	" / "	24.2 / 15.5- 31.3	44.9 / 28.0-55.1	" / "
Untreated control		++ / ++ - +++	96.4 / 93.2-100.0	63.2 / 52.0-72.1	++ / ++ - +++

Twenty mice in each series. Size of inoculum = 10^6 tumor cells.

Standards for estimate of fluid volume and implantation extent see *Material and methods.*

In series 1 and 5 tumor cells, mostly atypical, were counted only in mice containing sizable amount of fluid.

Non-neoplastic cells in fluid: granulocytes and small lymphocytes in controls; macrophages and large lymphocytes in pretreated mice.

8, 10) in the majority, and absence of any growth in a sizable number of pretreated mice. In each localization, extent of inhibition was at its maximum in animals pretreated a day before inoculation.

Sections from mediastinal pleura (serosa and subserosa), of the scalp, and of the leg muscle showed in pretreated mice edema of tissue and clumping of tumor cells within pre-

TABLE II. Inhibition of Malignant Growth and Spread of Subcutaneous and Intramuscular Inocula by Topical Pretreatment with Radioactive Chromic Phosphate (0.15 mc) in 9 Series.

Area of pretreatment and of tumor inoculation	Days between pretreatment and inoc.	Extent of tumor growth and spread on 8th day (avg / variation extremes)
Scalp	1	0 / 0 - +
"	3	" / "
Untreated controls		++ / + - ++
Skin of abdomen	1	+ / + - +++
Idem	3	" / "
Untreated controls		++ / ++ - +++
Left leg	1	+ / 0 - +
Idem	3	+ / + - ++
Untreated controls		+++ / ++ - +++

20 mice in each series.

Doses of 10^6 to 10^7 Krebs-2 ascitic cells were inoculated.

Scale of estimate: See *Material and methods.*

Doses of 0.15 mc of $CrP^{32}O_4$ were used in all experiments.

treated and inoculated area and their absence beyond its periphery, while in controls numerous proliferating cells and islands of organized tumor tissue were found scattered widely far beyond site of inoculation. The role of these phenomena in tumor inhibition by pretreatment will be discussed below.

Discussion. In our previous experiments (1) on growth inhibition of ascites tumors by intraperitoneal pretreatment with a very short-living isotope (Y^{90}, half-life 2.7 days), maximum effect was found in mice inoculated 3 days after pretreatment, *i.e.,* at the time when stored radioactivity had considerably decayed. These findings and microscopic evidence of inhibited tumor cell spread in pretreated tissue suggested that a decrease of tissue susceptibility (or, in other words, increase of its resistance) to cell invasion, rather than irradiation of tumor cells was responsible for the effect of pretreatment with Y^{90}. In our investigations reported here, a longer living isotope (P^{32}, half-life 14.7 days) was used, and it is understandable that amount of radioactivity in the pretreated tissue at time of inoculation was the major factor of tumor inhibition. Indeed, in averages of all experiments presented in Tables I and II, this inhibition was directly proportionate to doses of the isotope and inversely to interval be-

tween its injection and tumor inoculation. However, findings in sections of pleura and subcutaneous tissue pretreated with P^{32} were analogous to previously described changes in the peritoneum pretreated with Y^{90}. Accordingly, they were given similar interpretation. It may be presumed that irradiation with P^{32} decreased viability in tumor cells, and in the pretreated tissue, its susceptibility to invasion by these cells (or, in other terms, increased its resistance). This interpretation is in agreement with the experience of Andrews, Root and Kniseley(11) that "some unknown mechanism in action, possibly on surfaces not involved with tumor, may be important" for therapeutic action of Au^{198} in human peritoneal carcinosis.

Wide range in variations of results was found in series 7, Table I, and series 8, Table II, where radioactivity level in tissue was expected to be at its lowest. In these conditions, extent of tumor cell inhibition depended more considerably on tissue response to irradiation, which probably varied individually within the same series of mice.

In the same conditions of experiment, extent of inhibition by pretreatment was highest in scalp and lowest in subcutaneous tissue of the abdomen. These differences suggest the importance of tissue characteristics (loose or dense connective tissue, vascularization, etc.) and of its topographic localization in the mechanism of tumor prevention by pretreatment. Radioactivity doses of .15 mc achieved considerable preventive effect by all routes. This dose was found to be well below the maximum tolerated doses (.2 mc intrapleurally and .25 mc by other routes) and moreover below doses required for therapeutic use(7,8,12) in tumor-bearing mice. Accordingly, preventive use of the isotope offers advantages of greater safety than its therapeutic use.

Summary and conclusions. (1) Pretreatment of new mice with $CrP^{32}O_4$ in well tolerated doses for topical prevention of tumor growth and spread was applied in various topographic areas, *i.e.*, pleural cavity, scalp, abdominal wall and leg. (2) Reduction of tumor growth in all areas and of its spread in pleural and scalp were directly proportional to dose of radioactivity and inversely to the period between isotope injection and tumor inoculation. (3) These findings showed that amount of radioactivity stored in pretreated tissues at time of tumor inoculation was the major factor of the isotope's preventive effect. However, previous results(1) suggested also another effect of pretreatment, that of tissues increased resistance to invasion with tumor cells. (4) It was concluded that topical pretreatment with $CrP^{32}O_4$ may be attempted for prevention of postoperative growth and spread of tumor cells.

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