2. The examination of each specimen of peritoneal fluid consisted (a) in the microscopic assay of integrity and ability for multiplication (percentage of mitoses) of free tumor cells which multiplied in the peritoneal fluid before treatment and (b) in the biological assay of viability of tumor cells, *i.e.*, their ability to survive and multiply after transfer into new mice.

3. The results indicate that with a single exception (tumor strain C isolated from a treated mouse) all tumor cells suffered loss of viability even after treatment with the minimal dose (0.2 mc) of I^{131}. In most mice this effect was detected only after the failure of tumor cells from treated animals to grow in the peritoneal fluid of new animals. In other animals, where higher dose of radiation was used or, presumably, where nutrient environment was drastically changed, the tumor cells disintegrated in the body of the treated mouse.

4. Rapid disappearance (after 24 to 48 hours) from the peritoneal fluid of injected radioactive preparation suggested the differentiation between its early and primary effect (which may be the production of a "lethal mutation") and its later secondary effect (cell disintegration) may be due to additional factors (higher dose of radioactivity, drastic changes in the biological medium).

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Distribution and Effect of Colloidal Radioactive Gold in Peritoneal Fluid Containing Free Sarcoma 37 Cells.* (18001)

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Hahn and Sheppard(1-3) introduced the use of colloidal radioactive isotopes by intravenous administration in the treatment of diseases of the lymphoid macrophage system. Subsequently, they and their colleagues suggested the use of such colloids, especially that of the insoluble radioactive metallic gold sols, in the treatment of malignant tumors by direct infiltration(4). Administered by the intravenous route these particles are rapidly removed from the circulation through phagocytosis by the reticulo-endothelial cells of the liver and spleen, and to a much less extent by the bone marrow and lymph nodes (5). Given by the direct infiltration route, these particles, insoluble in body fluids, remain for the most part *in situ* except for a certain degree of drainage through the fascial planes and through the neighboring lymphatics. Thus, a certain degree of selectivity may be effected through the direction of the operator in the treatment of certain malignant disease processes. Distribution studies by Hahn, Sheppard and their associates(5) in humans and dogs, by Barrow and associates (6) in rabbits, and by Sheppard and Furth (7) in mice showed that intravenously injected gold colloids are stored in the organs rich in the reticulo-endothelial elements

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1. Hahn, P. F. and Sheppard, C. W., *So. Med. J.*, 1946, v39, 558.

2. Sheppard, C. W. and Hahn, P. F., *So. Med. J.*, 1946, v39, 562.

3. Sheppard, C. W., Goodell, J. P. B. and Hahn, P. F., *J. Lab. and Clin. Med.*, 1947, v32, 1437.

4. Hahn, P. F., Goodell, J. P. B., Sheppard, C. W., Cannon, R. O., and Francis, H. C., *J. Lab. and Clin. Med.*, 1947, v32, 1442.

5. Sheppard, C. W., Wells, E. B., Hahn, P. F. and Goodell, J. P. B., *J. Lab. and Clin. Med.*, 1947, v32, 274.

6. Barrow, J., Tullis, J. L. and Chambers, F. F., *Nav. Med. Rev. Inst.*, Bethesda, Md., Prog. AM007 039, rep. No. 24, July 25, 1949.

7. Sheppard, C. W., Furth, J. and Wish, L., *Fed. Proc.*, 1950, v9, 343.

(macrophages) mainly in the liver and spleen. The radioactive colloidal gold used in these studies was obtained by bombardment by slow neutrons in the chain reacting pile, the target material being 100% naturally abundant Au^{197}. The cross section of the latter to slow neutrons affords a highly economical end product for therapeutic use and the colloid(3) is extremely stable and meets very satisfactorily the criteria set up for therapeutic use of radioactive isotopes(8). The colloid as used contains about 4 mg of gold per cc.† In mice inoculated intraperitoneally with S-37 cells, the peritoneal exudate contains numerous, rapidly proliferating tumor cells, as well as leucocytes and macrophages (9). Thus, it could be used conveniently to study simultaneously the effect on tumor cells and the fate in macrophages of the colloidal gold injected intraperitoneally. The essential results of this study are reported below.

Technic. Our methods of inoculation of tumor cells and of assays of the results were described previously(9). A single intraperitoneal injection of gold containing Au^{198} was performed in inoculated mice. Doses of 0.2 to 0.42 mc were injected in a volume of 0.5 cc. Thus, the original preparation was diluted, according to its activity, 10 to 20 times. The control mice remained untreated or received various doses of inactive colloidal gold, *i.e.*, a preparation formerly containing Au^{198} stored several months to allow decay of its radioactivity. Some mice received as much as 0.25 cc of undiluted inactive gold. Following tests for integrity of macrophages and polymorphonuclears were used: (a) Diluted (1:10) mouse blood was injected (0.5 cc) in the peritoneal cavity of treated mice and controls; occurrence or absence of phagocytosis of red blood cells was recorded. Similar technic was used to test the phagocytosis of bacteria by polymorphonuclears. (b) Alcoholic 0.4% solution of neutral red (few drops) was evaporated on a slide, and a drop of peritoneal fluid was placed on stained area and covered with a cover glass; within 5 minutes all necrotic elements were diffusely stained; cells with only stained inclusions were considered as undamaged.

Results. (1) *Survival of mice.* Out of 48 mice inoculated *i.p.* with about 10,000 S-37 cells and treated, 2 or 3 days later, by the same route with 0.4 mc of gold only 15 survived less than 7 days. Out of 144 mice treated 2 to 5 days after inoculation with 0.2 to 0.33 mc of gold, 54 died or were sacrificed within the first 7 days, and the remaining animals survived at least 14 days (*i.e.* as long or longer than the control animals).

(2) *Effect on tumor cells.* In all 136 mice treated at the end of the 48 hour period after inoculation and in 19 out of 24 mice treated after 72 hours, only scarce and cytologically abnormal tumor cells were found in the peritoneal fluid examined 24 hours after

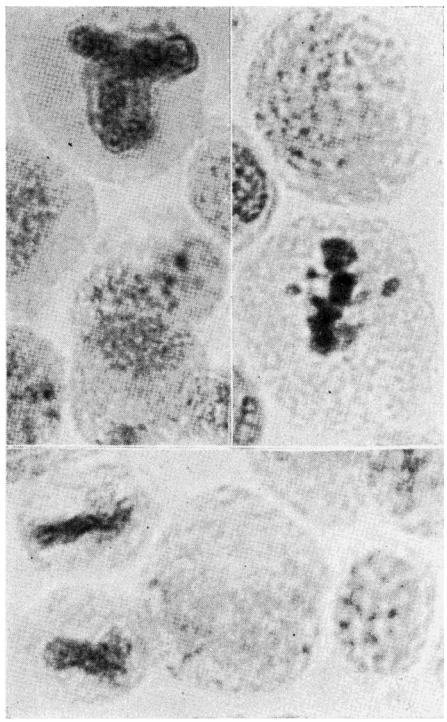

FIG. 1.
Sarcoma cells in the peritoneal fluid of the mouse, 6 days after i.p. inoculation of 10,000 sarcoma cells and 24 hr after i.p. inj. of colloidal radioactive gold (0.33 mc). Aceto-Orcein ×1000.

8. Hahn, P. F., A Manual of Artificial Radioisotope Therapy, Chapter III, Academic Press, New York, 1950.

† Colloidal radioactive gold containing Au^{198} was obtained from Abbott Laboratories, North Chicago, Ill.

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

treatment and at any later date. However, in 5 out of 24 mice treated on the 4th day and in all 32 mice treated (with 0.2 to 0.4 mc of gold) on the 6th day, many tumor cells were present in the fluid withdrawn 24 to 48 hours after treatment. These cells were mostly different from those in controls; in resting cells their cytoplasm was unusually large and the nucleus contained higher number of nucleoli; in dividing cells the chromosomes were asymmetrically arranged, often overcondensed in large clumps; they formed bridges between opposite poles and frequently showed delayed division (stickiness) in telophase and prophase (Fig. 1), as well as giant cells with small chromosomes or with 2 nuclei. In order to test the viability of these cells, they were transferred in 0.1 to 0.2 cc of peritoneal fluid from each of 30 mice *i.p.* into 30 new mice and subcutaneously into 15 mice. Repeated withdrawal of the fluid from the recipients did not reveal any growth or even survival of transferred cells, and no subcutaneous tumors were recorded. Moreover, the specimens of the peritoneal fluid obtained from the "donors" on the 3rd day after treatment contained only a few abnormal tumor cells or their debris and numerous macrophages. Twelve mice were inoculated with a higher number of S-37 cells (50,000) and treated, after 2 days, with 0.4 mc of gold; in all mice the tumor cells disappeared from the peritoneal fluid or became very scarce within 24 hours. In control mice, the inoculated sarcoma cells multiplied vigorously in the fluid (15 to 25% mitoses) until the 6th or 7th day; at that date the percentage of their mitoses began to decrease and dropped to a low level (about 5%) on the 10th day in the surviving mice. In all controls intraperitoneal and subcutaneous implants were found about the 7th day at the site of inoculation. The *i.p.* transfer of the peritoneal fluid from these mice, at any stage after inoculation, induced in the recipient the same growth cycle of tumor cells.

(3) *The presence of gold particles in macrophages.* The gold colloids used in these experiments were exceedingly highly dispersed with ultramicroscopic particles ranging in size from about 10 to 50 millimicra. Immediately following injection one would not expect to find microscopically visible particles in any tissues examined(5). Thus, the agglomeration noted below is of considerable interest. The intraperitoneal injection of colloidal gold preparations in amounts as small as those used in our experiments (0.08 to 0.15 mg) was followed after 24 to 48 hours (in an experiment with one specimen of the preparation within 18 hours) by the appearance of dot-like gold particles in the macrophages (Fig. 2). In this stage, these groups of spherical dots resembled a swarm of cocci, keeping closely together even when the cytoplasm was not outlined. If it were sharply outlined some particles were always found apparently attached to the outer side of the cell membrane. On the following days the gold dots were found replaced by clumps of irregular spherical shape, often adjacent to the cell membrane, until finally only one or 2 large black clumps were found in each macrophage. In control animals injected with similar amounts of inactive colloidal gold, the intracellular formation and aggregation of coarse gold particles inside the macrophages or on their membranes followed the same pattern, but the clumps showed the tendency to remain dispersed, without final

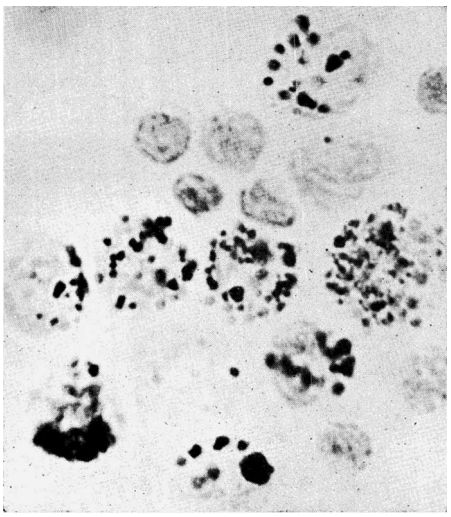

FIG. 2.

Macrophages in the peritoneal fluid of the mouse, 5 days after i.p. inoculation of 10,000 sarcoma cells and 3 days after i.p. inj. of colloidal radioactive gold (0.33 mc). Aceto-Orcein ×1000.

formation of few single clumps.

The neutral red test and the phagocytosis test revealed in samples of peritoneal fluid withdrawn 2 or 3 days after treatment that the majority of free macrophages loaded with small or large clumps of colloidal radioactive gold showed tinctorial characteristics of undamaged cells and maintained their phagocytic function. In the same samples of fluid (containing radioactive gold) many polymorphonuclears were able to absorb bacteria added to the fluid. Peritoneal fluid showed rapidly decreasing radioactivity during 3 days following the injection (only traces on the 4th day). This activity was concentrated in the cellular sediment of the peritoneal fluid and present only in traces in supernatant fluid.

Discussion. A full radiation effect on tumor cells was obtained by using the peritoneal fluid as growth environment for these cells, and colloidal gold containing Au^{198} as source of radioactivity. Since the half-path of the beta particles is about 0.4 mm in tissue, while for most gamma rays it is much longer, it may be presumed that the ionizing radiation responsible for radiation effect was derived to an extent of about 90% from the beta particles of gold suspension intimately mixed with tumor cells in the peritoneal fluid(10). The destruction of tumor cells was evidenced(1) by their advanced stage of distintegration or their absence in stained smears from peritoneal fluid; (2) by the absence of subcutaneous or intraperitoneal implants in treated mice at the site of inoculation and (3) by the lack of viability of treated cells, as shown by their inability to grow after *i.p.* or *s.c.* transfer into new mice. This marked radio-therapeutic effect of colloidal radioactive gold on peritoneal tumor cells in mice corresponds to recent clinical observations of Müller(11) in 8 female patients with carcinomatous peritonitis where it was claimed that the intraperitoneal administration of colloidal radioactive gold (100 to 150 mc) induced "undoubtful therapeutic results".

The cytological abnormalities observed in tumor cells as a result of radiation resembled closely those recorded by other authors and reviewed recently by Koller(12): fragmentation or over-condensation of chromosomes, prolongation of metaphase and of late prophase stages, lagging of chromosomes and increased number of nucleoli in resting cells.

The absence of microscopically visible gold dots or clumps in free cells other than macrophages suggests that ultramicroscopic gold particles are selectively absorbed by macrophages and do not simply adhere to any cell surface. On the contrary, the agglomeration into larger clumps may be purely physical phenomenon, analogous to those observed in several colloidal systems under similar physicochemical conditions. The tendency of inactive colloidal gold to remain dispersed at a certain level may be due to the difference in electric charges of radioactive and inactive gold particles.

Our demonstration of morphological and functional integrity of macrophages loaded with radioactive gold clumps indicates the remarkably high resistance of these cells to intracellular radiation effects. The absence or scarcity of floating free gold clumps in the fluid, even after transfer of gold bearing macrophages into the peritoneal cavity of new mice, suggests that the gold clumps from macrophages which eventually disintegrate are immediately absorbed or adsorbed by the tissue macrophages in the peritoneal wall. This is supported by extreme abundance (macroscopic grey patches) of gold clumps in the peritoneal tissue macrophages. Our data indicate that many polymorphonuclears in the peritoneal fluid were destroyed (or their life cycle was shortened) by the radiation effect, but those which escaped this effect maintained their functional integrity as shown by phagocytosis of bacteria.

Summary and conclusions. 1. Two to 5 days after intraperitoneal inoculation of 10,000 (in one series—50,000) free sarcoma-37 cells from peritoneal fluid, several series of CFW mice were treated with a single intra-

10. Peacock, W. C., personal communication.

11. Müller, J. H., *Bull. Schweiz, Akad. d. Med. Wissensch,* 1949, v5, 484.

12. Koller, P. C., *Brit. J. Cancer,* 1947, v1, 38; and Koller, P. C. The Behavior of Tumor Cells Under Normal and Experimental Conditions. Symposium on Biol. Science, Univ. of Milan, 1949.

peritoneal injection of 0.2 to 0.44 mc of colloidal radioactive gold. Control mice remained untreated or were treated with inactive (decayed) gold preparations.

2. On 3 consecutive days after treatment, specimens of peritoneal fluid were withdrawn by exploratory puncture from treated and control mice. Stained smears from these specimens revealed the absence of tumor cells or cytological abnormalities therein in gold treated mice, while in controls the tumor cells were numerous and showed high proportion of mitoses. The non-viability of morphologically alterated cells was demonstrated by their inability to multiply or even to survive after their transfer into new mice.

3. Ultramicroscopic colloidal gold particles mixed immediately after injection with the peritoneal fluid became condensed, after 24 to 48 hours, into dot-like particles in the cytoplasm of macrophages and during the following days into coarse clumps. The macrophages loaded with gold particles maintained their structural and functional integrity; lymphocytes and polymorphonuclears were partially and temporarily destroyed by radiation effect.

4. It is concluded that in the cellular peritoneal exudate from mice inoculated *i.p.* with S-37 cells, the tumor cells were highly sensitive to small amounts of radiation well tolerated by the mice themselves while macrophages showed very high resistance to the same agent. Thus, the complete destruction of free tumor cells in the peritoneal cavity of the mice may be described as a selective radiotherapeutic effect of radioactive colloidal gold.

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A Simple Chromatographic Procedure for the Separation of Angiotonin from Crude Mixtures. (18002)

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The purification of angiotonin (hypertensin) has been hampered not only by its lability but by its relatively high solubility in water and low solubility in ordinary organic solvents. In preliminary chromatographic experiments with the capillary-ascent test tube method of Rockland and Dunn(1), using 7% aqueous solution of phenol as the developing agent, it was found that angiotonin could be separated from materials that gave a positive ninhydrin color reaction. Although a satisfactory separation could be obtained with small quantities by this technic, as much as 800 to 4000 units of angiotonin could be isolated on a column prepared from No. 1 Whatman paper macerated in a Waring blendor with 7% aqueous phenol solution.

Methods. Preparation of column. Whatman No. 1 filter paper was macerated in a Waring blendor with 5% aqueous phenol (7 g reagent grade phenol in 100 cc distilled water) and transferred to a sulfur absorption tube of 30 mm diameter (Corning 39620) the fritted glass disc of which had been covered with a layer of glass beads. The tube was fitted into a suction flask by means of a one-hole rubber stopper. Suction was applied to the tube while the pulped paper was added in small portions, a wide-footed glass rod being used to aid in forming a tightly packed column. The column was washed with 7% phenol solution to remove any material possibly soluble in the solution.

Preparation of angiotonin. The angiotonin was prepared by Plentl and Page's modification(2) of the method of Page and Helmer(3) from renin substrate processed from hog serum and an angiotonase-free hog renin.

1. Rockland, L. B., and Dunn, M. S., *Science*, 1949, v109, 539.

2. Plentl, A. A., and Page, I. H., *J. Biol. Chem.*, 1945, v158, 49.

3. Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, v71, 29.