tional effect upon the endometrium is maximal.

The progestational property of progesterone has been considered highly specific in that it is known to be possessed by only few closely related steroids and has not been previously demonstrated in non-steroidal compounds. Desoxycorticosterone and 17-hydroxy-11-dehydrocorticosterone (Cpd. E. of Kendall) are known to possess limited degrees of progestational activity(4). Conversely, progesterone has a limited corticoid effect(4). In view of the progestational activity of Compound I, the potential corticoid activity of this and related substances adds interest to the further biological characterization of this series of compounds. We shall subsequently report on the chemistry and physiological activities of other substituted desoxybenzoins.

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Effects of Radioactive Iodine on Free Sarcoma 37 Cells in the Peritoneal Fluid of the Mouse.* (18000)

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In producing biological effects on living tissue cells, the gamma rays of radioactive isotopes expend their energy by ejection of high speed electrons. Consequently, it was presumed that the action of beta particles on cell complexes would be based on similar mechanism. However, it is known that beta particles do not penetrate significantly in the body or organ surface exposed to irradiation (Dobson and Lawrence)(1). Moreover it is difficult to distinguish the primary effect of beta particles on tumor cells from the secondary effect induced by the irradiated layer of malignant tissue in tumor stroma, blood vessels and deeper portions of the tumor. It seems reasonable to study the primary effect of beta particles on tumor cells, by introducing radioisotopes in body fluids containing free tumor cells. Thus, Evans and Quimby(2) injected radioactive sodium subcutaneously into leukemic mice and recorded the effects on blood counts. We have found that it is convenient to induce and to observe primary changes in tumor cells by injecting a radioactive isotope intraperitoneally into mice bearing viable free tumor cells in their peritoneal fluid. The viability of such cells has been demonstrated by several authors: by Graham(3) in human patients, by Warren and Gates(4) in rats and by several German authors(5) in mice. Recent experiments (Goldie and Felix)(6) have shown that it is possible to grow in the peritoneal fluid free tumor cells (sarcoma 37 cells in CFW mice, thymoma cells in dba mice) in serial intraperitoneal transfers. At each transfer the multiplication of tumor cells can be estimated quantitatively by counting cells and their mitoses first in the inoculated fluid and later on in specimens of peritoneal fluid from inoculated mice. This method was applied in the experiments reported below recording the changes in number and characteristics of free sarcoma 37 cells after intraperitoneal injection of radioactive iodine.

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Technic. (1) *Inoculation of tumor cells.* The number of tumor cells in the peritoneal fluid of a "donor" mouse was calculated by counting the total number of cells (tumor cells, leucocytes, peritoneal tissue macrophages) in the fluid (using an hemocytometer) and the percentage of tumor cells (differential counts in the smears stained with Wright's stain and by Aceto-Orcein method) in the same specimen. The requisite number of tumor cells was obtained by diluting the peritoneal fluid to the concentration of 10,000 cells (standard dose) or 50,000 cells (maximum dose) in 0.5 cc. The needle was thrust under the skin at the junction of the middle and the lower third of the abdominal axillary line and the peritoneum was pierced ½ inch lower (to avoid leakage). The mice used in the experiment were CFW strain of Carworth Farms. The tumor was a sarcoma 37 strain maintained in more than 70 intraperitoneal transfers.

(2) *Treatment.* Radioactive iodine (I^{131})† was injected intraperitoneally in doses of 0.2 to 0.4 mc, 2 or 3 days after inoculation of tumor cells by the same route. This interval of time was fixed after observation that late treatment (more than 3 days after inoculation) does not prevent the appearance of subcutaneous tumors at the site where the inoculating needle was thrust through the skin. The localization of free tumor cells in the connective tissue at the site of trauma by injection or exploratory puncture of the abdomen occurred, within 4-6 days, in all control mice, inoculated and untreated. Twenty-five control mice were treated with inactive iodine, *i.e.*, a specimen of I^{131} preparation which lost its activity. All animals survived at least 7 days after administration of the maximal therapeutic dose of iodine used in these experiments (0.4 mc, about 0.02 mc per 1 g weight).

(3) *Assays of the effect on tumor cells.* Amounts of 0.1 or 0.2 cc of peritoneal fluid were withdrawn from inoculated mice 1, 2 or 3 days after treatment, in some instances after 4, 7 or 10 days. In control mice the fluid was withdrawn at similar intervals after inoculation. The withdrawn material was used for microscopic assay (presence of tumor cells in the fluid) and for biological assay (viability of remaining tumor cells). (a) *Microscopic assay: morphological changes in tumor cells or their disappearance from the fluid.* A small drop (about 0.01 cc) of each specimen was smeared thinly on each of two slides: one was stained with Wright's stain (for differential study of tumor cells and leucocytes) and the other with Aceto-Orcein (for counting of mitoses). The results were compared with those in specimens from control mice. Disappearance of mitoses and eventually disappearance of tumor cells were considered as marking points in each experiment. (b) *Biological test: viability of tumor cells from treated mice after their i.p. transfer into normal mice.* Major portions of the fluid (0.05 to 0.2 cc) withdrawn at regular intervals after treatment were transferred intraperitoneally into new mice, and in these mice the fluid was examined by repeated withdrawals as above, but the period of observation lasted up to 20 days. If the microscopic test revealed the presence of mitotic tumor cells in the fluid of recipients, this fluid was transferred (2nd transfer) into new mice, from these eventually (3rd transfer) into new mice and so on. The number of transfers during which the tumor cells maintained their morphological integrity and ability to multiply (mitoses) was considered to be an index of their viability. (c) *Test for radioactivity.* The disappearance of injected radioactive preparation from the peritoneal fluid was checked by testing with a Geiger counter.

(3) *Results.* (a) *Tumor cells in the peritoneal fluid of control mice.* In more than 100 control mice (untreated or treated with decayed I^{131}) the inoculated 10,000 tumor cells multiplied, after a lag period of a few hours, and reached within 4 to 6 days the concentration of 40,000 to 60,000 per cc (the total amount of fluid in the peritoneal cavity varied approximately from 0.2 to 0.5 cc). The percentage of mitoses in these cells was of 15 to 25%. (Fig. 1). After the 6th or the

† Carrier-free I^{131} was obtained from the Isotopes Division, U.S.A.E.C., Oak Ridge, Tenn.

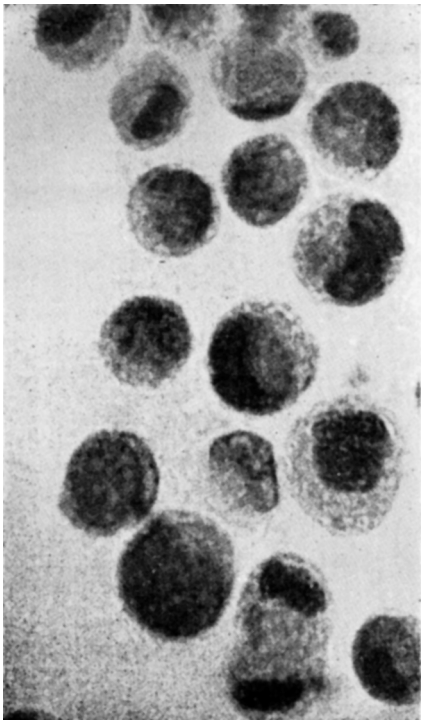

FIG. 1.
Sarcoma cells in the peritoneal fluid of the mouse 5 days after i.p. inoculation of 10,000 sarcoma cells. Aceto-Orcein ×1000.

7th day the number of intact tumor cells and the percentage of mitoses decreased slowly, while the percentage of necrotic cells increased as long as the mice survived. All animals developed solid implants at the site of intraperitoneal inoculation and died mostly within two weeks. The intraperitoneal transfer of the peritoneal fluid from the control mice into new mice resulted always in tumor cell multiplication and later on in growth of solid tumor at the site of inoculation. The subcutaneous inoculation of this fluid induced solid tumors. The serial peritoneal transfers of peritoneal fluid from control mice could be continued indefinitely without any decrease in the extent of tumor cell multiplication at each transfer.

(b) *Tumor cells in treated mice.* Twenty-four hours after treatment, no tumor cells were found in 9 out of 34 mice treated with 0.2 to 0.22 mc of I^{131}, (1st group) and in 51 out of 72 mice treated with 0.4 to 0.44 mc of the same preparation (2nd group). In remaining mice, some cells were apparently normal and showed mitoses, but many cells were distinctly altered; their cytoplasm was large and vacuolized, the chromatin separated in fragments. In apparently intact cells, the proportion of mitoses was quite high (10 to 20%), but within the next 2 days, it dropped to less than 5% and in most mice to less than 2%. The chromosomes in these mitoses were atypically arranged or abnormal (Fig. 2). The majority of surviving tumor cells appeared markedly changed on the 3rd day or the 4th day after treatment. Thus, the damage inflicted to tumor cells by the treatment was progressing for several days after the injection of I^{131}. As the final result, only 2 out of 106 treated mice showed in their peritoneal fluid mitotic tumor cells on the 8th day after treatment. This microscopic evidence of damage to tumor cells was strongly corroborated by the evidence of biological tests: Tumor cells appearing intact or con-

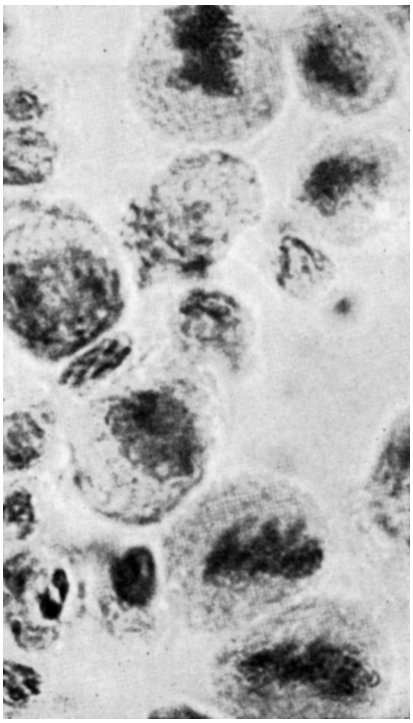

FIG. 2.
Sarcoma cells in the peritoneal fluid of the mouse, 5 days after i.p. inoculation of 10,000 sarcoma cells, 48 hours after i.p. injection of radioactive iodine (0.2 mc). Aceto-Orcein ×1000.

taining mitoses were transferred with the peritoneal fluid of 30 mice of the first group and 30 mice of the second group into 60 new mice; only 7 recipients showed the occurrence of tumor cells and mitoses in their peritoneal fluid after transfer, while in 53 recipients the transferred cells died more or less rapidly. The "second transfer" of fluid using seven positive recipients as donors resulted in cell multiplication in only one mouse. None of 106 treated mice developed a small nodule. Subcutaneous inoculation of 0.2 cc of fluid from 30 treated mice failed to induce tumors or even nodules in normal mice. Only one tumor strain isolated from a treated mouse (mentioned above) was able to induce regularly subcutaneous tumors. This strain (which we have designated as strain C) showed higher growth potency than the original sarcoma strain, it grew more vigorously (in 25 transfers up to the present time) and induced larger amounts of fluid and only a slight leucocytic reaction at each transfer. Two groups each of 12 mice were inoculated with 50,000 tumor cells and treated with 0.2 (1st group) or 0.4 mc (2nd group) of I^{131}. The tumor cells disappeared within 3 days in 9 mice of the first group and in 4 mice of the second group. In 9 out of 11 "positive" mice, the tumor cells disappeared after the first transfer and in remaining 2, after the second transfer.

(c) *Radioactivity of the peritoneal fluid.* The peritoneal fluid of all mice injected with I^{131} showed weak radioactivity at the end of the first 24 hours, but only in few mice at the end of the second day. In no instance, did the fluid remain radioactive at the end of the third day. After transfer of treated cells into new mice, the peritoneal fluid of recipients consisted in 70 to 80% of large macrophages vacuolized and stuffed with debris of tumor cells.

Discussion. Immediate morphological changes and, in several mice, rapid disappearance of tumor cells from the peritoneal fluid were observed after treatment with radioactive I^{131}, but not after injection of decayed I^{131}. Thus, the biological effect of I^{131} on tumor cells should be attributed to its radioactivity. The radioactivity test of the peritoneal fluid indicated that the injection preparation was immediately dissolved in the peritoneal fluid and distributed even in the "pockets" of the peritoneal cavity. Thus, the tumor cells, the leucocytes and the macrophages were suspended in a radioactive fluid medium. In these conditions the chief effect on the cells would likely be produced only by beta particles of the preparation. The radioactivity of the peritoneal fluid did not last more than 24 to 48 hours, while the signs of damage to cells continued to increase after that period. Thus, the primary action of beta particles was a "hit and run" blow to tumor cells, but it was followed by secondary effect of damage to these cells. The decrease in the viability of tumor cells was manifested by their inability or limited ability to multiply into serial intraperitoneal transfers and to induce sizable subcutaneous tumors. Since Lea(7) interpreted the killing of bacteria by radiation as a lethal mutation, it may be presumed that similar phenomenon is responsible for the loss of viability of irradiated tumor cells in the peritoneal fluid. The mutant tumor cell loses its essential characteristic of autonomic growth and therefore lacks the ability to survive and multiply after transfer into a new host. In the case of tumor strain C the tumor cells from treated mice continued to grow vigorously in serial transfers. This may be attributed to survival of particularly "tough" tumor cells after destruction of all "weak" individuals by radiation or to a mutation resulting in better adjustment of tumor cells to the host.

Summary and conclusion. 1. CFW mice of 25 g weight were inoculated intraperitoneally with requisite numbers (mostly 10,000) of S-37 cells and treated *i.p.* with radioactive iodine (I^{131}) (0.2 or 0.4 mc). The fate of tumor cells was studied by repeated withdrawal and examination of peritoneal fluid in each mouse and compared with their fate in control animals (inoculated with the same dose of cells, but untreated or treated with decayed I^{131}).

7. Lea, D. E., Actions of Radiation on Living Cells, N. Y., Macmillan, 1947, Chap. 9.

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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