Topical Localization in Mouse of Radioactive Silver Oxide [$(Ag^{111})_2O$] Introduced by Various Routes.* (22403)

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Previous work by West(1,2) has revealed that the short half-life radiosilver isotope Ag^{111}, injected in the form of a soluble salt (nitrate) intramuscularly or intravenously is taken up by blood leukocytes and carried into inflammatory body areas. It was presumed that combination of silver nitrate with tissue proteins resulting from the corrosive effect of this salt on tissues was a prerequisite for phagocytosis of radioactive material and its transportation in the blood stream by phagocytes.

It occurred to us that microscopic particles of an insoluble inorganic silver compound would be equally a target for phagocytes with the advantage of greater uniformity and of lack of corrosive action on tissues. Moreover, it was conceivable that this material introduced in the vicinity of tumor tissue will be carried by normal tissue lymphatics into lymph spaces of the tumor and stored by its phagocytic elements. Accordingly, we have injected radioactive silver oxide $(Ag^{111})_2O$ into subcutaneous tissue on the periphery of mouse tumors or into body serous cavities (peritoneal and pleural) containing solid tumor implants and exudate. Controls were normal mice injected with the same material in corresponding body areas by the same routes. The fate of injected material was investigated by taking autoradiographs from treated tumor bearing mice and controls and, moreover, by testing radioactivity (Geiger counts) of tumor tissue and of various organs from the mice of each series. The results illustrated the modification of radiosilver distribution in the body by the presence of tumor growth near the site of radiosilver injection.

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Material and methods. Radioactive Ag^{111} was separated in the form of nitrate from the radioactive palladium target (obtained from the pile at Oak Ridge A.E.C. Laboratories) by a procedure developed by William F. Clark; it was transformed into the oxide by treatment with sodium hydroxide. The material was washed several times with distilled water, suspended in the same at the ratio of 1.0 or 2.0 millicurie per 10 cc and vigorously shaken before each injection in order to break clumps into uniform particles. Doses of 0.1 or 0.2 mc were injected subcutaneously into mice carrying 7- to 15-day-old tumor growths on the back, the scalp or the hind leg; the dose was introduced in 5 fractions at various sites of subcutaneous tissue on tumor periphery and/or tumor bed. Similar doses were injected into pleural or peritoneal cavity bearing malignant tissue implants and exudate. The following tumor strains and mice strains were used: Sarcoma 180 and Sarcoma McGhee (isolated in our laboratories) in Swiss Albino strain, Carcinoma C_3H/BA in C_3H mice and Carcinoma EO771 in C57-6 mice. In controls (normal mice) similar doses were introduced into the same areas of the skin or the cavities. Twenty-four hours later, the animals were sacrificed: 2 or 3 mice of each series (10 to 15 mice) were used for autoradiography, while in the remaining mice the organs and tumor tissue were placed into tin cups, ground, suspended in a detergent solution, dried and tested by a scaling circuit in conjunction with a thin mica end-window Geiger tube. Subcutaneous tumors (mostly single spheric lumps) were cut each in 4 quarters, partly because they were bulky and contained large amounts of radioactivity and partly because this procedure afforded some information on distribution of radioactive material in the tumor tissue. Peritoneal and

TABLE 1. Distribution of Radioactive Ag^{111}_2O Injected by Various Routes into Normal and Tumor Bearing Mice.

Route of inj.	Geiger counts (avg of each series)											
	Liver	Spleen	Lung	Sto-mach	Small intes-tine	Colon	Kid-ney	Subcut. tissue at site of inj.		Tumor tissue (and intracavitary exudate)		
								1	2	3	4	
1. Subcut. in normal mice	1,465	140	31	53	45	46	39	4,569				
2. Subcut. into tumor periphery	461	67	99	97	116	109	102	604	3,028	2,517	7,060	6,974
3. Into normal pleural cavity	388	62	3,291	65	74	78	85	224				
4. Into pleural cavity with malignant growth	1,394	106	8,340	58	44	48	75	188		15,068		
5. Intraper. in normal mice	2,786	664	81	218	315	464	228	848				
6. Into peritoneal cavity bearing malignant growth	706	223	76	135	182	72	94	528		18,602		

Note: Counts in 3 groups each of 10 mice were pooled in each series. For technical reasons results are fully comparable within each series, but not between different series of mice (inj. by various routes).

pleural exudates were tested separately from intracavitary implants, but since this separation was not complete (exudate contained often fragments of implants) the results were pooled (as shown in Table I). For obtaining autoradiographs, each mouse was secured to a cardboard in a position that presented the best profile of the area of malignancy. It was then quick frozen to minimize the possibility of further metabolic activity. After freezing, the surface activity was measured by means of a precision counting rate meter in order to determine the required exposure time. The animals were then placed in pliofilm envelopes and secured to cardboard x-ray film. They were placed in a deep freeze for the required exposure time. At the end of this period each film holder and its contents were x-rayed intact; thus, the skeletal structure is included on the same film with the exposure due to isotope radiation. In other words, a photograph was taken showing localization of radioactive material on the background of an x-ray picture of the skeleton in the body of each animal.

Results. Autoradiographs 1-6 illustrate the tendency of radioactive silver from the oxide injected subcutaneously to be retained by the injected area with only a minor portion of material to be distributed into distant tissues and organs. However, the radiosilver injected into subcutaneous tissue on the periphery of a subcutaneous tumor (Fig. 2) was shown to be retained not only by the injected area but in a still greater proportion, by tumor tissue; the same material injected intraperitoneally or intrapleurally was retained within the cavity and mainly within tumor implants and exudate containing tumor cells (Fig. 4 and 6). Serous cavities of normal mice have equally retained the injected radioactive material but stored it mainly in the liver and other organs (Fig. 3 and 5). Geiger counts (Table I) provided interpretation for the data supplied by autoradiography: it appears from Table I that in normal mice (Series I) the subcutaneously injected radioactive silver oxide was transported by the blood stream into liver and spleen with a major portion of the material remaining in the

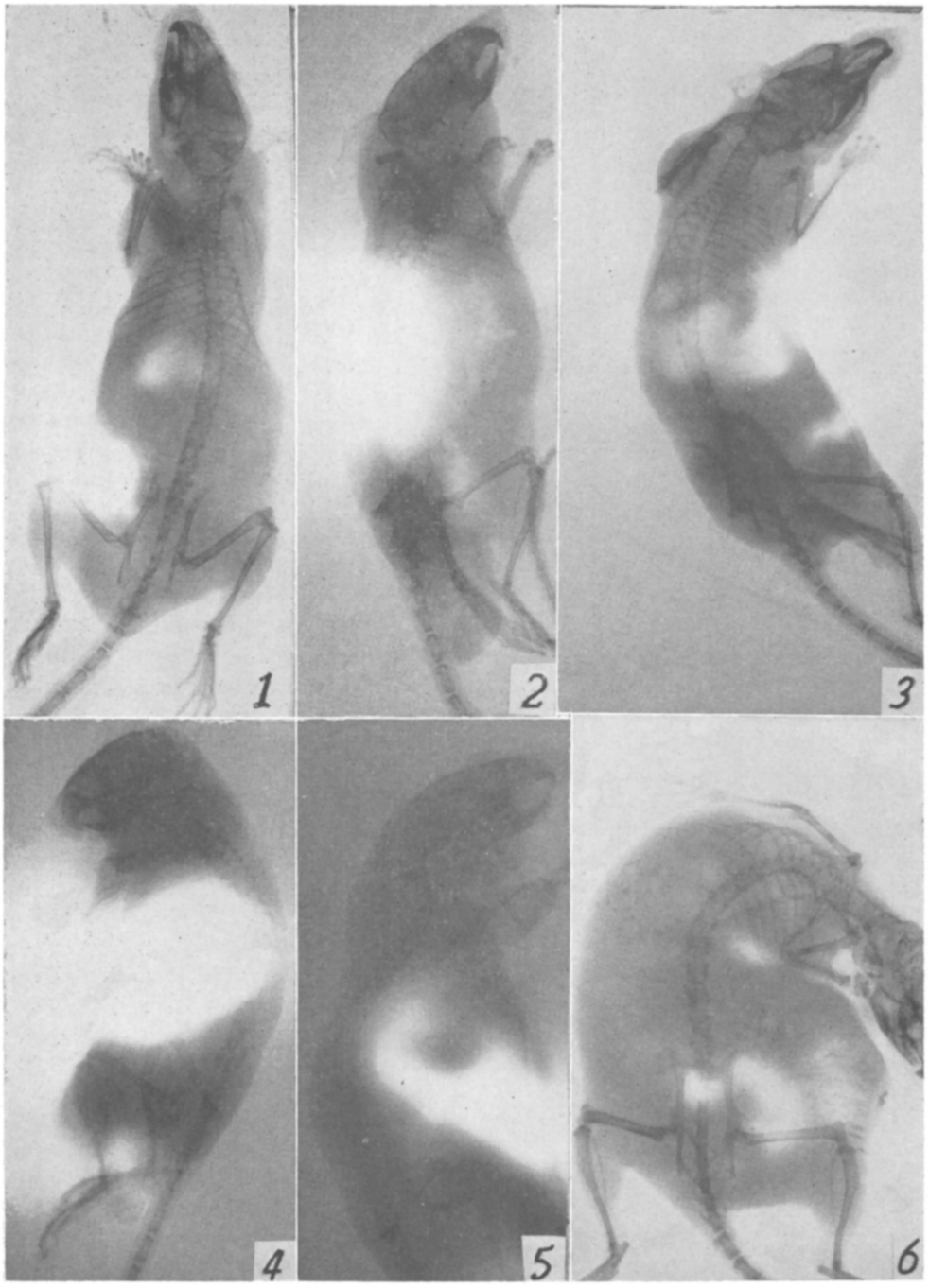

PLATE I. Distribution of $Ag^{111}O$ inj. by various routes into normal and tumor-bearing mice. (Autoradiographs.)

FIG. 1. Inj. into subcut. abdominal tissue of normal mice.
FIG. 2. Inj. into periphery of tumor on back.
FIG. 3. Inj. into normal pleural cavity.
FIG. 4. Inj. into pleural cavity containing malignant exudate and implants.
FIG. 5. Inj. into normal peritoneal cavity.
FIG. 6. Inj. into peritoneal cavity bearing malignant exudate and implants.

subcutaneous tissue at the injected area. However, the material injected into tumor bed and periphery (series 2, Table I) was stored in the tumor tissue in much higher proportion than in the injected subcutaneous tissue; only insignificant amounts were taken up by abdominal organs. In the pleural cavity of a normal animal (series 3) the radiosilver was stored mainly in the lung and the mediastinal nodes, but a significant portion of the material was translocated into the liver; a much larger portion of injected radioactive material was stored in the pleural cavity containing malignant growth (series 4), the storage taking place in the first line in tumor implants and exudate. Similar difference in distribution of radioactivity was recorded in mice with or without peritoneal malignant growth (series 5 and 6).

Microscopically, particles presumably of silver oxide were found inside macrophages of malignant exudates (pleural and peritoneal) of tumor tissue, but also abundantly as free particles in the exudate and tissue fluids; many lymphatics in the injected area of subcutaneous tissue were filled up with the material.

Discussion. Distribution of our radioactive material in normal mice reveals the role of tissue spaces, lymphatics and macrophagic elements in the storage of silver oxide particles; a large portion of material was found in organs rich in reticulo-endothelial elements (liver, spleen, lungs) and another portion was retained in the injected area, in particular after subcutaneous injection, presumably as a result of clogging of lymphatics. Tumors in the vicinity of an injected subcutaneous area drained the injected area by absorbing the particles into macrophages and interspaces of malignant tissue, thus decreasing the deposit at the site of injection and the output into the blood stream.

The competition between subcutaneous tumors and their hosts' tissues for peritumorally injected radioisotopes has been described in our report on the effect of radioactive Yttrium ($Y^{90}Cl^3$) in tumor bearing animals (3). The intracavitary retention of particulate isotopes and the role of macrophages in storage of their particles has been described for radiogold (Au^{198}) by Goldie, West *et al.* (4,5,6) and for radioactive chromic phosphate ($CrP^{32}O_4$) by Gabrielli(7) and by McCormick, Jaffe and Sied(8); the topical localization of radioactive Yttrium in the muscle and the cavities with only insignificant translocation into organs was described by Lahr, Olsen, Gleason and Tabern(9). Our present investigation adds an Ag^{111} particulate compound to the group of radioisotopes suitable for topical application. It should be noted also that Ag^{111} shares with the Au^{198}, P^{32} and Y^{90} the advantage of being short lived (half life 7.5 days) and with the latter two isotopes an additional advantage of being a pure beta (short wave) emitter, thus more suitable for selective topical application than isotopes with some proportion of gamma (long wave) irradiation which is liable to exert distant action on hematopoietic organs.

Summary and conclusions. (1) Radioactive silver (Ag^{111}) was injected in the form of the insoluble oxide into subcutaneous tissue and serous cavities of normal and tumor bearing (Sarcoma 180, Carcinoma C_3H/BA, Sarcoma McGhee, Carcinoma EO771) mice, in doses of 0.1 or 0.2 millicurie. Twenty-four hours later the animals were sacrificed; in each series some mice were used for autoradiography and others for testing radioactivity of their tissues and organs with Geiger counter. (2) Subcutaneously injected $(Ag^{111})_2O$ is stored in normal mice mainly in the injected area and in smaller proportion by liver and spleen; in subcutaneous tumor bearing mice, the major portion of silver oxide injected in vicinity of the malignant growth is taken up by malignant tissue. (3) $(Ag^{111})_2O$ injected into the normal pleural cavity is stored mainly by lungs and liver, and in tumor bearing cavity—by malignant exudate and implants. Similar difference in distribution was recorded for normal and tumor bearing peritoneal cavities. (4) It was concluded that radioactive $(Ag^{111})_2O$ deposited subcutaneously or inside serous cavities is drained by tissue or exudate fluid into adjacent malignant tissue where it is stored by intercellular fluid and by macro-

phagic elements.

1. West, H. D., Johnson, A. P., and Johnson, C. W., *J. Lab. and Clin. Med.*, 1949, v34, 1376.

2. West, H. D., Elliott, R. R., Johnson, A. P., and Johnson, C. W., *Am. J. Roentgenology*, 1950, v64, 871.

3. Goldie, H., and West, H. D., to be published in *Cancer Research*, 1956.

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

5. Goldie, H., Calvin, L. B., Nash, H., and Hahn, P. F., *ibid.*, 1951, v76, 480.

6. Goldie, H., West, H. D., Jeffries, B. R., and Butler, C. H., *ibid.*, 1952, v80, 327.

7. Gabrielli, E. R., *Acta Physiol. Scandinav.*, 1951, v23, 283.

8. McCormick, J. B., Mille, G., Jaffe, B., and Sied, L., *Arch. Path.*, 1954, v48, 187.

9. Lahr, T. N., Olsen, R., Gleason, G. I., and Tabern, D. L., *J. Lab. and Clin. Med.*, 1955, v45, 68.

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Distribution of Glucose in Blood of the Chicken. (22404)

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The whole blood sugar value for the domestic chicken varies over a wide range(1). A portion of this variation has been attributed to a sex difference. It has been reported(2) that immature fowls of both sexes and the mature hen have a similar glycemic level which is significantly higher than that of the adult male. Orchidectomy increases the whole blood sugar value to within the range of the hen(3). In a comparative study(4) with normal and testosterone propionate injected laying hens it was observed that androgen depressed the blood sugar level significantly. A sex variation in red cell volumes, counts, and hemoglobin values has also been reported for this species(5,6). Immature fowls of both sexes and the mature hen have a lower packed-cell volume, red cell count and hemoglobin percentage than the adult male. Coincident with maturation the cockerel shows a steady increase in packed-cell volume. The mature value represents an increase of approximately one-third. Sinistral poulards also have a high red blood cell value, whereas bilaterally ovariectomzied hens remain within the normal range for the hen(6). Androgen injection into immature chickens and into adult laying hens results in a marked and fairly rapid increase in red cell volume, red cell count, and hemoglobin level, all of which may attain the normal male adult range. Castration in the male results in values which do not significantly vary from those of the hen(7). This discussion suggests an intimate relationship between whole blood sugar levels and blood cell concentration. This association is supported by reports of an unequal plasma-cell glucose partition. One such report(8) for the hen indicates only 16.3% of the glucose in the circulating cells. Similar findings have been recorded in the pigeon(9). Little or no glucose has been found in the cells of swine, dog, cat, rat, guinea pig, and human(10,11).

It was decided to study plasma glucose levels in the chicken and to test the hypothesis that the sex variation reported for whole blood glucose values is a direct function of the cell concentration, and further that the androgen hypoglycemic effect is likewise a function of its red-cell-increasing capacity.

Materials and methods. Nineteen White Leghorn laying hens were used. Feed and water were offered *ad libitum*. Fifteen to 30 ml blood samples were taken in the morning after a 16-hour fast by heart puncture with a heparin-wetted syringe. The plasma was separated within 15 minutes. Packed-cell volumes were determined with Wintrobe tubes spun at 2500 rpm for 30 minutes. Since an error of approximately 10% is incurred by this low speed technic, high speed centrifuga-