TABLE I. Effect of Transthoracic Vagotomy on the Quantitative Acid Secretion from Heidenhain Pouches.

	Before, mEq.	After, mEq.	% increase
A-38	46	88	92
A-69	14	47	236
A-71	20	105	425
Avg	27	80	196

significantly. Thus, if food remains in contact with the gastric antrum for a longer period after vagus section than before, it is reasonable to assume that the antral phase of gastric secretion may be increased.

Conclusions. In the dog, section of the vagus nerves to the main stomach causes a marked increase in the quantitative 24 hour acid secretion from Heidenhain pouches.

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Intrapleurally Injected Colloidal Au^{198} and Growth and Implantation of Free Tumor Cells in Pleural Exudate.* (19613)

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Several authors(1) have reported clinical trials with intrapleurally injected colloidal Au^{198} in the treatment of malignant pleural effusions and thoracic neoplasms. However, to the best of our knowledge, no attempt has been made to treat similar conditions in experimental animals. Previously(2,3) we have described a method of testing the effect of radioisotopes on free tumor cells in the peritoneal fluid and on their implants in the abdominal wall. A similar method provided a tool for assaying the effect of intrapleurally injected colloidal Au^{198} on free tumor cells in pleural cavity. The results of this assay are reported below.

Material and methods. A. *Technic of tumor cell inoculation.* Requisite numbers (1 to 15 millions) of tumor cells (Sarcoma 37 in CFW mice, carcinoma in C3H and melanoma in dba)† were inoculated (in a volume of 0.2 to 0.5 ml) into the right phrenico-costal sinus (see details in (4)). Colloidal Au^{198} was injected in the same area a few days later. B. *Treatment.* Colloidal Au^{198}‡ was diluted with 0.85% NaCl solution to the concentration of 0.4 mc per cc, and doses of 0.5 ml of this solution were used. For each tumor, 3 series each of 20 mice were inoculated with tumor cell suspensions. On the 5th or 6th day after inoculation, 10 mice of each series were injected intrapleurally with 0.2 mc of Au^{198}, while 10 mice were kept untreated as controls. Three or four days later the pleural exudate was withdrawn from at least 5 mice of each group and assayed as described below. On account of slow growth of melanoma cells, mice inoculated with such tumor cells were treated on the 10th day and sacrificed on the 20th day. D. *Assay of the results.* Stained specimens of the pleural exudate from treated mice and from controls were examined for morphological characteristics of tumor cells and for percentage of their mitoses. In a few animals of each group this microscopic assay of tumor cells was supplemented by biological assay: failure of inoculation of pleural exudate (0.1 ml) from treated mice to induce tumors

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† CFW mice obtained from Carworth Farms, New City, N. Y. C3H and dba obtained from Roscoe Jackson Memorial Laboratory, Bar Harbor, Me.

‡ Colloidal Au^{198} obtained from Dr. D. L. Tabern, Abbott Laboratories, N. Chicago, Ill.

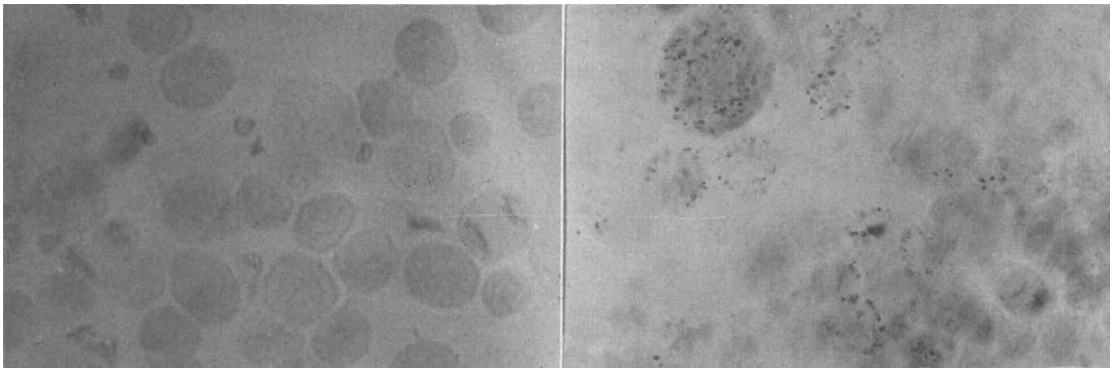

FIG. 1. Sarcoma-37 cells in pleural exudate of mouse 9 days after intrapleural inoculation of 10000000 cells. Aceto-Orcein × 600.

FIG. 2. Pleural exudate of mouse 9 days after inoculation of Sarcoma-37 cells and 4 days after intrapleural inj. of colloidal Au^{198} (.2 mc). Aceto-Orcein × 600.

in new mice was considered as evidence of nonviability of any tumor cells remaining in the exudate. Additional evidence about the effect of Au^{198} was obtained from the autopsy material. E. *Assay of Au^{198} distribution.* New CFW mice of 23 to 26 gr weight were injected intrapleurally with 0.002 to 2 mc Au^{198} and sacrificed at various intervals (see legends to Tables I and II). Their thoracic tissues as well as specimens of the liver and the spleen (for comparative study) were triturated and dried for estimation of their radioactivity. It was found that the weight of organs in sacrificed mice varied considerably, apparently on account of variations in degree of hyperemia and edema. For this reason, we used in each animal the whole amount of tissue (content of mediastinum, diaphragmatic and costal pleura), the whole organ (spleen or lung) or its fragment of certain size (one-twelfth of the liver). In order to obtain data on storage of colloidal Au^{198} in tumor tissue, similar experiments were carried out on mice bearing thoracic tumors of various strains and injected intrapleurally with doses of 0.1 mc of Au^{198}.

Results. Eight to 10 days after inoculation the peritoneal exudate withdrawn from the untreated controls contained 75 to 85% of tumor cells (S-37, S-180 or Carcinoma) 10 to 20% of which showed mitoses (Fig. 1). The transfer of this exudate (even diluted 1:10 or 1:20) into new mice resulted in their abundant multiplication. On the contrary in treated mice only scarce and cytologically abnormal tumor cells were found in the pleural exudate (Fig. 2), the amount of which was considerably lower (0.1 cc or less) than in controls (up to 0.7 cc). The cell abnormalities resembled those of free peritoneal tumor cells treated with radioisotopes(3). Transfers of these cells into new mice induced positive "takes" in only 6% of pooled results. The exudate of treated mice contained numerous macrophages loaded with tiny clumps of colloidal gold, resembling the pigment-loaded macrophages of melanoma exudate which, for this reason, were found unsuitable for study of its cellular composition in gold treated mice. The control groups each comprising 30 mice (3 groups of 10 mice for each tumor strain) showed, after 15 days only 12 survivals for S-37, 15 for S-180 and 13 for carcinoma. Tumors were found in all these animals. As a contrast, none of the treated mice died during the 15 days observation period. Only regressing nodules, sometimes consisting mostly of necrotic tissue, were found in the mediastinum of these mice. All melanoma controls survived the observation period of 30 days, at which date they were sacrificed. Large tumors, often spreading in the abdomen or in the subcutaneous tissues, were found in their pleural cavities. These tumors were absent in treated mice of the melanoma group, which carried however small perfectly round (1 or 2 mm in diameter) nodules of firm melanoma tissue.

It is obvious from distribution studies in new CFW mice (Table I) that a relatively

TABLE I. Distribution of Intrapleurally Injected Colloidal Au^{198} in Thoracic Cavity of Sacrificed Mice. Amount of radioactivity (counts/min).*

	First series (inj. dose = .1 mc†)	Second series (inj. dose = .3 mc†)
Right lung	5244 (1378-12951)	10942 (5360-22518)
Left "	817 (238-1526)	3772 (1417-5287)
Mediastinal tissue	13924 (4084-23150)	23805 (5960-38546)
Liver fragment (1/12 of organ)	10690 (6041-14397)	27938 (12282-37563)
Spleen	2777 (1558-4043)	24972 (15221-29721)
Diaphragmatic pleura	1447 (1027-1911)	8428 (3054-13920)
Right costal "	366 (305-428)	2472 (918-3411)

* Avg 30 mice and (in parentheses) individual extremes. Animals sacrificed 3, 7 or 14 days after inj. of Au^{198} into right pleural cavity. All specimens from same group of mice counted on same day.

† Millicurie.

large portion of radioactive material was stored in the mediastinum.

Microscopic examination of smears from mediastinal lymph nodes (Fig. 3) and from lung tissue revealed the presence of agglomerated colloid particles (described in (3) inside macrophages or in the lymph, but not inside lymphocytes. The pattern of radioactivity distribution was not modified by the use of C3H mice instead of CFW or by dilution of Au^{198} with inactive gold instead of saline solution. Moreover, it is evident from Table I that in spite of massive storage

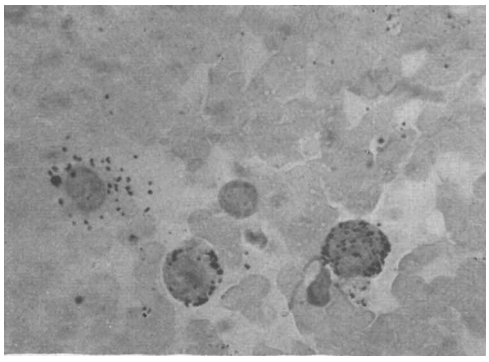

FIG. 3. Smear from a mediastinal lymph node 48 hr after intrapleural inj. of .3 mc of radioactive colloidal gold. Wright's stain × 600.

in the mediastinum of intrapleurally injected Au^{198}, the major portion of it escaped into the liver (an amount 12 times higher than that indicated in the table). The comparison of the results in two series suggested that Au^{198} distribution between the thoracic and the abdominal tissues can be modified by variations in the size of injected dose. Accordingly several groups each of 10 mice were injected with increasing doses (0.002 to 2.0 mc) of Au^{198} and sacrificed after 2 or 3 days for estimation of radioactivity in their tissues. The data of each group were used for comparison of the average radioactivity in the thoracic material (T) with that of the abdominal material (A), thus computing the ratio

$$\frac{T}{A} = \frac{\text{mediastinum + lungs + diaphragmatic and costal pleura}}{\text{spleen + 1/12th of the liver}}$$

as an index of radioactivity distribution between thoracic and abdominal tissues. It should be emphasized that this ratio is purely conventional, since only a fragment of the liver is included in the denominator, in order to avoid large differences between numerator and denominator. The results are presented in Table II.

It is shown that even a slight increase in the dose of Au^{198} was paralleled by the decrease of the $\frac{T}{A}$ ratio *i.e.* by the increase of the portion of injected dose deposited in the abdominal organs.

In an attempt to evaluate the prospects of intrapleural Au^{198} treatment of thoracic tumors (10 to 12 days old), the radioactivity per gram tissue of these tumors was compared with the radioactivity in the equal amount of mediastinal tissue (M) or liver (H) in the same mouse. The ratios $\frac{T}{M}$ and $\frac{T}{H}$ were calculated for 3 groups of 10 mice, each group bearing different tumors. The results varied considerably for various tumor strains: for Carcinoma $\frac{T}{M}$ was 0.61 and $\frac{T}{H}$ –0.61; for Melanoma $\frac{T}{M}$ –0.36 and $\frac{T}{H}$ –0.85, but for

TABLE II. Influence of Dose of Intrapleurally Injected Colloidal Au^{198} on Its Distribution Between Thoracic and Abdominal Tissues (Ratio $\frac{T}{A}$).

Dose inj., mc	Ratio $\frac{T}{A}$
.002	2.2
.005	1.5
.02	1.2
.05	1.17
.1	.98
.3	.93
.5	.53
2	.35

Samples of thoracic tissues comprised both lungs, mediastinal content, costal and diaphragmatic pleura; samples of abdominal tissue—fragment of 1/12 of liver and whole spleen. Each ratio is computed from averages in 10 mice, sacrificed 2 or 3 days after injection.

S-37 – $\frac{T}{M}$ was 2.37 and $\frac{T}{H}$ –2.95. Considering that intrapleurally injected colloidal Au^{198} is stored mainly in the mediastinum and in the liver, the portion of this isotope intercepted by tumor tissue is significant.

Discussion. We have used peritoneal and pleural exudates as growth media and peritoneal and pleural cavities as incubators to grow tumor cells of various strains as free cells and to study the effect of Au^{198} on these cells. We have found in both fluids the same essential signs of damage to the tumor cells by radiation: distortion of mitotic figures and loss of viability followed by disintegration of cell structure. These findings agree with the observations by Haddow(5) and Koller(6) on the close association of mass appearance of abnormal mitoses in tumor tissue with the loss of its viability, as the effect of radiation or of nitrogen mustards.

Free tumor cells were affected with small doses of Au^{198} (0.2 mc) more extensively in the pleural exudate than in the peritoneal fluid (as reported in(3)). This difference may be accounted for by the much smaller volume of pleural exudate and smaller dimensions of the pleural cavity. Obviously the concentration of the injected dose was higher in the pleural exudate and the ratio "amount of radiation per unit surface" was considerably higher than in the peritoneal cavity. In our current experiments we attempt to study separately, on quantitative lines, the direct (tumor cell degeneration) and the indirect (atrophy of the tumor bed) inhibiting effect of radiation on the tumor growth.

It is known(1,3) that colloidal Au^{198} is taken up from body fluids by macrophages, lymph nodes and lymphatics. These tissues are abundant in the mediastinum which therefore is the center of colloidal Au^{198} storage in the thoracic cavity. A lesser site of storage is found in the lungs. Since no marked changes of the distribution pattern were found in animals sacrificed at various intervals after injection, it is evident that the bulk of colloidal Au^{198} reached its ultimate destination within 3 days after its intrapleural administration.

Our results on the distribution of intrapleurally injected gold colloid agree closely with the data on the pattern of absorption of other types of particulate material (carmine, lampblack) from the pleural cavity of the dog(7,8).

Tables I and II show that reduction of the dose did not prevent the escape into the liver and spleen of the major portion of intrapleurally injected gold, but it changed the ratio between the thoracic and the abdominal storage of gold in favor of the former. Since the complications of treatment with colloidal Au^{198} result from extensive damage to the liver and the spleen by relatively high doses of radioactivity, it seems reasonable to avoid or to minimize this damage and to deposit, at the same time, sufficient amounts of Au^{198} in the thoracic cavity by using repeated low doses rather than a single massive dose.

Summary and conclusions. (1) Mice inoculated intrapleurally with suspensions of free tumor cells of various strains (S-37, S-180 in CFW mice, carcinoma in C3H mice, Melanoma in dba) were treated 4 or 5 days later, by intrapleural injections of colloidal Au^{198} (0.2 mc). After 3 or 4 days, specimens of pleural exudate were withdrawn, stained and compared, as for cellular composition, with those of untreated controls. (2) It was found that in all controls the pleural exudate contained numerous growing (mitoses) tumor cells. Their transfer (intrapleural or intraperitoneal) into normal mice resulted in renewed vigorous growth. However, in treated mice tumor cells were scanty, abnormally shaped, with few

and abnormal mitoses. They were non-viable as demonstrated by their failure to grow after transfer into new mice. Moreover, the exudate of treated mice contained numerous macrophages loaded with clumps of colloidal gold(3). In untreated controls the growth of free tumor cells in the exudate resulted, as a rule, in their implantation into mediastinum and later on in abundant growth of these implants. Only small nodules, often consisting of necrotic tissue, were found in treated animals. (3) Intrapleurally injected colloidal Au^{198} was largely stored in the thoracic cavity (in macrophages and in lymphatic tissue), mainly in the mediastinum, but nevertheless it reached the liver and the spleen in considerable amounts. However, it was found that ratio $\frac{T}{A}$ (index of Au^{198} distribution between thoracic and abdominal tissues) could be increased by the use of small doses for intrapleural injection of Au^{198}(5). Infiltration of thoracic tumors with intrapleurally injected Au^{198} was noted. (4) It is concluded that irradiation with intrapleurally injected Au^{198} induced degeneration and disintegration of free tumor cells in the pleural exudate. It is presumed that inhibition of tumor cell implantation was the result of radiation damage to implanted tumor cells (direct effect) and to the tumor bed (indirect effect on tumor growth).

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Pulmonary Edema in Oxygen Poisoning.* (19614)

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In the majority of investigations on pulmonary edema associated with oxygen poisoning, the criteria for the evaluation of the degree of the edema have consisted of gross and microscopic examination of the lungs or lung sections removed at autopsy(1-3). The gross examination has consisted of an approximate estimate of the size and texture of the extirpated lungs, the color, the presence of foam in the airways and the determination of whether or not fluid exudes from a cut surface. In order to supplement this usual pathological examination and to provide quantitative numerical data suitable for statistical evaluation, a method of lung analysis has been devised which will evaluate factors useful for determining the degree of pulmonary edema (4). This new method, which consists of a chemical and physical analysis of lungs extirpated at necropsy, has been used to determine quantitatively the degree of pulmonary edema following exposure to pure oxygen at ground level barometric pressure.

Methods. A group of 7 to 9 healthy guinea pigs of approximately the same size were selected and placed on the same dietary regime in the same laboratory for a period of 2 to 3 weeks. At the end of this period, 2 animals (controls) were killed and their lungs ana-

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