tality in mice inoculated i.p with S-37 and treated successively with NCG and RCG. This phenomenon was obtained only by combination of the following 4 conditions: (1) tumor cell multiplication in the peritoneal fluid; no influence of pre-treatment with NCG was noted in RCG treated normal mice or mice with subcutaneous tumors; (2) strain of tumor—no influence of NCG on the effect of RCG in mice inoculated i.p with malignant lymphoma; (3) strain of mice—this influence was particularly marked in dba mice, less in CFW; (4) time factor—administration of NCG before and not after RCG.

Since it has been found that RCG condensed in clumps and stored in macrophages acted on tumor cells more slowly than the same dose of the original RCG preparation (perhaps, because it has to be disintegrated and resuspended in the fluid in order to exert an effect on cells), the attenuating influence of NCG pre-treatment on RCG effect may be attributed to a shift of a large proportion of injected RCG from the fluid into numerous macrophages. This interpretation was tested in a special series of experiments which will be reported separately.

Summary and conclusions. (1) Sarcoma 37 and malignant lymphoma were cultured as free cells in the peritoneal fluid of mice by serial transfers of these cells in the peritoneal fluid. Requisite number of tumor cells (100,000 or 1,000,000) were inoculated i.p into several groups of mice (S-37 in CFW or dba strain; lymphoma in dba mice only) and treated, after 2 to 14 days, with various doses (0.05 to 0.3 mc) of radioactive colloidal gold, either intraperitoneally or intravenously. In several series of experiments the mice were pre-treated with inactive (decayed) colloidal gold (0.1 or 0.2 cc) before the treatment with active gold. Control experiments investigated the growth characteristics of S-37 and lymphoma cells in untreated mice, the effect of the treatment with active or inactive gold in normal mice and the effect on tumor cells of radioactive gold stored in macrophages. (2) The experiments have outlined the role of the following factors in production of a radiotherapeutic effect of the radioactive colloidal gold on free tumor cells growing in the peritoneal fluid; (a) strain of the tumor; (b) strain of mice; (c) size of the inoculum; (d) interval between the inoculation and the treatment; (e) route of treatment; (f) dose of radioactivity; (g) pre-treatment with inactive gold. The effect was estimated by checking on the 6th day after the treatment, the percentage of surviving mice and the presence of tumor cells in the peritoneal fluid of treated mice and of controls. (3) The results were interpreted in the light of the data on growth characteristics of tumor cells and macrophagic reaction in the peritoneal fluid.

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Radioactive Colloidal Gold in Macrophages and Serous Exudate in Peritoneal Fluid of Sarcoma Bearing Mouse.* (18530)

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Studies by Hahn, Sheppard and their associates(1) in humans and dogs, by Barrow and associates(2) in rabbits and by Sheppard and Furth(3) in mice showed that intravenously injected gold colloids are stored mainly in the macrophages (reticulo-endothelial element) of the spleen and the liver.

* This work was carried out under Contract At-(40-1)269 with the Division of Biology and Medicine, U. S. Atomic Energy Commission.

1. 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.

2. Barrow, J., Tullis, J. L., and Chambers, F. F., *Nav. Med. Rev. Inst.*, Bethesda, Md., Prog. AM007039, rep. No. 24, July 25, 1949.

This intracellular storage is recognized also by microscopic examination of tissue section of these organs: the injected ultramicroscopic colloidal gold particles appear condensed in large clumps inside the liver and spleen macrophages (Barrow and associates)(2). This phenomenon of intracellular condensation of colloidal gold (either radioactive or non-active through decay) was studied recently in free macrophages which appear in large number in the peritoneal fluid of the mouse inoculated intraperitoneally with sarcoma cells and treated with radioactive or inactive gold (Goldie and Hahn)(4). In no instance were the gold clumps found in other cells of the peritoneal fluid, *i.e.*, polymorphonuclears, lymphocytes and tumor cells. Thus, the intraperitoneally injected radioactive colloidal gold is evidently distributed between serous exudate (where the introduced ultramicroscopic colloidal particles are resuspended) and macrophages (where they are condensed and stored). Recently(5) it has been found that a preparative intraperitoneal injection of inactive (decayed) colloidal gold decreased the radiotherapeutic effect of radioactive colloidal gold on free sarcoma cells in CFW mice and even more in dba mice. It appeared therefore that distribution studies of radioactive colloidal gold in the peritoneal fluid of the mouse may contribute to the understanding of the therapeutic effect of this radio-isotope on free tumor cells growing in the peritoneal fluid.

Material and methods. (A) *Radioisotope preparation.* The sol of radioactive metallic gold used was that introduced by Hahn and Sheppard(6) for treatment of malignant tumors and of leukemias.† This preparation will be referred to below as RCG. A standard dose of 0.1 mc was used throughout in most experiments: this dose destroyed all peritoneal sarcoma cells only in a minority of CFW mice, but damaged a considerable number of tumor cells in most mice(4,5). The dose of 0.3 mc was used in 2 experiments for purpose of comparison. The preparation was injected either intraperitoneally or intravenously. The RCG preparation was used when it was completely decayed by aging as inactive colloidal gold (NCG) (containing approximately 3 mg Au per ml) in doses of 0.1 or 0.2 cc injected always intraperitoneally. (B) *Biological test material.* Two different strains of mice, CFW and dba, were used for comparative study of RCG distribution: both strains were susceptible to grow free Sarcoma 37 cells in their peritoneal fluid and to produce an abundant macrophagic reaction during this growth and after radioactive treatment. The technic of culture of free tumor cells by serial transfers in the peritoneal fluid and the use of this technic for screening the effect of radioactive isotopes on tumor cells were described elsewhere(4). Standard dose of 100,000 sarcoma cells was used throughout for intraperitoneal inoculation. (C) *Technic of experiments.* In each experiment, groups of CFW and dba mice were used on the 5th or 6th day after inoculation when they had accumulated a large amount of peritoneal fluid containing numerous tumor cells and macrophages. Each experiment included 4 groups of 10 mice each: (1) CFW mice treated i.v or i.p with RCG without preparation with NCG (CFR group); (2) CFW mice prepared intraperitoneally with NCG before (i.p or i.v) injection of RCG (CFN mice); (3) dba mice treated with RCG alone (dbR group); (4) dba mice prepared by NCG and treated by RCG (dbN group). The experiments varied according to the route of RCG administration, intraperitoneal or intravenous. For checking the results, small amounts (about 0.05 cc) of peritoneal fluid were withdrawn from injected mice with capillary glass pipettes. Each specimen was immediately diluted with 5% sodium oxalate solution and centrifuged in order to separate the cellular content of the specimens from its diluted

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

4. Goldie, H., and Hahn, P. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 638.

5. Goldie, H., Watkins, F. B., Powell, C., and Hahn, P. F., to be published.

6. Hahn, P. F., and Sheppard, C. W., *Southern Med. J.*, 1946, v39, 558.

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

serous exudate remaining in the supernatant. The two fractions were separated, each placed in a tin cup and dried. The amount of radioactivity in each specimen was estimated by a Scaling circuit in conjunction with a thin mica end-window Geiger-Muller tube. It might be presumed that centrifugation will precipitate not only cellular elements of the peritoneal fluid but also a significant number of colloidal gold particles suspended in a protein solution. In order to test this hypothesis we have added test doses of RCG (0.3 mc) to a 10% solution of cell free exudate from the peritoneal fluid. This solution was centrifuged and the obtained fraction was tested for radioactivity. Specimens of peritoneal fluid were withdrawn from injected mice according to a standard schedule after ½ hour, 1 hour, 3, 5, 7, 10 and 24 hours. In some experiments sampling was done only after 24 and 48 hours, since frequently repeated withdrawal of the fluid from the same mouse limited its usefulness. In at least one experiment on each type of treatment (i.p or i.v) specimens of peritoneal fluid taken from treated mice were examined partly for radioactivity and partly for integrity of tumor cells and for condensation of RCG in macrophages.

The aim of the experiment was to determine the relative portion of radioactivity in each of 2 fractions of centrifuged fluid, *i.e.*, to calculate the ratio P/S (radioactivity of precipitate/radioactivity of supernatant) for each specimen, independently from the absolute radioactivity content in this specimen. For this reason, in all collected specimens from the same series of mice (same experiment), the radioactivity was tested on the same day and approximately on the same hour: absolute values of radioactivity were decreased in all specimens by progressive decay of the radioisotope, but the ratios remained unchanged.

Results. Centrifugation precipitated from RG suspension in diluted serous exudate only an insignificant amount of radioactive material which could not influence our results showing nearly always a ratio much higher than 1.0.

TABLE I. Distribution of Radioactivity Between the Precipitate and the Supernatant (Ratio P/S) in the Peritoneal Fluid of the Mouse 24 and 48 Hours After Intraperitoneal or Intravenous Injection of RCG.

Groups of mice*	Intrap. after 24 hr	Intrap. after 48 hr	Intrav. after 24 hr	Intrav. after 48 hr	Remarks
			Dose of 0.1 mc		
CFR	12.8/1	21.8/1	1.5/1	3.5/1	Each ratio
CFN	26/1	28/1	4.5/1	10.5/1	represents
dbR	24/1	33/1	3.5/1	7.5/1	avg for
dbN	40/1	47/1	18.5/1	35.0/1	10 mice
			Dose of 0.3 mc		
CFR	52/1	71/1			
CFN	60/1	68/1			for
dbR	88/1	130/1			5 mice
dbN	182/1	212/1			

* See technic of exp.

The ratio P/S for specimens of peritoneal fluid withdrawn 24 and 48 hours after i.p. or i.v injection of RCG in different groups of mice is shown in Table I.

The results of Table I were partly interpolated by studying for similar experimental groups of mice, the changes of P/S ratio in the peritoneal fluid at various intervals during the first 24 hours after injection of RCG. The data obtained were plotted in graphs (Fig. 1 and 2).

Microscopic examination of peritoneal fluid repeated at hourly intervals revealed that both after intraperitoneal and intravenous injection of RCG, the damage to tumor cells (swelling, clumping of chromosomes, abnormal mitoses) appeared regularly after 4 to 5 hours and increased progressively in proportion to the dose injected, showing also individual variations in various mice.

Discussion. Dilution and centrifugation of the colloidal preparation used in these experiments did not result in precipitation of any significant amount of radioactive material. Thus, the relatively high content of radioactivity in the precipitate of the peritoneal fluid should be attributed to absorption (or adsorption) of the colloid by cellular elements of the fluid. It has been found that RCG is selectively absorbed and stored by macrophages of the peritoneal fluid in amounts so large as to be visible with low power microscope. Any adsorption of ultramicroscopic colloidal particles on the surface of other fluid

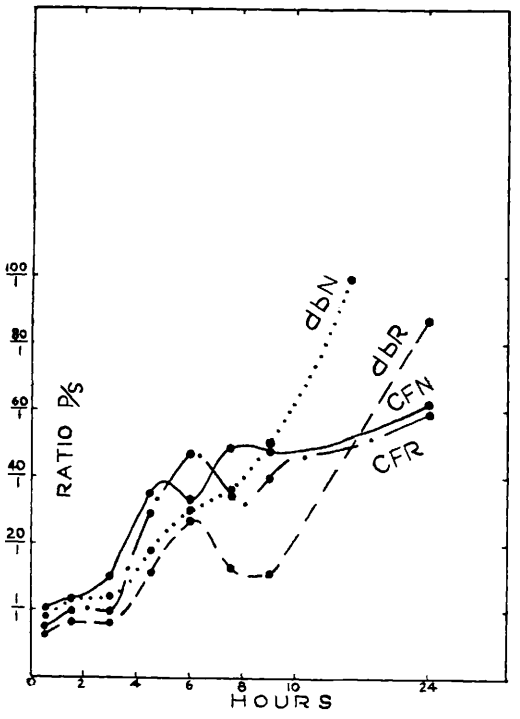

FIG. 1.

Distribution of radioactivity between precipitate and supernatant (ratio P/S) of the peritoneal fluid at various intervals after intraper. inj. of 0.1 radioactive colloidal gold into the mouse. *CFR and dbR*: groups of CFW and dba mice treated with radioactive gold only. *CFN and dbN*: groups of CFW and dba mice prepared with inactive gold before the treatment with radioactive gold.

cells appears negligible by comparison with copious storage of large gold clumps in macrophages. Thus, for all practical purposes, the P/S ratio of the peritoneal fluid (or of the blood) probably reflects the distribution of radioactive material between macrophages and serous exudate.

It should be emphasized that RCG suspended in the form of ultramicroscopic particles in the peritoneal exudate diffuses rapidly into the blood and vice versa, while RCG stored in macrophages remains localized with these cells in the peritoneal cavity. Thus, the increase of P/S in the peritoneal fluid may reflect an increased activity or/and number of macrophages absorbing the colloid from the serous exudate or a decreased diffusion of RCG from this serous exudate into the blood, or both phenomena simultaneously. On the other hand, a decrease of P/S may be attributed to a decrease in activity of macrophages or to an increase in RCG diffusion from the peritoneal fluid into the blood or to both phenomena.

It is obvious that intraperitoneally injected RCG disappears steadily and at a progressively increasing rate from the serous exudate through absorption by macrophages and simultaneously through diffusion into the blood. These two parallel phenomena account sufficiently for steady increase of P/S in all groups injected intraperitoneally (Fig. 1). On the contrary, the results of intravenous injection of RCG were determined by the balance of two opposite phenomena: RCG supply from the blood to the fluid and absorption of supplied radioactive material by macrophages. For dba mice (dbN and dbR) (Fig. 2) this balance was, during the first 4 hours, in favor of absorption by macrophages as shown by the sharp rise of P/S. This initial rise was followed by a temporary fall of P/S, reflecting presumably a transient de-

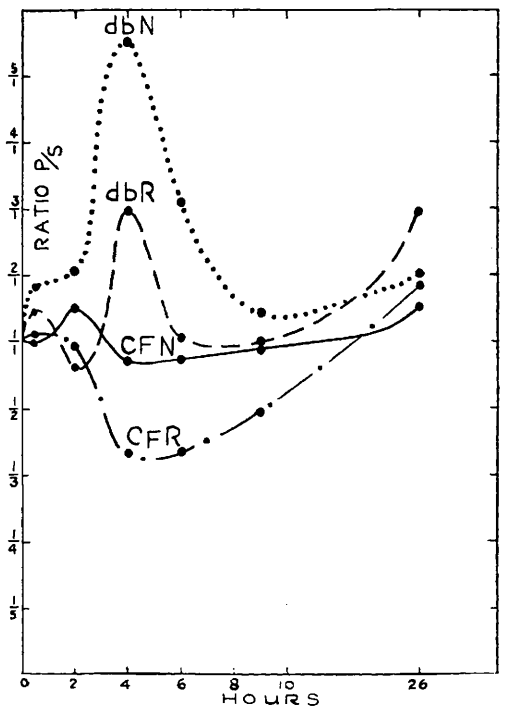

FIG. 2.

Distribution of radioactivity between precipitate and supernatant (ratio P/S) of peritoneal fluid at various intervals after intrav. inj. of 0.1 radioactive colloidal gold into the mouse. See Fig. 1.

crease in the activity of macrophages. For CFW mice (CFN and CFR) the absorption was evidently balanced by diffusion during the first 24 hours. Between 24 and 48 hours the decreased supply of RCG from the blood and the increased activity of macrophages in the peritoneal fluid raised P/S for all groups of mice to higher level (Table I).

Table I and Fig. 1 and 2 indicate clearly that higher levels of P/S were induced consistently in mice by the following factors: (1) high dose of injected RCG; (2) strain characteristics of dba mice and (3) preparation by NCG. It is easier to explain the effect of these factors by stimulation of macrophages or increase in their number than by increased diffusion of RCG into the blood. Moreover, microscopic examination showed higher percentage of macrophages in dba mice than in CFW and in mice prepared with NCG than in unprepared. Whatever may be the interpretation of effect of these factors, their role indicates the importance of various biological conditions rather than purely physical conditions in distribution of RCG in the peritoneal fluid. This interpretation is in agreement with the data of H. P. Smith(7) on the distribution of colloidal dyes between blood and tissues.

Summary and conclusions. (1) CFW and dba mice were inoculated intraperitoneally with Sarcoma 37 cells from serial intraperitoneal transfers. After formation of copious peritoneal exudate containing tumor cells and macrophages, these mice received intraperitoneally or intravenously 0.1 mc (in 2 series 0.3 mc) of radioactive colloidal gold. At various intervals (½ hour to 48 hours) after injection, specimens of peritoneal fluid were withdrawn from these mice and centrifuged. Radioactivity of precipitate (P) and of supernatant (S) in each specimen was determined by Geiger counter. (2) Ratio P/S was accepted as index of radioactivity distribution between macrophages and serous exudate of the peritoneal fluid. Data illustrating trends in changes of P/S in various groups of mice were plotted in graphs and discussed. These data were interpreted as the combined effect of two parallel phenomena responsible for disappearance of radioactive colloid from the peritoneal serous exudate; (a) absorption and storage of colloid particles into macrophages; (b) their diffusion into circulating blood; (3) The ratio P/S in various specimens of peritoneal fluid from the same mouse remained consistently on higher levels after (a) the use of higher doses of radioactive colloidal gold; (b) the use of dba mice and (c) the preparation with inactive (decayed) colloidal gold before treatment with radioactive gold. The effect of these factors is attributed to the increase in the number and in the activity of macrophages.

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Pneumoperitoneum in Preoperative Preparation for Total Gastrectomy. (18531)

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Inasmuch as pneumoperitoneum, employed in the therapy of human tuberculosis, produces a rise of the diaphragm and a visceroptosis, it seemed logical that pneumoperitoneum might be of value in making the stomach and the lower segment of the esophagus more accessible for the procedure of total gastrectomy, if it were employed prior to the exploratory laparotomy. However, it was not certain that this alteration of organ position would persist after the peritoneum had been entered. Experimental studies were thus undertaken.

Eight adult mongrel dogs were employed in this study. Four dogs were employed as