ance is caused in rats by the intravenous injection of as little as 25 mg per kg every other day for 4 weeks, although blood sugar levels remain normal while the rats are on a normal diet(5).

Inhibition of bartonella multiplication is effected physiologically by the presence of the intact spleen, and artificially by arsenicals(6), by aureomycin and terramycin(7), and by alloxan. Arsenicals and alloxan are known to inactivate thiol groups. In spite of extensive study, the mechanism by which the spleen inhibits the growth of *Haemobartonella muris* has not been discovered(8).

In future work, the possibility that alloxan may have chemotherapeutic activity in infections other than bartonellosis will be explored, and a variety of closely related compounds will be tested. The observation reported here suggests the possibility that compounds formed naturally in the course of nucleic acid metabolism may be chemotherapeutically effective.

Conclusions. Alloxan in relatively low and presumably nondiabetogenic dosages was found to be effective in preventing the development of murine bartonellosis, which invariably follows splenectomy in untreated carrier rats. The activity of this agent probably depends on its reaction with SH groups.

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Factors Influencing Effect of Radioactive Colloidal Gold on Free Tumor Cells in Peritoneal Fluid.* (18529)

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In a previous note(1) a method has been outlined for screening quantitatively the effect of radioactive isotopes on tumor cells in mice: cells of Sarcoma 37 were grown serially as free cells in the peritoneal fluid of CFW mice and tested for their resistance to various doses of a radioactive isotope injected intraperitoneally. Using this technic in experiments with radioactive colloidal gold(2) it has been found that sarcoma cells grown in the peritoneal fluid of a CFW mouse 2 to 5 days after inoculation of 10,000 cells, disintegrated and completely disappeared within 48 hours after i.p injection of 0.3 to 0.42 mc of radioactive colloidal gold. In a new series of experiments with several groups of mice, we have analysed this radiotherapeutic effect† by varying in each group of mice one of the conditions of the experiment.

Material and methods. Two strains of tumors were used in this work: (1) Sarcoma 37 was grown in 2 strains of mice—CFW and dba; (2) malignant lymphoma (isolated originally from the thymus of a dba mouse) in dba mice. Thus, dba mice were used for carrying 2 different tumors. Both tumors were propagated in serial transfers in the peritoneal fluid. The technic of inoculation with a requisite number of tumor cells and the method for estimating the therapeutic results

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

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

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TABLE I. Effect of Radioactive Colloidal Gold on Free Tumor Cells in the Peritoneal Fluid of the Mouse Under Various Experimental Conditions.

Dose of RCG, mc	Route	Strain of tumor	Strain of mice	Cells inoculated, No.	Days between inoculation and treatment	Inoculated mice surviving after treatment with: RCG alone, No.	RCG and NCG, No.	Controls, No.	Inoculated mice showing disappearance of tumor cells in perit. fluid after treatment with: RCG alone, No.	RCG and NCG, No.	Controls, No.
.3	I.p.	S-37	CFW	100000 to	5 or 6	63/2	48/17.	12/53	63/2	41/24	0/65
	I.v.			1000000		36/4	33/7	12/53	32/8	33/7	
.1						38/27	32/33	12/53	21/34	24/31	0/40
						14/26	18/22	12/53	12/28	16/24	
.05						14/26	12/28	8/32	12/28	10/30	1/39
						5/5	4/6	1/9	2/8	1/9	
.3			dba			23/32	13/42	7/23	21/34	13/42	3/27
						11/29	8/22	7/23	9/31	16/34	
.1						15/35	10/40	5/25	15/35	9/41	2/38
						18/32	22/28	5/25	16/24	12/28	
.05						8/12	8/12	6/14	2/18	2/18	1/19
						9/11	6/14	6/14	3/17	1/19	
.3		Malignant lymphoma		100000	7	20/0	18/2	4/16	20/0	18/2	2/18
						20/0	20/0		19/1	20/1	
.1						18/2	16/4	4/16	15/5	13/7	1/19
						13/7	15/5		13/7	12/8	
.05						10/10	8/12	5/15	10/10	8/12	
						7/13	8/12		7/13	7/13	
.3	I.p.				14	8/12	10/10	2/18	8/12	9/11	0/20
.1						5/15	7/13		2/18	7/13	
.05						2/18	2/18		1/19	2/18	
.3				1000000	7	5/15	4/16	3/17	4/16	2/18	0/20
.1						4/16	5/15	3/17	2/18	1/19	
.05						2/18	1/19	3/17	0/20	1/19	
.25 RCG stored in macrophages		S-37	CFW	10000	5	9/1		4/6	4/6		0/10
.1 as above						5/5		3/7	2/8		0/10

All results were checked 5 or 6 days after treatment.e colloidal gold.
RCG = Radioactive colloidal gold. NCG = Inactiv

were described elsewhere(1,3). Since it was found previously(2) that intraperitoneally injected colloidal gold (either radioactive or inactive) is condensed and stored by numerous macrophages of the peritoneal fluid, it was considered necessary to study the influence of the storage of radioactive gold in macrophages on tumor cells. For this purpose, the amount of radioactivity in a suspension of macrophages containing gold (separated from serous fluid by centrifugation) was determined in a Geiger counter (using as standard for comparison an aliquot of the original preparation), and doses of suspension containing 0.1 or 0.25 mc were injected i.p into 20 mice previously inoculated with S-37 cells. Furthermore, it seemed reasonable to assume that the preparative intraperitoneal injection of a massive dose of inactive (decayed) colloidal gold (designated below as NCG) would modify the conditions for distribution of subsequently injected radioactive colloidal gold (designated below as RCG) in the peritoneal fluid, and therefore, its effect on peritoneal free tumor cells. For this reason, doses of 0.1 or 0.2 cc of inactive (decayed) colloidal gold (NCG) were injected i.p into inoculated mice 1 or 2 days

before their treatment with RCG, while other groups of similarly inoculated mice were treated with RCG alone, and controls remained untreated.

Results. Table I given below reviews results in experimental animals, *i.e.*, bearing tumor cells in the peritoneal fluid and treated with RCG. Only those findings are recorded in the table where the change in a condition of the experiment resulted in the change of the result. The conditions are indicated in the left side column; the therapeutic result is estimated by survival of treated mice (as compared with controls) and by disappearance of tumor cells from their peritoneal fluid. For convenience of comparison these results are shown in the same column for 3 series of mice, treated with RCG only, with NCG and RCG successively and untreated controls.

The data of Table I revealed the following variations in the radiotherapeutic effect on tumors: (1) The intensity of the effect was distinctly proportionate to the dose of injected RCG. The administration of 0.3 mc was responsible for disappearance of tumor cells from the peritoneal fluid and for extension of the life span in a significant proportion of treated mice (nearly all CFW with S-37 and dba inoculated with 100,000 lymphoma cells), while the dose of 0.1 mc produced this effect only in a relatively small number of mice of both strains and the dose of 0.05 mc only in a few mice with S-37. (2) Intravenous administration of RCG was less efficient than intraperitoneal treatment, but this difference was not striking. This finding is in agreement with our repeated observation that after treatment with 0.3 mc by either route the first marked signs of tumor cell disintegration (swelling, distorted mitoses) in the peritoneal fluid appeared consistently at the same time, *i.e.*, after about 4 hours. (3) For the same strain of tumor S-37, and the same procedure of inoculation and treatment, the effect in CFW mice was strikingly better than in dba mice. However, it was noted that dba mice showed higher percentage of subcutaneous tumors at the site of inoculation. (4) The effect on sarcoma cells was independent of the size of the inoculum within wide limits (10,000‡ to 1,000,000 cells) and to a certain extent (2 to 5 days) from the length of the interval between inoculation and treatment. However both factors modified significantly the effect on lymphoma, when the inoculum varied from 100,000 to 1,000,000 cells and the interval from 7 to 14 days. (5) The pre-treatment with NCG decreased significantly the effect of therapeutic doses (0.3 and 0.1 mc) of RCG on sarcoma in CFW mice and even more strikingly in dba mice, but not on lymphoma in dba mice. (6) The action of RCG stored in macrophages on sarcoma cells in peritoneal fluid appeared delayed.

Discussion. The intrinsic tendency of Sarcoma 37 cells to regression after initial multiplication in the peritoneal fluid(3) was a factor limiting the number and the vitality of tumor cells and thus contributing to the similar effect of the radiation, while this factor was absent in the treatment of free lymphoma cells in the peritoneal fluid(3). Their unchecked proliferation accounts for decrease of their response to the radiotherapeutic effect in conditions increasing cell multiplication (large size of the inoculum, long interval between inoculation and treatment), while for S-37 in the same conditions the increased cell multiplication is automatically countered by spontaneous cell regression. Similar differences in growth potency of tumors may account for striking differences in the results of radiotherapy in various cases of human tumors.

Our control experiments indicated higher invasiveness of free S-37 cells for tissues of dba mice: this difference may account for lower level of the radiotherapeutic effect of RCG on free S-37 cells in dba mice. Even for different mice of the same strain, variations in this effect may result from different invasiveness of the same tumor cells for different mice. Thus, the variations of the radiotherapeutic effect are manifestations of various combination of the action of radiation with biological factors inherent in tumor strain, mouse strains or individual mice.

A similar combination of factors was obviously responsible for the increase of mor-

‡ The results with 10,000 and 50,000 sarcoma cells were reported in a previous paper(1).

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.

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