fusion of 5HT through the heart *in vitro* causes the release of NE (unpublished work of Nash, Costa and Brodie).

The data presented here indicate that the emetic action of 5HTP is mediated through release of catecholamines; thus the emetic action is abolished by pretreatment with reserpine and is considerably reduced by pretreatment with α-MMT or α-methyl DOPA.

It is possible that the locus of action of NE is the hypothalamus, for Hess(9) caused emesis in cats after electrical stimulation of this brain area which is rich in NE. Alternatively, NE might also act at the area postrema for Borison(10) has demonstrated that ablation of this area abolishes the emetic action of catecholamines. However, other loci may also be involved in the action of 5HTP.

Summary. 1. 5-Hydroxytryptophan (5-HTP) given parenterally to cats exerts emesis. This effect is enhanced by various monoamine oxidase inhibitors and blocked by pentobarbital. 2. The emetic effect of 5HTP is antagonized by chlorpromazine and by compounds that deplete brain catecholamines (reserprine, α-methyl DOPA and α-methyl-m-tyrosine) suggesting that 5HTP acts through release of norepinephrine.

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Action of Isoimmune Serum on Peritoneal Cells *in vitro.** (29261)

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It has recently been shown that peritoneal macrophages from mice are capable of phagocytizing tumor cells *in vitro* in the presence of isoimmune serum(1,2). The isoimmune serum and peritoneal macrophages were invariably obtained from mice of the same inbred strain and the immune serum was directed against the isoantigens of the tumor cells. The isoantibody would therefore be expected to combine with the antigens on the surface of the tumor cells but not with those of the peritoneal cells. In this report experiments are described in which the isoimmune serum was directed against the peritoneal cells, and the properties of these cells were examined in both cytotoxic(3) and phagocytic tests(1,2). It was found that peritoneal macrophages when placed in tissue culture were characterized by a high resistance to the cytotoxic activity of isoantibody and complement under conditions where peritoneal lymphocytes, eosinophils, neutrophils and mast cells were readily lysed. Furthermore, in the presence of immune serum directed against the isoantigens of both the macrophages and the tumor cells, peritoneal macro-

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phages were found capable of phagocytizing isogenic ascites sarcoma or leukemia cells.

Materials and methods. The following strains of mice, bred in our colony, were used: C57BL/6, BALB/c, A and C3H/An, and the (C3H/An × I)F_1 hybrid.

The tumors used in the phagocytic test were: a BALB/c ascites sarcoma, Meth A (4), a C57BL ascites leukemia, EL4(5), and a C57BL/6 radiation-induced leukemia, ERLD(6).

Isoimmune sera were prepared in mice by 4 or more subcutaneous and intraperitoneal (i.p.) inoculations of histoincompatible tumor cells over a period of 2 to 3 months. Pooled guinea pig serum, the complement source, was obtained by heart bleeding. The sera were stored at —70°C.

Unless otherwise stated, the population of peritoneal cells was collected 3 or 4 days after the mice had received an i.p. injection of 1 ml of a 3% starch suspension in normal saline (Starch Hydrolyzed, Connaught Medical Research Laboratories, Toronto, Canada). The cells were obtained by rinsing the peritoneal cavity of each mouse with 2 to 3 ml of "199" (Mixture 199, Microbiological Associates, Bethesda, Md.) containing 5 units of heparin, 100 units of penicillin, and 0.1 mg of streptomycin per ml. The cells were kept in an ice bath after collection and were used within 30 minutes.

Cytotoxic tests were performed either with suspensions of freshly collected peritoneal cells or with tissue culture preparations following attachment of these cells to a glass surface. 1) The cytotoxic test described by Gorer and O'Gorman(3) was used in a modified form. To serial doubling solutions of isoimmune serum prepared in 0.1 ml of 199 in small test tubes in an ice bath were added 0.1 ml of a *suspension* of peritoneal cells (1 × 10^6 cells/ml) and 0.1 ml of the complement source (a ⅓ dilution of guinea pig serum). The contents were mixed and the tubes were incubated in a water bath for 45 minutes at 37°C. Finally 0.1 ml of a 0.16% trypan blue solution(7) was added, and the viability of the peritoneal cells (unstained by trypan blue) was determined microscopically. 2) *Tissue cultures* of peritoneal cells were prepared by placing 1 ml of a suspension of a counted number of peritoneal cells into Leighton tubes each containing a glass cover slip. After incubating for 2 hours at 37°C, the indicated amounts of isoimmune serum and undiluted guinea pig serum were added, and the tubes were incubated for an additional 1, 2, 4, or 24 hours. The cover slips were removed, and the cells were stained by the May-Grunwald-Giemsa method. An atmosphere of 5% CO_2 in air was introduced into each Leighton tube before incubation.

TABLE I. Cytotoxic Titrations of BALB/c Peritoneal Cells in Suspension with C57BL/6 Anti Meth A (BALB/c) Serum.

Dilution of C57BL/6 anti Meth A (BALB/c) serum:	BALB/c peritoneal cells from Normal mice	Starch-injected* mice
	% cells stained by trypan blue	
1/10-1/320	>95	>95
1/640	60	"
1/1280	22	63
1/2560	<10	20
1/5120	"	<10
No antiserum	"	"

Complement source: guinea pig serum diluted 1/3 with mixture 199.

* Donor mice received 1 ml of 3% starch i.p. 3 days before collection of peritoneal cells.

The phagocytic test has been described in detail(1,2). Monolayers of peritoneal macrophages were prepared by adding 3 × 10^6 peritoneal cells in 1 ml of medium to Leighton tubes each containing a glass cover slip. After 2 hours' incubation, during which the macrophages adhered to the cover slips, the supernatant fluid was removed and replaced with 1 ml of fresh medium containing 10% normal mouse serum. The tubes were then incubated overnight. The supernatant fluid was again removed and replaced with 1 ml of medium containing isoimmune serum, 100,000 tumor cells and 10% normal mouse serum. Following an additional 1½ hours' incubation the cover slips were removed, and the cells were stained.

Results. Effect of isoantibody and complement on peritoneal cells in vitro. When peritoneal cells were maintained in *suspension* after collection, the entire population was fully susceptible to the cytotoxic activity of isoimmune serum and complement *in vitro* (Table I). Peritoneal cells from both nor-

mal and starch-injected mice were lysed to an essentially similar degree at the same antiserum dilution.

In *tissue culture* preparations made by adding 3×10^6 peritoneal cells in 1 ml of medium to Leighton tubes, the macrophages spread out and formed angular cytoplasmic processes. In this circumstance macrophages from both normal and starch-injected mice were resistant to immune lysis after addition of 0.1 ml each of isoimmune serum and guinea pig serum. Morphologically, the macrophages showed no abnormalities other than a slight retraction of cytoplasmic processes. By contrast, the peritoneal lymphocytes were lysed when incubated under similar circumstances with isoimmune serum and guinea pig serum. After incubation for one hour lymphocytes had dense, shrunken nuclei and irregular, indistinct cytoplasm; many were phagocytized by macrophages (Fig. 1). Following 4 hours' incubation only fragments of lymphocytes remained (Fig. 2). Neutrophils, eosinophils and mast cells were also completely lysed. After 24 hours' incubation, the cultures consisted entirely of macrophages. In control preparations in which peritoneal cells were incubated with either guinea pig serum or isoimmune serum alone, no lysis of cells was noted. A greater percentage of peritoneal cells invariably adhered to the cover slip in the presence of isoimmune serum than when no immune serum was added (Fig. 3).

The resistance of macrophages to cytolysis was further investigated in experiments in which different quantities of isoimmune serum and guinea pig serum were added to 3×10^6, 7.5×10^5, or 3×10^5 peritoneal cells in Leighton tubes. The results with BALB/c peritoneal cells from starch-injected mice and C57BL/6 anti Meth A (BALB/c) serum are summarized in Table II. Peritoneal macrophages were lysed in the presence of isoimmune serum when the proportion of guinea pig serum was increased with respect to the number of peritoneal cells. This was accomplished either by increasing the amount of guinea pig serum added or by decreasing the number of peritoneal cells present. The other cells of the peritoneal population were invariably lysed in the presence of isoimmune serum and guinea pig serum, except in the instance when 0.3 ml of isoimmune serum and 0.1 ml of guinea pig serum were added to

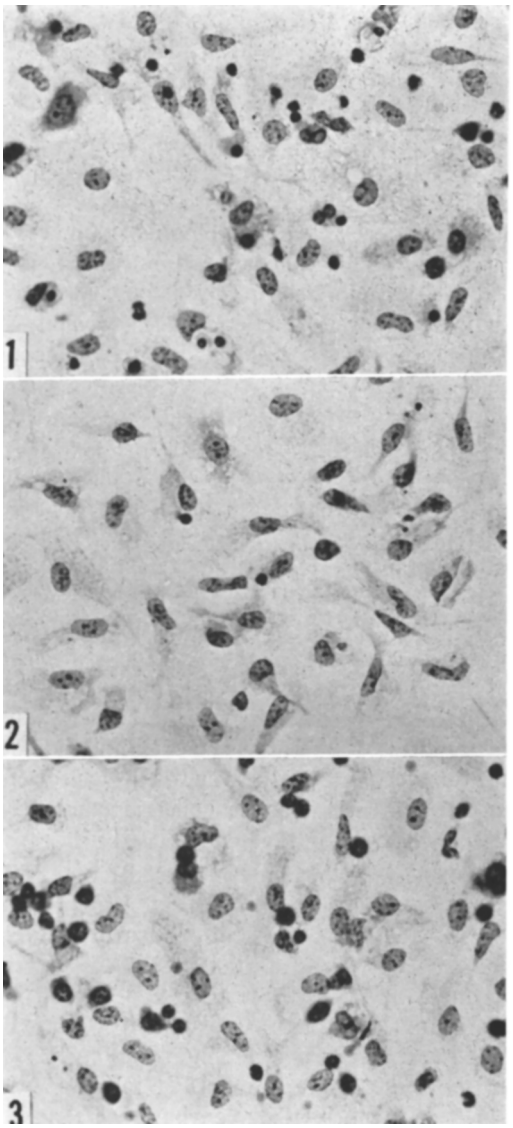

FIG. 1. BALB/c peritoneal cells in tissue culture with 10% C57BL/6 anti Meth A (BALB/c) serum and 10% guinea pig serum (complement source). After incubation for one hour lymphocytes are lysed while macrophages remain morphologically unaltered. × 394.

FIG. 2. After 4 hours incubation with isoantiserum and complement, lymphocytes have almost entirely disappeared. × 394.

FIG. 3. BALB/c peritoneal cells incubated for 4 hours with isoantiserum but without guinea pig serum. No lysis of macrophages or lymphocytes is noted. × 394.

TABLE II. Effects of C57BL/6 Anti Meth A (BALB/c) Serum and Complement on Tissue Culture Preparations of Peritoneal Cells from Starch-Injected* BALB/c Mice.

No. of BALB/c peritoneal cells in tissue culture	Amount of C57BL/6 anti Meth A (BALB/c) serum added	Amount of guinea pig serum added (complement source)	Degree† of lysis of	
			Macrophages	Lymphocytes and granulocytes
	.0 ml	.4 ml	0	0
	.3	.0	0	0
	.01	.1	0	3+
3×10^6	.1	.1	0	4+
	.1	.2	1+	4+
	.1	.4	3+	4+
	.3	.1	0	2+
7.5×10^5	.1	.1	2+	4+
	.1	.2	3+	4+
	.0	.4	0	0
	.4	.0	0	0
3×10^5	.1	.1	3+	4+
	.1	.2	4+	4+
	.1	.4	4+	4+

* Donor mice received 1 ml of 3% starch i.p. 3 days before collection of peritoneal cells.
† Degree of lysis: 0 = no lysis, 1+ = occasional lysed cell, 2+ = many lysed cells, 3+ = nearly complete lysis, 4+ = complete lysis. Incubation period—2 hr.

TABLE III. Phagocytosis of Tumor Cells by Isogenic or Allogeneic Peritoneal Macrophages in the Presence of Isoimmune Serum.

Isoimmune serum	Tumor cells for phagocytic test	Donor strain of peritoneal macrophages	Dilution of isoimmune serum				
			1/10	1/100	1/1000	1/10,000	No immune serum
			Phagocytized tumor cells in 40 high power fields				
(C3H/An x I)F_1	EL4	(C3H/An x I)F_1	423	298	253	173	0
anti EL4	(C57BL)	C57BL/6	124	59	163	62	0
C57BL/6 anti	Meth A	C57BL/6	360	237	171	22	0
Meth A	(BALB/c)	BALB/c	222	179	169	28	1
A anti ERLD	ERLD	A	321	245	184	183	1
	(C57BL/6)	C57BL/6	239	158	333	150	1

3×10^6 peritoneal cells. The incomplete lysis in this situation undoubtedly represents a prozone(7). There was no lysis of cells in control preparations where either immune serum or guinea pig serum was omitted. Thus macrophages in tissue culture possess a relative resistance to lysis, although in the presence of sufficient complement they are also fully susceptible to cytolysis. Similar results were obtained with peritoneal cells from normal BALB/c mice and from both normal and starch-injected C57BL/6 mice.

Phagocytosis of tumor cells by peritoneal macrophages in vitro. In the phagocytic test as originally described(1,2) the isoimmune serum and the peritoneal cells were obtained from mice of the same strain, and the isoimmune serum was directed against the allogeneic tumor cells. Since peritoneal macrophages were found to be relatively resistant to the cytotoxic effect of isoantibody and complement, it seemed possible that these cells could phagocytize isogenic tumor cells in the presence of isoantibodies directed against the macrophages and tumor cells. Phagocytic tests were therefore performed to measure the comparative phagocytic activity of macrophages in the presence of isoimmune serum directed against the tumor cells, with macrophages derived from the strain of origin of either the isoimmune serum or the tumor. Thus, as shown in Table III, the degree to which the BALB/c tumor Meth A was phagocytized in the presence of C57BL/6 anti Meth A serum was measured with macrophages from either C57BL/6 or BALB/c

mice. Similarly, the phagocytosis of EL4 (C57BL) with (C3H/An × I)F_1 anti EL4 serum was measured with both (C3H/An × I)F_1 and C57BL/6 macrophages, and the phagocytosis of ERLD (C57BL/6) with A anti ERLD serum was measured with both A and C57BL/6 macrophages. In every instance, the tumor cells were phagocytized by macrophages from the donor strain of either the immune serum or the tumor. Thus, tumor cells were phagocytized by isogenic macrophages in the presence of isoimmune serum, although the degree of phagocytosis was generally less than when macrophages originated in the same strain as the immune serum.

Differential counts on peritoneal populations. It has been noted above that in the presence of isoimmune serum directed against the donor strain of the peritoneal cells, a greater percentage of the peritoneal population in tissue culture attached to the cover slip. When immune serum was omitted from the incubation medium, approximately 25% of the cells remained unattached, whereas non-adherent cells amounted to less than 10% of the total inoculum when immune serum was present. Differential counts of the stained cells adhering to the cover slips after incubation with immune serum indicated that the proportion of the cell types varied in both normal populations and starch-induced exudates in the various strains tested. The normal population consisted of 45-55% lymphocytes, 40-50% macrophages, and the remainder of mast cells and eosinophils. The starch-induced populations contained 60-80% macrophages, 10-30% lymphocytes, and the remainder, eosinophils and neutrophils.

Discussion. The results indicate that the resistance of peritoneal macrophages in tissue culture to immune lysis is not absolute, and that in the presence of sufficient antibody and complement, macrophages are completely susceptible to lysis. In this respect, it once appeared that Sarcoma 1 was entirely resistant to cytolysis *in vitro* by isoimmune serum and complement and that ascites sarcomata such as BP8 contained a proportion of resistant cells(8). More recently both Sarcoma 1(9) and BP8(7) were shown to be fully susceptible to immune cytolysis under optimal experimental conditions. These results with Sarcoma 1 and BP8 in addition to the present findings with macrophages, emphasize the necessity of providing adequate experimental conditions for demonstrating the susceptibility of different cell populations to cytolysis with isoantibody and complement (7,10,11).

Why peritoneal macrophages allowed to spread in tissue culture are relatively resistant to cytolysis with isoantibody and complement has not been determined. This resistance may well be peculiar to macrophages, since several tumor cell lines derived from chemically-induced sarcomas maintained in tissue culture in this laboratory were fully susceptible to immune cytolysis (unpublished observations). One possible explanation is that the cell membrane of macrophages is capable of repairing cytolytic lesions as they occur, but that with large amounts of complement the extent of damage is irreversible. Alternatively, antigenic sites on macrophages that have spread in tissue culture may be too distant from one another to permit effective fixation of complement. In this respect, studies of immune lysis of sheep erythrocytes indicated that fixation of complement occurred only when 2 antigen-antibody combination sites were closely adjacent(12).

As an extension of these observations, it was found that peritoneal macrophages phagocytized isogenic tumor cells *in vitro* in the presence of immune serum. It may be that macrophages possess resistance to cytolysis *in vivo* and that the growth of isogenic tumors within the peritoneal cavity may be suppressed by injection of isoimmune serum.

Our differential counts and those of other workers(13,14) indicate that the peritoneal population is heterogenous, whether the cells are obtained from normal mice or from mice receiving a provoking agent. In view of the immunological capacities of the lymphocyte and possibly also the macrophage(4,14-17), the properties of whole peritoneal populations, whether normal or induced, do not necessarily reflect the activities solely of macrophages, but rather of a mixture of cells. The relative resistance of macrophages to

cytolysis by isoimmune serum and limited amounts of complement may provide a method for preparing populations of peritoneal macrophages free of other cell types and suitable for immunological studies(17).

Summary. The entire population of peritoneal cells maintained in suspension is susceptible to cytolysis by isoantibody and complement *in vitro*. In tissue culture, the macrophages spread on glass and become resistant to cytolysis by isoantibody and limited quantities of complement. This contrasts with the marked susceptibility of the other peritoneal elements (lymphocytes, eosinophils, neutrophils and mast cells). In the presence of isoimmune serum and additional complement, however, macrophages are also lysed. Peritoneal macrophages are capable of phagocytizing isogenic tumor cells *in vitro* in the presence of isoimmune serum directed against both macrophages and tumor cells.

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Flocculation of Unsensitized Bentonite Particles by Cancer Serum.* (29262)

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Unheated sera from healthy individuals and from those suffering from cancer flocculate bentonite particles to which no antigenic material has been adsorbed. If, however, both types of sera are heated at 50°C for 30 minutes flocculation is observed with the cancer sera while normal sera reacts negatively or with a much reduced titer. This report concerns the quantitative differences in the above reaction when cancer and other sera are heated to 50°C.

Materials and methods. A. Bentonite suspension was prepared according to the method described by Bozicevich *et al*(1) without use of a dye. The suspension was kept at 6°C and prepared fresh every 5 to 7 days.

B. Serum samples. Two hundred and thirty-eight serum samples from the same number of individuals suffering from various types of cancer were obtained from the Laboratory of Clinical Chemistry; Tumor Surgery

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