tically significant. There were no differences between the 2 groups of animals in their mean plasma levels of fibrinogen (factor I) and proaccelerin (factor V), which are the 2 other blood clotting factors possibly produced in the reticulo-endothelial system. All rats received the same daily dose of vit. K_1 in their food. The germfree rats, however, showed a higher mean plasma level of antihemophilic B factor (factor IX) and Stuart-Prower factor (factor X) than the control rats, the difference in the latter factor being statistically significant at the 5% level. No other differences were detected between the 2 groups of animals as far as known blood clotting factors are concerned.

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Mouse Tissue Isoantigen Detectable by Immunoprecipitation.* (29605)

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Isoantigenic variants of mouse gamma globulin (allotypes) as well as other mouse serum isoantigens have been demonstrated by immunoprecipitation(1-3). *Mouse heteroantigens* of cellular origin have also been shown to react with appropriate antibodies from a foreign species in gel-diffusion tests(4,5). Mouse cellular *isoantigens,* however, have to our knowledge been detected only by other techniques, such as hemagglutination, hemagglutination-inhibition, leukocyte agglutination, immuno-fluorescence, cytotoxicity, induction of tumor homograft enhancement or accelerated homograft rejection. We now report that certain mouse sera obtained after viral oncolysis of a transplanted Ehrlich ascites tumor contain an antibody which precipitates with aqueous extracts from certain mouse tumors and similar extracts from normal organs of several mouse strains.

Materials and methods. Inbred A2G mice and random-bred ICR mice were purchased from Dublin Laboratories, Dublin, Va. Other inbred mice and F_1 hybrids were purchased from Jackson Laboratories, Bar Harbor, Maine. Coisogenic B10.A and C3H.K mice were obtained from Dr. G. D. Snell, Jackson Laboratories.

Ehrlich ascites tumor and the conditions for oncolysis by WSA influenza virus were as previously described(6). The Krebs 2

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ascites tumor was obtained from Dr. T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. The Sarcoma 180 ascites tumor was obtained from Dr. K. Sugiura, Sloan Kettering Institute, New York. The Sarcoma I ascites tumor was obtained from Dr. G. D. Snell.

The immune fluid #24, with which most of the present work was done, had the following history. An A2G mouse, resistant to the lethal action of neurotropic influenza A virus(7), was inoculated on day 0 with 10^6 Ehrlich ascites tumor cells and on day 6 with the WSA strain of tumor-adapted neurotropic influenza A virus. The virus induced oncolysis and the mouse survived(6). On day 86, the mouse was challenged with 10^4 Krebs 2 cells; no tumor developed. On day 300, the mouse was rechallenged with 10^6 Ehrlich cells. Ascites slowly developed. On day 316 the mouse was killed; ascitic fluid and blood serum were collected separately. Both serum and ascitic fluid (after removal of the fibrin clot) behaved in the tests to be described below in much the same manner. For reasons of convenience, most tests were done with the ascitic fluid.

Aqueous tumor and tissue extracts were prepared from washed tumor cell sediments or from finely chopped normal mouse organs by disruption in a French pressure cell at 250 Atm, by homogenization in a Virtis "45" homogenizer, or by sonication at 10 kc/sec for 1 min. The homogenates were frozen and thawed twice and centrifuged at 10,000 *g* for 15 min; the supernatants were used as antigens in gel-diffusion tests.

Double diffusion gel precipitation tests and immuno-electrophoresis were done in 3 mm thick layers of agar gel on microscope slides (0.8% Ionagar No. 2, 0.05 M sodium barbital buffer pH 8.6).

Results. When fluid #24 was tested in an Ouchterlony type agar gel-diffusion assay against an aqueous extract of Ehrlich tumor cells, a single precipitation line formed between the 2 wells. Extracts of Krebs 2 and Sarcoma 180 ascites tumor cells gave reactions identical to those of Ehrlich cells. Extracts from the strain-specific Sarcoma I ascites tumor showed no precipitation under the same conditions (Fig. 1).

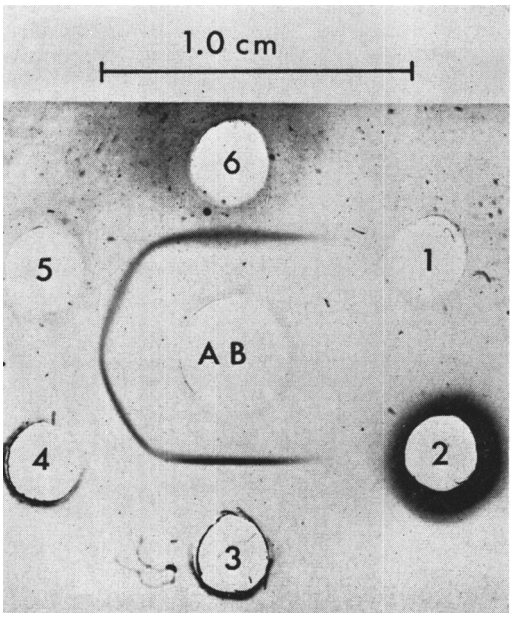

FIG. 1. Agar gel immunoprecipitation of mouse isoantibody with aqueous mouse tissue extracts. Center well (AB): No. 24 fluid. Numbered wells contain aqueous extracts from the following: 1. Sarcoma I ascites tumor cells; 2. C3HeB/FeJ livers and spleens; 3. C57BR/cdJ livers and spleens; 4. 129/J livers and spleens; 5. Ehrlich ascites tumor cells; 6. Krebs 2 ascites tumor cells.

The antibody contained in #24 fluid moved on immuno-electrophoresis with the anodic half of the gamma region of mouse serum proteins. The antigen in aqueous tumor extracts could not be sedimented at 105,000 *g* within 1 hour. After shaking with an equal volume of chloroform, the aqueous phase still precipitated with antibody.

Extracts from normal mouse organs were similarly assayed for precipitation with #24 fluid. Non-inbred ICR mice contained antigen in all organs tested: liver, spleen, pancreas, kidney, lung, brain. Whole ICR embryos of 15 days' gestation likewise had antigen. Pooled liver and spleen extracts from each of a number of inbred mouse strains also gave positive gel-precipitation with #24 fluid, whereas similar extracts from the A2G and several other mouse strains were inactive (Fig. 1, Table I). Normal mouse serum did not precipitate with #24 fluid.

First generation offspring (F_1) from

TABLE I. Distribution of ϵ-24 Antigen Among Various Strains of Mice and Mouse Tumors. Aqueous tumor cell or organ (liver and spleen) extracts were assayed in an immunodiffusion test against #24 antibody.

No precipitation	Precipitation
A2G	BALB/cJ
A/J	B10.A/Sn*
A/HeJ	C57BL/6J
AKR/J	C57BL/10J
CBA/J	C57BR/cdJ
C3H/HeJ	C57L/J
C3HeB/FeJ	C58/J
C3H.K/Sn†	DBA/1J
RF/J	DBA/2J
Sarcoma I ascites tumor	SJL/J
	SWR/J
	129/J
	ICR (non-inbred)
	B6D2F1/J‡
	B6AF1/J§ (weaker reaction)
	C3D2F1/J‖ (" ")
	Ehrlich ascites tumor
	Krebs 2 ascites tumor
	Sarcoma 180 ascites tumor

* H-2^a on C57BL/10 genetic background.
† H-1^b on C3H genetic background.
‡ (C57BL/6 × DBA/2)F1.
§ (C57BL/6 × A)F1.
‖ (C3H × DBA/2)F1.

crosses between strains possessing the antigen gave reactions identical to those of the parent strains. F_1 offspring from crosses where only one of the parents carried the antigen gave distinct, but weaker reactions. Thus, extracts from C57BL/6J livers could be diluted up to 1:8 and still give a faint line of precipitation. Extracts from livers of F_1 hybrids between C57BL/6J and A/J (B6AF1/J), however, could not be diluted beyond 1:2 to 1:4. Table I shows the reactions obtained with 19 inbred mouse strains, one non-inbred strain, 2 coisogenic lines, 3 F_1 hybrids and 4 mouse tumors.

Discussion. We have screened more than 30 postoncolytic sera and ascitic fluids obtained from A2G mice after varied challenge schedules. All of these agglutinated Ehrlich tumor cells(8) and contained detectable amounts of antibodies directed against the virus used for oncolysis. Only a few of the sera and fluids gave lines of precipitation in double diffusion tests with aqueous tumor and organ extracts. One fluid, #24, which was available in relatively large amounts, was used in the work described above. Three other fluids which gave identical precipitation patterns could have been used instead. Trial bleedings of some postoncolytic mice, however, revealed sera which seemed to produce at least 2 lines of precipitation with Ehrlich tumor extracts. Therefore, certain postoncolytic immune fluids may contain isoantibodies directed against several soluble cell constituents; #24 and similar fluids happen for some reason to reveal only one antigen, which we provisionally call ϵ-24.

The antigen ϵ-24 is water soluble and readily diffuses through agar gels. Its distribution among inbred mouse strains is different from that of the better known histocompatibility antigens. In particular, there is no obvious relationship to the strong H-2 histocompatibility complex. For instance, strain A mice, which are H-2a, lack ϵ-24, whereas B10.A mice, likewise H-2a and coisogenic with C57BL/10, contain ϵ-24. Similarly, H-2k is represented in mice which have ϵ-24 and in mice which lack it. The strain distribution of ϵ-24 is also different from that of recently described serum isoantigens related to complement(2,3) and from that of the H-2 associated serum protein(10). Several strains lacking ϵ-24 are closely related: Thus, CBA and C3H are of similar derivation, as are AKR and RF, and A2G and A. All were derived at some time from Bagg albino mice (9).

The strain distribution of ϵ-24 makes it likely that its presence is under genetic control. F_1 offspring from crosses where only one of the inbred parents contained the antigen seemed to have roughly half as much of the precipitating material as homozygous, antigen-positive mice. Segregation was observed in preliminary tests on individual mice born from backcrosses between F_1 and antigen-negative parents.

Viral oncolysis is known to result in unusually potent tumor immunity(6,8,11). Possible roles of the virus in initiating this immunity have already been discussed(6). The present work shows that the immune response following viral oncolysis can be used to uncover isoantigenic specificities which might be otherwise difficult to detect.

Summary. An immune fluid was obtained

from an A2G mouse which had recovered from Ehrlich ascites tumor after viral oncolysis. This fluid precipitated in gel-diffusion assays with aqueous extracts from 3 unspecific tumors and from organs of mice belonging to several inbred strains, but not with similar extracts from other strains. The antigen involved has been provisionally called ϵ-24.

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Comparative Effects of Benzquinamide and P-2565 on Cardiac Rate. (29606)

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The central effects of 2-hydroxy-3-diethylcarbamyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzoquinolizine acetate (benzquinamide) and its alcohol analogue (P-2565) have been described by Weissman and Finger (1), Feldman(2), Smith(3), and Hashkes (4). Chemically both structures are closely related although their potentials to release amines centrally are not parallel(1). Many of the hypotensive agents which release or deplete the brain of norepinephrine (NE) and 5-hydroxytryptamine (5HT) also release or deplete peripheral organs of norepinephrine(5,6,7). The present report describes the effect of benzquinamide and P-2565 on heart rate and on NE levels of the heart.

Materials and methods. Benzquinamide solution was prepared by dissolving 40 mg in 4 ml of water with a drop of glacial acetic acid. P-2565 is readily soluble in water. In the experiments in which the NE levels were determined, the rats were sacrificed by decapitation and the heart tissue was rapidly removed and analyzed by the single extraction method of Shore and Olin(8).

The cardiovascular measurements were carried out on male cats and Sprague-Dawley rats anesthetized with pentobarbital 40 mg/kg i.p. In some experiments rats were anesthetized with a mixture of chloralose 100 mg/kg and urethane 500 mg/kg i.p. Arterial pressure from the carotid artery was recorded by the Statham transducer model 5 Grass Polygraph assembly. Heart rate was taken from the pressure record. The effects of benzquinamide and P-2565 were also tested in adrenal demedullated rats as well as in vagotomized animals. Benzquinamide and P-2565 were administered intraperitoneally. The effect of P-2565 on the isolated cat heart was studied in the Anderson heart perfusion apparatus. A polyethylene tube (No. 50) was introduced into the apparatus through a sidearm to allow drugs to be administered directly into the aorta close to the origin of the coronary arteries. The total volume of the catheter was only 0.1 ml.

Results and discussion. The bradycardia produced in rats treated with P-2565 50 mg/kg i.p. persisted for a longer period than