mentally infected with *Anaplasma,* a rickettsial parasite of erythrocytes of cattle, were ascertained at various phases of experimental infections. The percentage increase of free sialic acid in the sera of 4 calves, 2 of which were splenectomized, ranged between 25 and 150 while the bound sialic acid ranged from 6.3 and 41. In 2 splenectomized calves, the percentage increases of free sialic acid were 150 and 137 and the percentage increases of bound sialic acid were 41 and 15 respectively. In contrast, the percentage increases of free sialic acid in 2 nonsplenectomized calves were 25 and 46 and percentages of bound sialic acid in the same calves were 6.3 and 23 respectively. The percentage increase in free sialic acid was approximately the same regardless of the quantity of inoculum of infected blood, *i.e.,* 5.0 ml or 220.0 ml, used. The maximal increase in free sialic acid occurred during the early invasive stage of the disease. The possibility that the free sialic acid in serum might have originated from the receptor substances of the erythrocytes is discussed in the light of a hypothesis concerning the cycle of development of the causative agent, according to which the invasive mechanism of *Anaplasma* involves penetration of the erythrocytic membrane by initial bodies of this organism.

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Induced Resistance to Electroshock and Audiogenic Seizures in Inbred Strains of Mice.* (28703)

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Attempts to identify physiological mechanisms and the sites of neurological lesions implicated in genetic audiogenic seizures in rodents have largely employed two methods: (1) Tests for the effectiveness of various biochemical compounds and drugs that change the characteristic seizure pattern in susceptible animals; (2) Ablation experiments to analyze the effect of discrete neuroanatomical lesions on seizure pattern and incidence in rodents genetically predisposed to audiogenic convulsions.

The results of these studies have implicated metabolic intermediates(1) steroid hormones(2) and several depressant, sedative and analgesic drugs(3). Extensive ablation of cortical and some subcortical structures re-

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vealed no modification of audiogenic seizure pattern in genetically susceptible strains(4, 5).

The first aim of this study was to test the effect on 2 types of seizures, namely audiogenic and electroshock convulsions of some compounds that were neither depressants, metabolic intermediates, and not characterized as steroid substances, but for which some evidence was available that they had a special affinity for specific brain areas. The agents chosen were the compounds 2,4 dichlorophenoxyacetic acid (2,4 D) and the vital dye trypan blue. Our choice was dictated by the observation of one of us that 2,4 D has an excitatory effect on the spontaneous electrical activity of the hypothalamus (unpublished data) and that it acts as a thermolytic agent in rodents(6), coupled with the finding that this substance protects against audiogenic seizures in mice without exerting any barbiturate-like or analgesic effects(7). The dye trypan blue was chosen because this dye protects against audiogenic seizures(8) and similar dyes have been reported to prevent strychnine induced convulsions(9).

The second aim of this study was to test the hypothesis that mice genetically predisposed to audiogenic seizures might also have a lower threshold to another type of convulsion, *e.g.*, electroshock convulsions, for which some evidence exists, that they are cortical in origin.

Materials and methods. Male mice of the Swiss Webster strain (commercial source: Miss Flora O'Grady, New York) and the DBA/2 strain (Roscoe B. Jackson Laboratory, Bar Harbor, Maine) which weighed 13-20 g were used. The DBA/2 strain is inbred for susceptibility to audiogenic seizures and the genetics of this trait in this strain has been analyzed(10). In the Swiss Webster strain 2 sublines, a seizure resistant line and a line susceptible to audiogenic seizures, were selectively bred.

In the first series of experiments designed to test the effect of 2,4 D and trypan blue on electroshock seizures, mice of the Swiss Webster and the DBA/2 strain were given intraperitoneal injections of 2,4 D in dosages of 125 mg/kg body weight dissolved in sterile distilled water so that the volume injected ranged between 0.065-0.10 ml. Controls were injected with an equivalent volume of physiological saline. The dosage was selected because in higher concentrations this compound exerts thermolytic as well as myotonic effects. Trypan blue injections consisted of .75-1 ml of 1% dye solution. One to 3 hours‡ after injection mice were subjected to electroshock delivered through corneal electrodes for 0.2 second at current level of 11.0, and 12.5, milliamperes (ma) from a Hans model 2 C electroshock seizure apparatus, 60 cps.

In the second series of experiments designed to test the effect of these compounds against audiogenic seizures the same protocol was used. Swiss Webster and DBA/2 mice were tested for audiogenic seizure susceptibility 24-48 hours prior to treatment. Audiogenic seizure was induced in a wire-enclosed cage 30.5 cm in diameter placed 12.7 cm below a bell which produced a 90 db sound. The time was manually recorded by means of stop watch from the beginning of bell ringing to the end of running and the onset of clonus. The auditory stimulus was stopped at the onset of the convulsive attack, or at 60 sec if no seizure was induced. Positive-pressure artificial respiration was administered to convulsing mice until spontaneous respiration was reestablished.

The last series of experiments were undertaken to test the hypothesis that mice genetically predisposed to audiogenic seizures might have a lower threshold to electroshock convulsions. Two sublines of the Swiss Webster strain, selected for resistance and susceptibility to audiogenic seizures, respectively, were used. Response to electroconvulsive stimulation given at 11 and 12.5 ma was recorded in mice of these 2 sublines. An additional series consisted of audio-susceptible mice exposed to brief auditory stimulation resulting in preconvulsive wild running but not convulsions. This treatment was given immediately prior to electroshock stimulation at 8, 9 and 11 ma. The EST of mice treated

‡ Trypan blue treated mice were shocked 3 hours after injection to permit sufficient time for the dye to circulate.

in this manner was compared with that of controls not exposed to auditory stimulation prior to electroshock treatment.

Results. 2,4 D in dosage of 125 mg/kg of body weight was effective in conferring almost complete protection against audiogenic seizures as well as against electroshock seizures in mice of both DBA/2 and the Swiss Webster strain (Table I). The effective anticonvulsant dosage of this drug was not accompanied by any thermolytic effects in these mice tested at room temperature (26°C). The protective effect of 2,4 D against electroshock seizure was most apparent at current levels 11 and 12.5 ma (Table I). At higher current levels (15 ma and 17.5 ma) no protective effect was observed.

TABLE I. Protective Effect of 2,4 D and Trypan Blue on Electroshock and Audiogenic Seizures in Mice.

Strain	Group	Electroshock convulsions at given current levels						Audiogenic convulsions			
		11.0 ma				12.5 ma					
		% convuls.	(n)	X^2	P	% convuls.	(n)	X^2	P	% convuls.	(n)	X^2	P
Swiss Webster	Experimentals 2,4 D inj	13	(15)	4.2	<.05	45	(20)	.6	<.005	10	(20)	18.8	<.001
	Controls Saline inj	64	(42)			77	(48)			92	(35)		
	Experimentals Trypan blue inj	7	(28)	4.3	<.05	71	(14)	.04	<.980	27	(22)	8.7	<.005
DBA/2	Controls Saline inj	95	(22)	5.2	<.025	100	(11)	—	—	60	(20)	12.0	<.001
	Experimentals 2,4 D inj	47	(21)			100	(7)			0	(20)		

Trypan blue also conferred protection against the 2 types of seizures, (Table I) but it was less effective than 2,4 D. It protected against electroconvulsions only if the stimulus applied was of low magnitude (11 ma). The protection conferred by the dye against audiogenic seizures was also more limited than that of 2,4 D. The effective dosage of trypan blue was close to the lethal concentration. Fifty per cent of trypan blue injected mice died within 48 hours following treatment.

A comparison of the minimal electroshock threshold between the 2 sublines of mice selected, *e.g.*, the audio-seizure susceptible and the audio-seizure resistant line, revealed that the genetic constitution that predisposes mice to audiogenic seizures does not lower significantly their threshold to electroshock convulsions nor does a preconvulsive response to auditory stimulation just prior to electroshock affect the electroconvulsive threshold in mice (Table II).

Discussion. The experiments demonstrated that 2,4 D in dosages of 125 mg/kg protected mice against both audiogenic seizure as well as electroconvulsions. The protective effect of this compound against audiogenic seizures in one strain of mice had previously been reported(7).

Little is known about the physiological action of 2,4 D except for its thermolytic properties, which have been tested in rodents (6). The present study demonstrates convincingly that its effectiveness as an anticonvulsant is completely independent of and unrelated to its thermolytic action. This plus the observation that it has no overt depressant or sedative or analgesic effects, in the dosage used, might suggest a possible clinical usefulness for this compound. The thermolytic property of 2,4 D, and recent unpub-

lished results of one of us demonstrating hypothalamic excitation following injection of this compound in minute concentration suggests the possibility that this compound may be exerting pharmacological effects *via* the hypothalamus.

Less is known about the mechanism of action of trypan blue, and its protective effect on seizures is therefore difficult to interpret. Vital dyes are generally assumed not to have depressant effects on the nervous system when administered in single low doses. Trypan blue is prevented from entering the brain tissue proper by the blood brain barrier, the existence of which was originally deduced from the observation that dyes such as trypan blue fail to appear in adult nervous tissue after injection into the blood stream. The hypophysis and certain hypothalamic structures such as the mammallary body, are exceptions in that they are accessible to the injected dyes presumably because of the absence of the blood brain barrier in those regions. The possibility that the accumulation of trypan blue in hypothalamic or hypophyseal areas is related to its anticonvulsant effect needs to be tested.

TABLE II. Comparison of Electroshock Seizure Threshold Between 2 Sublines of Swiss Albino Mice Resistant and Susceptible to Audiogenic Seizure and Between Susceptible Mice Exposed to Auditory Stimulation Prior to Electroshock and Non-exposed Controls.

Group	% convulsions at given current levels											
	8-9 ma				11 ma				12.5 ma			
	% convuls.	(n)	X^2	P	% convuls.	(n)	X^2	P	% convuls.	(n)	X^2	P
Audiogenic seizure resistant	—				50	(10)			79	(29)		
			—	—			.85	<.30			.01	>.90
Audiogenic seizure susceptible	20	(20)			80	(20)			90	(30)		
			.08	<.75			.31	<.70			—	—
Audiogenic seizure susceptible—pre-exposed to auditory stimulation resulting in preconvulsive response	18	(17)			100	(10)			—			

Our failure to affect electroseizure threshold in mice susceptible to audiogenic seizures prior to brief exposure to sound stimulation, as well as the failure to detect any differences in electroshock seizure threshold (EST) between high audio-seizure susceptible and resistant lines of the same strain suggests that audiogenic and electroshock convulsions probably spread from distinct foci of the central nervous system.

The physiological mechanisms and events associated with audiogenic seizures have not been identified, in spite of numerous attempts to do so. The impression gained from this study is, that the genes "for audiogenic seizures" act in a very specific manner and affect different sites from those mobilized in electroshock convulsions. The demonstration by Beach and Weaver(4) that extensive ablation of the neocortex, and the demonstration by Koenig(5) that bilateral interruption of the primary auditory pathways at the level of the medial geniculate nuclei has little effect upon frequency of sound induced seizures in mice, strengthens the hypothesis that the neural events leading to audiogenic seizures in rodents originate probably in subcortical structures.

On the other hand the excitatory threshold of the different neural pathways mobilized in the 2 types of convulsions may be affected by similar or identical mechanisms.

Summary. A protective effect against both audiogenic and electroshock convulsions in mice has been demonstrated for the compound 2,4 dichlorophenoxyacetic acid, and for the vital dye trypan blue. The mechanism involved was not revealed but attention was directed to the finding that both substances are not depressants, and that they are probably centrally acting. The threshold to electroshock convulsions was compared in 2 sublines of the Swiss Webster strain of mice, selected for susceptibility and resistance to audiogenic seizures, respectively. No significant difference in electroshock threshold between the two lines was observed.

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Attempts to Interrupt Virus Tumorigenesis by Immunization Using Homologous "Bjorklund-Type" Antigen.* (28704)

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Investigations to date have established quite conclusively that certain malignant tissues may differ antigenically from those of corresponding normal host tissues(1-6). Such differences† may consist of apparent addition of a cellular antigen or of deletion of an antigen. Whether these differences represent appearance *de novo* of new antigen and total deletion of old antigen or whether they reflect only quantitative shifts in relative amounts of antigens present both in normal and corresponding malignant tissues is not clear. The possibility for existence of antigens unique to neoplastic tissue and against which a specific immunity may be induced provides a theoretical potential for eventual prophylaxis and therapy of cancer by immunologic procedures.

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† This refers to cellular antigens resulting from malignant transformation and is not related to the specific antigens of viruses which might have been involved in induction of the malignancy.

There has been considerable interest in the studies of Bjorklund and his co-workers (7-10). Bjorklund formulated and applied the concept that if malignant tissues of man contain a cancer-specific antigen *in common,* then the pooling of a variety of tumors from a number of persons would provide a material in which the cancer antigen would not be diluted whereas the normal host antigens, unique to the individuals, would be diluted in proportion to the number of such individuals in the pool. Thus, a selective concentration of cancer-specific antigens would be effected. Horses were immunized over a long period with such pooled antigen and it was reported that the antiserum, following absorption with human plasma, caused lysis of human cancer cells in culture but did not damage normal human cells grown under similar conditions. Complete removal of such cytotoxic effect was reported to occur following absorption with cancer cells but not following absorption with normal tissues. The malignant antigen was found to be a heat