On the basis of these circumstances, it was found in the present work that α-2-macroglobulin always binds insulin, when the insulin is incubated in serum at 4°C or at 22°C. Moreover the β-2-M-globulin probably can bind insulin, when the incubation occurs at 22°C.

Summary. Immuno-electrophoresis combined with autoradiography revealed insulin-J^{125} by *in vitro* experiments to be bound to α-2-macroglobulin at 4° and 22°C and to β-2-M (γ-1-M) at 22°C.

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Strain Differences in Seizure Responses and Brain Cholinesterase Activity in Rats.* (28169)

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Strain and sex differences in electroshock and picrotoxin seizure responses in 2 strains of rats have been reported(1,2). Greater brain excitability, as shown by lower seizure thresholds, was observed in both males and females of one (S_1) strain than in males and females of another (S_3) strain. Also, within the same strain, females demonstrated greater convulsability than males. Because the S_1 strain has higher brain acetylcholine (ACh) concentration and cholinesterase (ChE) activity than the S_3, it was suggested that strain differences in convulsability might be related to differences in either ACh concentration or ChE activity or both(1). To explore further the relation between convulsability and brain ACh-ChE levels, electroshock seizures were measured in additional strains of rats specifically bred for differences in cortical ChE(3) and also characterized by differences in brain ACh(4). Seizure responses were correlated with brain ACh and ChE in males only, as the latter data were not available for females. However, because of the strong central excitatory action of estradiol in rats(5,6), seizures were also measured in females to determine if sex, as well as strain, differences could be detected. Objectives of this investigation then were to compare electroshock convulsions in 6 strains (3 pairs of strains) of rats, to determine if sex differences are present, and to try to correlate convulsive responses with corresponding values of brain ACh-ChE.

Methods. Animals. Six strains of rats—S_1, S_3, RCH, RCL, RDH, and RDL—known to differ in brain ChE activity and ACh concentration were used. The first 2 strains are descendants of Tryon's maze-bright (S_1) and maze-dull (S_3) rats(7). The remaining strains were selectively bred by Roderick

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TABLE I. Electroshock Seizure Thresholds (EST) and Forebrain Cholinesterase (ChE) Activity and Acetylcholine (ACh) Concentrations in 6 Strains of Rats.

Strain	EST, milliamperes		P: † males *vs* females	Forebrain* ChE	Forebrain ACh
	Females	Males		Males	
S_1	21.1‡	23.4	<.01	168.3	27.3
S_2	22.0	24.3	<.01	153.5	24.1
P: S_1 *vs* S_2	<.02	<.05		<.01	<.001
RCH	20.0	24.2	<.001	163.5	24.5
RCL	22.9	30.6	<.001	133.4	22.5
P: RCH *vs* RCL	<.001	<.001		<.001	<.05
RDH	21.7	22.5		146.5	23.1
RDL	24.8	30.8	<.001	122.7	23.0
P: RDH *vs* RDL	<.001	<.001		<.001	

* ChE and ACh values are for all of brain anterior to pons and cerebellum. ChE activity is expressed in moles ACh $\times 10^{10}$ hydrolyzed/min/mg fresh tissue, and Ach concentration in mμM/g fresh tissue. Data are reproduced from reference(4).

† P values are based on *t* tests.

‡ Data presented are means of 8-19 determinations of individual rats.

from rats of Dempster and Castle foundation stocks for differences in ChE activity in cerebral cortex(3). These strains are the RCH (Roderick Castle high brain ChE), RCL (Roderick Castle low brain ChE), RDH (Roderick Dempster high brain ChE), and RDL (Roderick Dempster low brain ChE). A total of 164 rats of approximately 120 days of age was divided into 12 strain-sex groups of about 13 rats each.

Electroshock seizures. Seizures were elicited with a 60-cycle a-c electroshock apparatus(8). The stimulus was delivered through corneal electrodes and had a duration of 0.2 sec. Convulsive parameters measured were: minimal electroshock seizure threshold (EST), maximal electroshock seizure, and post-seizure depression.

EST was defined as the minimum amount of current in milliamperes (ma) which resulted in a barely detectable clonic convulsion of ears, jaws, and forepaws. This convulsion lasted a few seconds only and was not associated with loss of equilibrium. EST is considered to be inversely related to brain excitability(8). Theshold determinations were performed 3 times on each animal with 48-hour intervals between tests. Table I presents values obtained during the third test.

A maximal electroshock seizure produced in adult rats by a supramaximal stimulus of 150 ma is characterized by the following sequence of phases: tonic hind-leg flexion, tonic hind-leg extension, and whole-body clonus. Duration of each phase was timed in seconds by automatic timers connected to the stimulator. Increased convulsability or increased intensity of seizures is associated with increased duration of tonic extension, or decreased duration of tonic flexion, or both(8,9).

Following a maximal seizure a rat undergoes a period of depression during which he lies quietly on his side. After several minutes the animal spontaneously assumes his normal upright posture. Time from termination of maximal seizure to recovery of normal posture is a measure of post-seizure depression and indicates the time required to regain, at least partially, normal brain function(8,9).

Maximal seizures were produced and durations of post-seizure depression were measured only once in each animal.

Neurochemical measures. Brain ChE activity and ACh concentration for adult males of these strains have been determined previously by some of us(4) and are compared here with electroshock responses.

Results. Electroshock seizure threshold. From the data presented in Table I, significant strain and sex differences in seizure threshold are apparent. For example, the lowest thresholds, which were found in the female RCH rats, were 65% of the highest thresholds found in the RDL males. Strain differences in EST were particularly striking in RDH-RDL and RCH-RCL males. Values for paired groups are clearly separated. Ranges of EST in ma for these 4 groups are

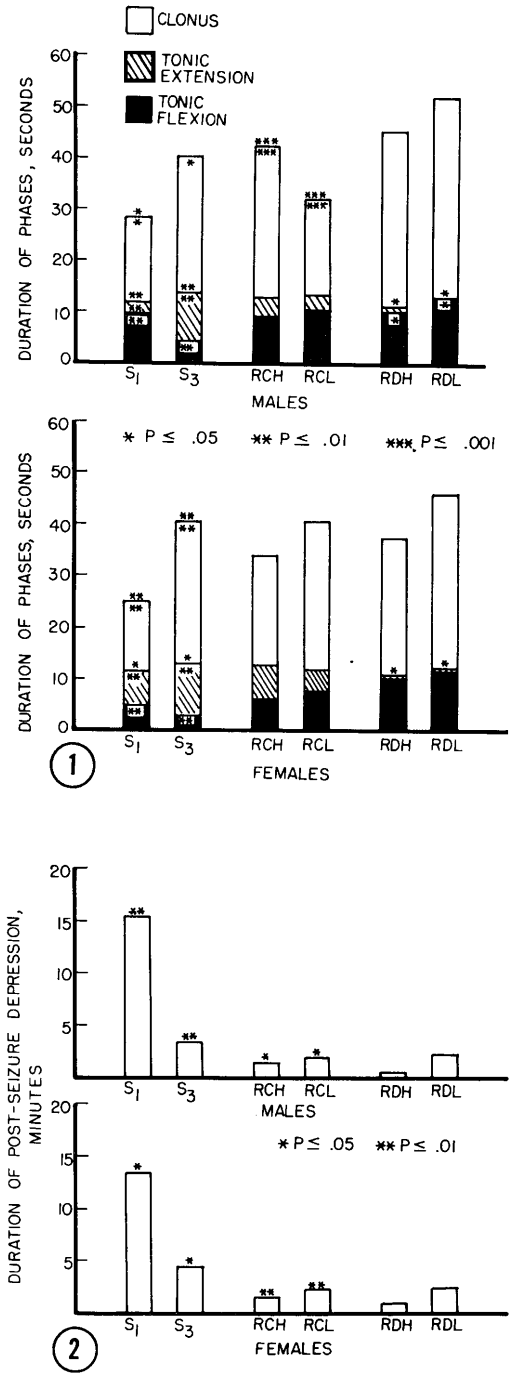

FIG. 1. Duration in seconds of phases of maximal seizures in males and females of 6 strains of rats. Phases for paired strains are compared. Asterisks represent P values based on *t* test and are placed at the top of and within the barogram sections for phases which differ significantly. Significant differences in duration of total tonus (flexion and extension) and of total seizure are represented by asterisks placed above the respective parts of the barogram. For example, S_1 - S_3 males differ significantly in durations of tonic flexion ($P \leq .01$), tonic extension ($P \leq .01$), total tonus ($P \leq .01$), clonus ($P \leq .05$), and total seizure ($P \leq .05$).

FIG. 2. Duration in min of post-seizure depression in males and females of 6 strains of rats. Depression was measured as time required for the rat spontaneously to right himself following a maximal seizure. Asterisks represent P values based on *t* test for significance of differences between paired strains.

as follows: RCH, 22.0-26.0; RCL, 29.0-32.5; RDH, 21.5-23.5; and RDL, 27.5-33.5. RCL and RDL thresholds were unusually high, as judged by our previous experience with other strains of rats of similar or greater age(6).

In each pair of strains from the 3 foundation stocks, males with the higher ChE activity had the lower EST. There is an interesting parallelism between significance of differences in EST and ChE activities: the S_1-S_3 rats differed least in seizure thresholds and brain ChE, whereas the RCH-RCL and RDH-RDL pairs differed markedly in both parameters. On the other hand, seizure thresholds appear to have varied independently of ACh concentration: differences in EST were small for the S_1-S_3 pair and large for the RCH-RCL and RDH-RDL pairs, whereas differences in ACh concentration exhibited the reverse relation.

In 5 of the 6 strains, females had significantly lower electroshock seizure thresholds than males. This agrees with reports that female rats or mice have lower thresholds than males for acid fuchsin(10), electroshock(1), audiogenic(5), and picrotoxin(2) seizures. EST and ChE activities were not compared in females because brain chemical determinations for this sex are not yet available.

Maximal seizures and post-seizure depression. Both males and females showed consistent strain differences in maximal seizure pattern and post-seizure depression (Fig. 1 and 2).

The S_3 rats exhibited the most convulsant or intense type of seizure (longest tonic extension) and the RDL and RDH, the least convulsant type (shortest tonic extension). The RDL males were unique in that not one of the 10 animals in the group exhibited tonic extension, which is considered to be a typical

component of maximal seizures in most animal species, including the rat(8,9). Only about 4% of adult Sprague-Dawley rats fail to show tonic extension(9). The abolition of tonic extension and the prolongation of tonic flexion and clonus are typical early signs of effective anticonvulsant activity of drugs(8, 9). The RDL males, then, had the most anticonvulsant type of pattern, because they had the longest periods of tonic flexion and clonus and no tonic extension (Fig. 1).

The S_1 rats, both males and females, required a far longer time to recover normal brain function following a maximal seizure than did any of the other groups (Fig. 2). In fact, post-seizure depression lasted 16 times longer in the S_1 males than in the RDH males.

Although sex differences in maximal seizures were not as pronounced as strain differences, the data presented in Fig. 1 indicated that females had a more convulsant type of seizure, as shown by the longer tonic extension and shorter tonic flexion, than males.

Unlike EST, maximal seizure and subsequent depression did not appear to be related to either ACh concentration or ChE activity.

Discussion. Comparison of electroshock seizure responses in rats disclosed pronounced strain and sex differences. Although a hyperexcitable strain of mice showing spontaneous seizures has been described(11), there are no reports of strains showing less than "normal" convulsability. The present findings support the hypothesis of a strain-dependent continuum in brain excitability, and demonstrate that there are hypoexcitable, as well as hyperexcitable, strains of rats; for example, the RDL males have a less than "normal" convulsant response.

Experimental seizures are frequently used to assess central nervous system activity and development. The present results emphasize that strain differences in seizure responses must be considered in evaluating the effects of physiological and pharmacological agents on convulsive susceptibility, for example, when comparing results from different laboratories.

Females have a greater convulsive response than males as measured by threshold and pattern of seizures. This difference may be explained by the high levels of estradiol in females and the excitatory action of this hormone(5,6). However, durations of post-seizure depression are similar in males and females of the same strain and appear to be independent of sex steroids.

A partial explanation for the strain differences in seizure responses may be sought in brain ACh and ChE differences. A relation between seizure susceptibility and activity of brain ACh-ChE system has been suggested by observations that ACh and ChE inhibitors applied to the cortex induce convulsions in animals(12). Also, free ACh was found in cerebrospinal fluid of epileptic patients following seizures, but not in the fluid of normal individuals(12). Furthermore, epileptogenic cortex showed decreased ability to bind ACh and increased ChE activity(12). The more advanced the epilepsy, the greater was the ChE activity of focal cortex(13).

The increased seizure susceptibility (lowered seizure thresholds) associated with increased brain ChE activity reported herein resembles the situation in epilopetogenic cortex and offers further evidence that the ACh-ChE system is basically involved in convulsability. Heightened ChE activity in strains with lower seizure thresholds and in epileptogenic cortex may represent an adaptive increase in response to greater ACh production. Determinations of brain cholineacetylase activities in the strains are underway to test this hypothesis. The present lack of correlation between brain ACh concentrations and convulsability is consistent with the finding that the initial ACh concentration of epileptogenic cortex did not differ from normal, although the production of "free" ACh during incubation was significantly greater than normal(12).

Increased convulsability is indicated by lower seizure thresholds, by more intense seizures, especially as measured by longer tonic extension, and by shorter post-seizure depressions(8,9). The present results clearly show that these 3 measures may vary independently of each other. For example, in males the RDH have the lowest thresholds of the 6 groups (Table I), whereas their maximal sei-

zure pattern is clearly anticonvulsant (Fig. 1). The absence of a relation between intensity of maximal seizures, post-seizure depression, and brain ACh concentration and ChE activity, whereas seizure thresholds appear to be related to ChE activity, is further evidence of the independent control of these 3 measures of convulsive responses.

Summary. Marked strain differences in minimal electroshock seizure threshold, maximal electroshock seizure pattern, and duration of post-seizure depression were found in adult males and females of 6 strains of rats. Seizure thresholds in one group averaged only 65% of thresholds in another group. Duration of tonic extension during maximal seizures and duration of post-seizure depression showed 10- to 16-fold variations among strains tested. Females in general showed greater convulsive susceptibility than males of the same strain, probably because of the excitatory action of estradiol. In males, differences in seizures were compared with values for brain acetylcholine (ACh) concentrations and cholinesterase (ChE) activities reported previously for these strains. In each of the 3 pairs of strains, selectively bred from 3 foundation stocks, the strain with the lower seizure threshold had the higher ChE. On the other hand, intensity of seizures and post-seizure depression could not be related to brain ACh or ChE.

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Role of the Thymus in Recovery of the Immune Mechanism in the Irradiated Adult Mouse. (28170)

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It is now well established that the thymus is essential for the provision of cells with immunological competence during development(1,2,3,4,5). Mice thymectomized at birth characteristically show (a) the development of a fatal wasting disease between 1 and 4 months of age (actual age depending on the strain(2,3,6), (b) a marked deficiency of small lymphocytes in blood and tissues (1,2,3), (c) a diminished ability to produce serum antibodies to some antigens(2,7), and (d) a permanent failure to reject skin grafts not only from foreign strains but also from rat donors(2,3). Thymectomy after 3 weeks of age is not associated with any marked defect except for a reduction in lymphoid cell population(8). This might at first suggest that the specific function of the thymus is restricted to the period of life before it involutes. Experiments performed in this laboratory(9) have, however, clearly shown that the thymus in adult life is essential for com-