Evidence of an Eocene age for volcanism on Guam is consistent with evidence on age of volcanism from other Mariana islands south of 16° north latitude (Fig. 1). Conclusive evidence of post-Eocene volcanism in the Marianas has not yet been found on islands to the south of the young volcanic chain that ranges northward from Anatahan. Rocks of Oligocene age are as yet unrecognized in these islands, volcanic materials of known Miocene age can be interpreted as reworked from Eocene deposits, and younger rocks and sediments in no way suggest volcanic affinities. If, however, volcanic rocks younger than Eocene, or any rocks of Oligocene age, are to be found in the Marianas, Guam is a good place to look for them. The reticulate camerinid of the fauna here noted has an "Oligocene flavor," and succeeding rocks in a normal sequence might grade to the Oligocene.

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Audiogenic Seizure and the Adrenal Cortex

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This study was designed to test the hypothesis that ACTH injections sufficient to increase adrenal cortical size modify the susceptibility of rats to audiogenic seizure (hereafter designated AGS). The rationale of the use of ACTH in relation to AGS susceptibility is that both endogenous and exogenous ACTH and adrenal cortical hormones have been shown to be important in the adjustment and adaptation of mammals to stress of physical, physiological, and psychological origin. The behavioral relevance of adrenal cortical function in stress adaptation has been discussed by Hoagland (*1*). The AGS situation can be conceived as stressful from two points of view: first, the environmental conditions used to produce it—exposure to intense sound while closely confined; and second, from the standpoint of the behavior of the rat displaying a complete audiogenic seizure. Such an animal releases tremendous amounts of energy in a severe bout of running and a subsequent tonic-clonic convulsion. Furthermore, the catatonic state which sometimes terminates the abnormal behavior probably overlies a state of increased energy mobilization.

In the experiment described here, 80 male and female hooded rats, approximately 40 days old, and obtained from a colony maintained at the Louisiana State University psychology laboratory, were used.

The seizure box was 48 in. high by 26 in. wide and was constructed of double layers of plywood insulated in the middle with asbestos. A double glass window in the top permitted observation of the subjects at all times. Within the box was the actual seizure chamber, a metal cylinder 28 in. high, with a diameter of 14 in. The sound source, consisting of two 3-in. electric bells operating off a 6-v transformer, was placed under the perforated floor of the cylinder 6 in. from the rats. The entire apparatus was constructed in such a fashion as to reduce extraneous stimuli to a minimum. Light was supplied by a 6-v bulb in the top of the box, so arranged as to maintain a constant visual field overhead; hence, distractions from the observation window were reduced to a minimum. All electric controls for the seizure box were located on an instrument panel on top of the box.

The adrenocorticotrophic hormone used in this study was equal to the Armour Standard.[1]

PHASE A

1. *Test for AGS susceptibility.* Seizure susceptibility was determined by one diagnostic exposure to sound stimulation. Preliminary experience with the particular strain of animals used indicated that the level of AGS susceptibility within the strain was approximately 50% with respect to the stimulus used. A 50% incidence of AGS was desirable, since it presented an equal opportunity for the detection of increasing or decreasing AGS frequency in the experimental animals. The diagnostic exposure was given 4 days before ACTH injection and was the animal's first experimental exposure to intense sound stimulation. The time interval was chosen to rule out as far as possible any spacing effects of seizures. There is evidence of refractoriness to seizures following massed seizures, but any such effect should dissipate in 4 days (*2*).

2. *Injection of ACTH.* Susceptible and nonsusceptible animals were equated with respect to weight and sex and assigned to one of four experimental groups. Group I consisted of susceptible animals that received injections of ACTH, Group II of susceptible animals that received injections of water, Group III of nonsusceptible animals that received injections of ACTH, and Group IV of nonsusceptible animals that received injections of water. Ten animals were assigned to each group. The animals receiving ACTH were given 2 mg standard ACTH subcutaneously, and the control animals were given an equal volume of water subcutaneously. The procedure was repeated at 4-hr intervals until three doses totaling 6 mg of standard ACTH had been given.

3. *Seizure test.* Two hr after the last injection of ACTH or water the animals in Phase A were subjected to 2 min of sound stimulation. If a seizure oc-

[1] We are indebted to Irby Bunding, of the Armour Laboratories, for providing the ACTH used in this study.

curred within this period of stimulation, it was allowed to go to completion and the latency, duration, and type of seizure were recorded. Seizure latency was defined as the interval from the activation of the sound source until a seizure began. A seizure was defined as beginning with either explosive running or convulsions. Duration of seizure was defined as the time interval elapsing between onset of seizure as defined above and exhaustion or coma. Seizures were typed with respect to severity and presence or absence of catatonia and coma.

4. *Gland assay.* Ten hr postseizure (12 hr following the last injection of ACTH or H_2O) all animals were killed, and their adrenal glands removed. Immediately following removal, the glands were cleaned of the adhering fat and connective tissue and weighed. This was done immediately after removal in an effort to minimize variability resulting from loss of moisture after removal from the animal.

Phase B

Phase B of the study was carried out to discover the effect on size of the glands of a seizure test 12 hr prior to removal of adrenal glands. The only difference between Phase A and Phase B is that, in Phase B, AGS-susceptibility tests (Step 3) were omitted.

Results

In Phase A, all the susceptible animals that got ACTH had seizures upon presentation of the auditory stimulus. Eight of the 10 susceptible animals that received water had an AGS when sound was presented. In no case did an animal that had been previously classified as nonsusceptible have a seizure when sound stimulation was presented. The results indicate that the amount of ACTH used in this study has little effect upon the incidence of audiogenic seizure when administered to animals of known seizure susceptibility. This conclusion is further supported by the fact that no difference in latency, duration, or severity of seizures was noted between susceptible animals that received ACTH and those that received water.

Table 1 gives the means and standard deviations of the percentage body weights of the adrenal glands of the eight groups of rats. Percentage body weights (total adrenal wt/body wt $\times$ 100) were used in order to correct for differences in body weight. It will be noted in Table 1 that the ACTH-injected rats possessed larger adrenal glands than the control rats, the susceptible rats appear to possess larger adrenal glands than the nonsusceptible animals, independent of ACTH injection, and Phase A gland sizes do not differ systematically from Phase B gland weights. Each of these interpretations was tested by analysis of variance, the results of which are presented in Table 2. Table 2 shows that the ACTH gland weights

TABLE 2

Analysis of Variance of Percentage Body Weights of Adrenal Glands for Eight Groups of Subjects Tested under Different Conditions

Source of variation	S.S.	df	Mean square	f*
ACTH vs. H_2O	24,657	1	24,857	8.39
Susceptible vs. nonsusceptible	21,550	1	21,550	7.28
Seizure–no seizure test	195	1	195	
Interaction seizure test $\times$ susceptibility	10.31	1	10.31	
Interaction seizure–nonseizure test $\times$ ACTH-H_2O	18.12	1	18.12	
Interaction suscuptible-nonsusceptible $\times$ ACTH H_2O	1.09	1	1.09	
Interaction susceptible-nonsusceptible $\times$ ACTH-H_2O $\times$ seizure test	43,240	1	43,240	14.60
Within groups	154,006	72	2,962	
Total	243,677	79		

* The value of f, was not computed where the mean square between groups was obviously smaller than the mean square within groups. Only those f ratios in excess of the 1% level are given.

differ significantly from the control glands, thus indicating the efficacy of the ACTH injections as stimulants of the adrenal cortices. Table 2 also reveals that a significant difference exists between the adrenal weights of susceptible and nonsusceptible rats. The analysis of variance indicates no statistically significant difference between the glands of animals under-

TABLE 1

Means and Standard Deviations of Percentage Body Weights of Adrenal Glands

Group	Phase A $N=40$				Phase B $N=40$			
	Seizure test following injection				No-seizure test following injection			
	Susceptible		Nonsusceptible		Susceptible		Nonsusceptible	
	ACTH	H_2O	ACTH	H_2O	ACTH	H_2O	ACTH	H_2O
	I	II	III	IV	V	VI	VII	VIII
Mean percentage body weight of adrenal glands	.0405	.0313	.0331	.0280	.0333	.0350	.0337	.0322
Standard deviation	.0037	.0042	.0050	.0027	.0050	.0063	.0035	.0037

going two seizures in a 4-day period (Phase A) and rats undergoing only one seizure 4 days before gland removal (Phase B). Having a seizure 12 hr before gland removal did not contribute to gland weight. Finally, it will be seen that the only significant interaction was the triple interaction among susceptible-nonsusceptible, ACTH-water, and the Phase A-Phase B variables. This calls for an intensive experimental analysis of the relatedness of these three sets of factors.

The initial problem set for this investigation was the determination of the effect of adrenal cortical stimulation on the AGS susceptibility of laboratory rats. This problem was attacked in Phase A of the present study, and results indicate that ACTH injections sufficient to increase the weight of the adrenal gland produce no reliable change in the AGS susceptibility of either susceptible or nonsusceptible rats.

It is of interest that the adrenal glands of the AGS-susceptible groups are heavier than those of the nonsusceptible groups. It is possible that rats undergoing AGS have larger glands as a result of the seizure. The seizures induced in these animals are characterized by severe explosive bouts of running, followed by tonic-clonic convulsions, and terminating in a state of catatonia. This tremendous, though short-lived, energy release may be sufficient to produce adrenal cortical hypertrophy. It will be recalled that the adrenal glands were removed from half the rats (Phase A) 12 hr after the second of two seizure tests (the first having been 4 days before the second), and from half the rats (Phase B) 4½ days after a single seizure test. If a seizure-induced adrenal cortical hypertrophy exists, it is doubtful if 12 hr, or even 4½ days would result in a complete return to normal adrenal cortical weight. On the other hand, if seizure-induced cortical hypertrophy does occur, there might be evidence of a summation of the effects of the two separate seizures. No such trend was found in these data. Examination of Tables 1 and 2 reveals no significant or suggestive signs of a summation of the two seizures. Absence of a summation effect following two seizures does not permit the assumption that the first seizure did not produce gland hypertrophy, however. If seizure behavior produces an increase in gland weight it may well be that this increase in weight reaches a limit after one seizure. Subsequent seizures might act as a similar physiological stimulant of the gland without producing further increments in gland weight. The possibility of seizure-induced cortical hypertrophy cannot be conclusively evaluated within this study. Future studies in this laboratory will be directed toward the question of whether larger adrenal glands precede or follow audiogenic seizures. If the latter is true it will be of biological interest that such a transient, though severe, bout of activity is sufficient to produce increased adrenal cortical weight. If future evidence indicates that AGS-susceptible rats have larger adrenal cortices than nonsusceptible rats, independent of seizure occurrence, it will be an interesting and provocative correlation of a biological and behavioral characteristic. Other studies have suggested a relationship between the adrenal cortex and convulsive seizure threshold, frequency, and severity. Griffiths (*3*) observed that adrenalectomy reduced either seizure incidence or severity in rats. Woodbury and Sayers (*4*) have shown that both ACTH and cortisone lower the electroconvulsive threshold in rats pretreated with desoxycorticosterone. These studies suggest the need for exploration of the possibility that seizure-susceptible rats have larger adrenal cortices than nonsusceptibles, independent of any immediate effect of the seizure.

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Nutritive Value of Rust-infected Leaves

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The habit of certain snails, slugs, beetles, and insect larvae of eating the rusted areas of bean, broad bean, or snapdragon leaves or stems in preference to the healthy portions of these plants suggested that rusted tissues might have unique nutritive properties. The reported salutary effect of rusted tissues on farm animals and man (*1*); the greater invasiveness of a powdery mildew (*2*), of several viruses (*3*), and of Fusaria (*4*), in rusted than in normal tissues; the collection of rust spores by bees (*5*); and the high carotene content of rusted leaves (*6*), all support this idea.

Microbiological assay (*7*) of primary pinto bean (*Phaseolus vulgaris*) leaves infected with rust (*Uromyces phaseoli*) revealed a higher pantothenic acid content in rusted than in healthy leaves (Fig. 1). The pantothenic acid content of inoculated leaves increased up to at least 12 days after inoculation, at which time it was about 10 times as great as that of normal leaves. Rust uredospores alone showed 45 γ pantothenic acid/g spores, and it might therefore seem that the pantothenic acid of rusted leaves was primarily localized in the rust mycelium and spores. No clear difference between healthy and rusted leaves with respect to thiamin, riboflavin, folic acid, or niacin content was detected in a preliminary test.

Snails (*Helix aspersa*) ranging from 0.3 to 8 g in weight were confined singly in pint jars or large Petri dishes with healthy bean leaves and/or with bean leaves inoculated 6–10 days previously with