CONFIRMATION OF THE ROSENOW ANTIBODY-ANTIGEN SKIN REACTION IN IDIOPATHIC EPILEPSY

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Rosenow (1947b) has reported the isolation of an immunologically identifiable strain of alpha streptococcus from patients with idiopathic epilepsy. He also reports that the intradermal injection of antibodies specific for this strain elicits an erythematous skin reaction in patients harbouring this organism, and in a high percentage of other epileptic patients as well. This paper presents an investigation and a statistical verification of this reaction in epileptic patients in England; antibodies prepared in the United States from organisms isolated there were used.

Materials and Methods

Two groups of patients were tested at the National Hospital; one consisted of patients considered to have idiopathic epilepsy; the other was made up of unselected patients from the out-patient clinic. The number and sex distribution of the test groups was similar, but the average and mode age of the epilepsy group was younger. It will be shown that this does not affect the results or conclusions. A comparison of the two groups is given in Table I.

TABLE I
COMPOSITION OF THE TEST GROUPS

	Control Group	Epilepsy Group
Total ..	105	117
Sex :		
Men ..	53	58
Women ..	52	59
Age spread ..	4-77 years	3½-72 years
mean ..	47.5 years	26.9 years
mode ..	4th-5th decade	2nd decade

Specific antibodies for strains of alpha streptococci isolated from the nasopharynx of patients with idio-

pathic epilepsy, multiple sclerosis, schizophrenia, arthritis, and migraine were made by Dr. E. C. Rosenow (1947a and b; 1948b, c, and d) in Cincinnati, Ohio, U.S.A., and sent by air mail to England. He also prepared and sent a control test solution of 0.9% NaCl containing 0.02% phenol.† For purposes of discussion, the diseases of the patients from whom the alpha streptococci were isolated will be used to identify the test antibodies.

All patients were tested with all antibodies whenever possible. The volar surface of the forearm was found to be the most convenient area for the test, though other areas would serve as well. After the skin was washed lightly and allowed to dry, 0.03 ml. of the test material was injected intradermally, and the maximum flare which usually appeared within five minutes was outlined with ink or skin pencil. The area of erythema was measured in square centimetres by superimposing circles of known area drawn on cleared radiograph film. All tests were done by the same person, who, when this was possible, had no knowledge of the clinical diagnosis.

In considering the results, the difference between the reaction produced by the saline phenol control solution, and that produced by the test antibody has been used as the measure of reactivity to the antibody.

Results

The mean reaction of the epilepsy group to the "epilepsy" antibody was 9.42 cm.² with a spread of 0.0 to 19.62 cm.², and a standard deviation of 4.72 cm.² The mean control group reaction to this antibody was 1.87 cm.² with a spread of 0.0 to 13.89 cm.², and a standard deviation of 2.09 cm.² It at once appears that this is a true difference. Calculation shows that the probability of this being a chance variation is, for practical purposes, zero.

Comparison of the other means shows a significant difference between the mean reaction of the two groups to the "migraine" and "arthritis" anti-

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† This concentration of phenol was used as a preservative in the antibody solutions.

TABLE II

COMPARISON OF THE ERYTHEMATOUS REACTION OF EPILEPTIC AND NON-EPILEPTIC PATIENTS TO ANTIBODIES ISOLATED FROM PATIENTS WITH VARIOUS DISEASES

Alpha Streptococcus Isolated from Patients with	No. Tested		Difference in Area (in cm. ²) of Reaction between Test Antibodies and Saline-phenol control								Comparison of the Means	
			Mean		Spread		Standard Deviation from Mean		Standard Error of Mean			
	Control Group	Epileptic Group	Control Group	Epileptic Group	Control Group	Epileptic Group	Control Group	Epileptic Group	Control Group	Epileptic Group	Critical Ratio	Probability
Idiopathic epilepsy ..	105	117	1.87	9.42	0-13.9	0-19.6	2.09	4.72	.204	.442	15.56	≈ 0
Migraine	102	77	2.51	3.54	0-12.6	0-12.6	2.84	2.87	.289	.326	2.39	≈ .02
Disseminated sclerosis	101	77	2.01	2.13	0- 8.8	0-12.5	1.75	2.38	.175	.270	0.26	≈ 0.8
Arthritis	101	80	1.68	2.50	0- 7.9	0- 8.0	1.66	2.63	.167	.293	2.43	≈ .02
Schizophrenia ..	68	78	1.70	2.40	0- 8.9	0-10.7	1.82	2.29	.227	.261	2.02	≈ .05

bodies, but these means are too close to be of importance in any one patient. These results are summarized in Table II.

In order to determine any effect the age difference of the two groups might have had, a study was made of those patients from the fourth and fifth decade, the mode of the control group. Thirty-one epileptic patients and 45 control patients fell into this age range. There were about half from each decade, and they were evenly split between the sexes. The mean epileptic group reaction to the epilepsy antibody was 8.14 cm.² with a standard deviation of 3.69 cm.², while the control group mean was only 1.43 cm.² with a standard deviation of 1.66 cm.² Comparison of the means gives a critical ratio of about 10, which indicates that the probability of such a chance variation is again zero for practical purposes. There were too few patients in the control group whose age was at the mode of the epileptic group to make a similar comparison at that age.

If a reaction greater than the mean reaction of the control plus two standard deviations is taken as significant (i.e. 6.05 cm.²), 78.8% of the patients with idiopathic epilepsy had positive reactions to the "epilepsy" antibody. This is somewhat smaller than the figure of 96% positive reaction given by Rosenow (1948c). This difference has resulted from the different criteria used for a positive reaction, because the mean reaction given by Rosenow

(1948c) of 9.63 cm.² in epileptic patients is within chance variation of the 9.42 cm.² mean of this series. Rosenow (1948c) states without giving statistical data that a reaction greater than 5 cm.² was significant. This is somewhat smaller than the critical figure of 6.05 cm.² found by statistical analysis of my results. However, it must be pointed out that this is a purely arbitrary value derived by mathematical methods which were designed to eliminate the chance inclusion of any normals in the positive group, but, because of the overlap of the amount of reaction in the two groups, some positives will be excluded.

A comparison with some of Rosenow's data is given in Table III.

Effect of Medication.—Almost all the epileptic patients tested were taking medication in the form of either phenobarbitone, diphenylhydantoin (dilantin, epanutin), tridione, or a combination of these drugs. However, a few patients were also taking benzedrine or a bromide, and some were without any medication. No correlation has been found between any one drug or combinations of drugs and the reaction to the intradermal injection of the "epilepsy" antibody.

Epileptic Patients with Negative Reactions to "Epilepsy" Antibody.—A comparison of the clinical picture of the epileptic patients who had negative reactions with the positive group did not reveal any marked difference in sex, age, age of onset, duration of disease, type of seizure, or medication. The interval since the last

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TABLE III

A COMPARISON BETWEEN PATIENTS WITH IDIOPATHIC EPILEPSY TESTED BY ROSENOW (U.S.A.) AND THOSE OF THIS STUDY (ENGLAND)

Source	Reaction to Antibodies Isolated from Patients with							
	Epilepsy				Schizophrenia		Arthritis	
	No. Tested	Mean Reaction	Size Reaction Considered Positive	Considered Positive (%)	No. Tested	Mean Reaction	No. Tested	Mean Reaction
Rosenow (personal communication and 1948d) ..	118	9.61 cm. ²	5 cm. ²	96	118	5.18 cm. ²	118	2.45 cm. ²
This study (England)	117	9.42 cm. ²	6.05 cm. ²	78	78	2.40 cm. ²	80	2.50 cm. ²

seizure before the skin test was made seemed important. The incidence of negative reactions was significantly higher in the group who had not had a seizure for a month or more, and was very low among patients who had had a seizure less than a week before the skin test was done.

The electroencephalograms (EEG) were suggestively better in the negative group, but the number of observations was small and statistically unimportant.

Seven patients had reactions that were significantly negative. As this is about twice the number expected on the basis of a normal distribution, a separate examination of their clinical status was indicated. Three of these patients had normal electroencephalograms, three had generalized EEG abnormalities, and one had not had an EEG. Of the three who had abnormal EEGs, one was dark skinned which makes the skin test difficult, one was a man of 56 with epilepsy of late onset, and one was a boy of seventeen. All these patients were having seizures regularly in spite of medication. The three patients who had negative EEGs had not had any seizures for one month, two years, and eight years respectively. The patient who had not had an EEG had not had a seizure for five years before the skin test was done.

Repeated Tests on the Same Patient.—Three people were tested four, 11, and four times over a period of

six weeks. The reaction never varied more than one division on the area measuring device. Several other patients had two skin tests at intervals up to two months. Here again the tests were uniformly similar in result, and only if the reaction was near the critical area was there a variation between positive or negative.

Patients in the Control Group with Seizures.—There were four patients in the control group who were having seizures associated with some known organic disease. Their diagnosis included parietal tuberculoma, pre-senile dementia, hypertensive encephalopathy, and C.N.S. syphilis. All these patients had negative skin reaction to the "epilepsy" antibody. This agrees with Rosenow (1948c).

Patients with Post-Traumatic Epilepsy.—Three patients with post-traumatic epilepsy were tested. One was negative, and two reacted strongly to the "epilepsy" antibody. Each of the positive patients was retested and again found to be positive.

Patients in the Control Group with Positive Reactions.—There were seven patients (6.7%) of the control group without a history of ever having a seizure, but who had positive reactions to the "epilepsy" antibody. Their diagnosis included facial palsy, chronic anxiety

TABLE IV

A COMPARISON OF THE ERYTHEMATOUS REACTION OF A GROUP OF NON-EPILEPTICS LIVING WITH EPILEPTICS AND ONE UNASSOCIATED WITH EPILEPTICS TO ANTIBODIES ISOLATED FROM PATIENTS WITH IDIOPATHIC EPILEPSY

	No. Tested	Reaction to Antibody Isolated from Patients with Idiopathic Epilepsy		Comparison Between Groups		
		No. Positive Reactions	Positive Reactions (%)	Standard Error of Difference	Critical Ratio	Probability
Non-epileptics living with epileptic patients ..	15	4	26.6	8.2	2.4	.016
Non-epileptic people unassociated with epilepsy ..	105	7	6.7			

state, cerebral thrombosis, traumatic spinal injury, carotid aneurysm, no disease, and hypertensive encephalopathy. The patient who had had a traumatic spinal injury also reacted to the "arthritis" antibody, and the patient with the facial palsy also reacted to the "migraine" antibody. In the other patients the reaction was limited to the "epilepsy" antibody.

Skin Reaction of Families of Epileptic Patients.—The testing of those living with epileptic patients was done on a few people towards the end of the study. A statistically significant increase in the incidence of positive reactions was found among these people as compared to the general control group. If this reaction indicates the presence of a specific organism, particularly one in the nasopharynx, an increased incidence of positive reactions might be expected among people intimately associated with those harbouring the organism.

Table IV gives the precise data.

Discussion

The association of idiopathic epilepsy with a skin reaction to certain alpha streptococci antibodies is definite. However, the meaning of this reaction is not clear. Speculation as to the significance of this test leads immediately to two possibilities: either the alpha streptococcus is aetiologically significant, or the host has a specific effect on the antigenic structure of the organism.

Rosenow (1948c) states that shortly before a seizure, the skin reaction to the epilepsy antibody rises, indicating an increase in circulating antigen; and that directly after a seizure the skin reaction to antibody falls sharply, showing a disappearance of the circulating antigen. He also states that if antigen is used as a skin testing agent, there is often a positive test after a seizure, showing the presence of circulating antibody. This suggests a local sensitization in the brain with circulating antigen as a trigger mechanism. Such a method has been used by Kopeloff and others (Kopeloff, Barrera, and Kopeloff, 1942 and 1944) for the experimental production of epilepsy. Though in the present study insufficient observations were made at or near the time of seizures to shed any light on this question, a definite association was found between the occurrence of seizures and a positive skin reaction to the "epilepsy" antibody.

This study was designed simply to verify the validity of the skin reaction as described by Rosenow (1947b), and no effort was made to investigate the aetiological or pathological significance of the alpha streptococcus in the patients tested. However,

the high incidence of positive reactions among people living with epileptic patients suggests a transmissible agent.

Summary

One hundred and seventeen patients with idiopathic epilepsy and 105 non-epileptic patients were tested in England with an intradermal injection of antibodies made in the United States to strains of alpha streptococci isolated from patients in the United States with divers diseases, including idiopathic epilepsy.

The results of the tests have been submitted to statistical analysis which shows the incidence of positive reactions of the epileptic patients to the "epilepsy" antibody to be well beyond any chance variation.

There were 78% positive reactions among the epilepsy patients to the "epilepsy" antibody, and 6.7% positive reactions to this antibody in the control patients. This has been compared with Rosenow's data.

There was definite correlation between the epileptic patients who had negative reactions to the "epilepsy" antibody, normal electroencephalograms, and time since last seizure before the skin test.

All patients with known organic cause for their seizures were negative to the "epilepsy" antibody except two patients with post-traumatic epilepsy.

A statistically higher incidence of positive reaction to the epilepsy antibody was found among non-epileptics living with epileptic patients than in the control group.

No evidence as to the relationship of this reaction to the disease has emerged from this study.

I am glad to have this opportunity to thank Dr. E. A. Carmichael for making this study possible and for his constant encouragement and advice. I also want to thank Dr. E. C. Rosenow for his willing cooperation and for supplying the test materials so liberally.

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