9.4- and 36-percent dilutions for the extracts from schizophrenic and nonschizophrenic patients, respectively.

These values strongly indicate that the observed differences are due to quantitative rather than qualitative factors.

One-dimensional descending chromatograms of the concentrated stock solution [Whatman No. 1 paper; 24° to 27°C; solvent: isopropyl alcohol, ammonium hydroxide, and water (40:1:9, vol/vol)] revealed several isolated spots under ultraviolet light of short wavelength. The volume of the water extracts for each of these spots was reduced to a proper amount and the extracts were injected into mice. The substance (or substances) from a blue spot (approximate R_F value, 0.69) produced pattern 4; the substance (or substances) from a purple spot (R_F value, 0.56) produced patterns 1, 2, and mainly 3, but with more frequent jumps (80 to 100 per minute) and a longer period of jumping behavior (20 to 30 minutes). From another area of the chromatograms, substances were obtained that produced violent scratching for 15 to 30 minutes, or Straub's tail phenomenon, lasting 20 to 40 minutes in the mild case. Water extracts from other areas produced only slight effects. The chromatographic separations were confirmed by several replicate runs.

Work is continuing on the identification of the active substances, which seem to be relatively stable and simple molecules, and on their significance in schizophrenia (*7*).

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7 June 1961

Epileptogenic Cerebral Electrical Activity and Serotonin Levels

Abstract. Chronically epileptic cats and monkeys showed marked activation of paroxysmal electrographic abnormalities both in the original focus and in a number of structures with secondarily altered functional states after intraperitoneal injection of the serotonin precursor 5-hydroxytryptophan (10 to 25 mg/kg) plus vitamin B_6. Much less 5-hydroxytryptophan was required to produce such epileptogenic activation if the animals had previously been treated with Marsilid, a monoamine oxidase inhibitor. Marked paroxysmal activity in epileptic animals was also produced by injection of Marsilid alone or of Marsilid in combination with reserpine. Since all of the activating agents used have been shown by others to elevate brain serotonin levels, the epileptogenic activation may be correlated with such high levels. Since the effects were at least partially blocked by atropine, such "serotonin-induced" activation may possibly involve some cholinergic mechanism.

During recent years, increasing significance has been attached to the alteration of the brain levels of neurohumoral agents such as serotonin and the catecholamines in the modification and organization of behavior. However, very little attention has been paid to the possible effect of such neurohumoral agents on epileptogenic electrical activity. In a recent study it was concluded that elevation of the serotonin level was responsible for the decrease in electroencephalographic abnormalities in epileptic patients after administration of Marsilid (*1*).

It is the purpose of this study (*2*) to show that, on the contrary, measures which are known to elevate serotonin levels cause increased electroencephalographic abnormalities in epileptic animals.

Three monkeys (two normal, one epileptic) and six cats (two normal, four epileptic) were used. All the animals had 42 to 50 electrodes permanently implanted in both the cortical and the deep structures (*3*). The epileptic animals had been made epileptic by placing aluminum hydroxide in one sensorimotor or frontal polar cortex at least 3 to 4 years prior to the experiment under discussion (*4*). A number of agents, or combinations of agents, which have been shown by others to increase the brain levels of physiologically active serotonin were used. These were (i) the serotonin precursor DL-5-hydroxytryptophan (5-HTP) (10 to 50 mg/kg), plus the decarboxylase coenzyme vitamin B_6 (50 mg/kg) (*5*); (ii) the monoamine oxidase inhibitor Marsilid (100 mg/kg), alone (*6*) or with 5-HTP (*7*); and (iii) Marsilid plus reserpine (0.1 mg/kg) (*7*). All the agents were given intraperitoneally. The electroencephalographic recording was made at least once every hour up to 24 hours after administration of any single agent. The time sequence of the administration, when more than one agent was given, is specified in the legend to Fig. 1.

None of the measures used had a notable effect on either the behavior or the electroencephalogram of the normal animals, except that some drowsiness and electrographic slowing was noted in normal animals given the larger doses of 5-HTP (25 mg/kg) or the smaller doses of 5-HTP (15 mg/kg) 17 to 20 hours after administration of Marsilid.

In epileptic animals, on the other hand, all of these agents produced marked activation of spontaneously occurring epileptic abnormality. Augmented paroxysmal discharges, of identical morphology and localization of spontaneous abnormality, occurred almost continuously up to 8 hours after administration of 5-HTP (15 to 25 mg/kg) plus vitamin B_6 (50 mg/kg) (Fig. 1, *d–f*). However, such electrographic evidence of epileptogenic activity was not accompanied by any behavioral seizure phenomena. When the animal was injected with Marsilid 17 to 20 hours before the administration of 5-HTP, as little as 10 mg of the latter per kilogram produced a similar but greatly intensified effect (Fig. 1*j*). Electrographic abnormalities, somewhat less marked but of almost identical morphology and localization, were produced in epileptic animals by administration of Marsilid or reserpine alone (Fig. 1*i, k, l*), or, more strongly, by Marsilid plus reserpine (Fig. 1, *m,n*). While the early effects of reserpine alone (Fig. 1*k*) and of 3,4-dihydroxyphenylalaine (DOPA) plus 5-HTP (Fig. 1*g*) were very similar, later, at 24 hours (Fig. 1*l*), the effect of 5-HTP was clearly predominant (Fig. 1, *d–f* and *h*). This finding is compatible with the view (*8*) that reserpine simultaneously releases physiologically active serotonin and catecholamines in the brain but that, because of the very rapid synthesis of serotonin (*9*), the effect of free serotonin is predominant in later reserpine action. No such epileptogenic-activating effect was noted when vitamin B_6 was given, either alone or with DOPA (25 to 100 mg/kg), the precursor of the catecholamines. The latter had eliminated all the spontaneously occurring epileptogenic ab-

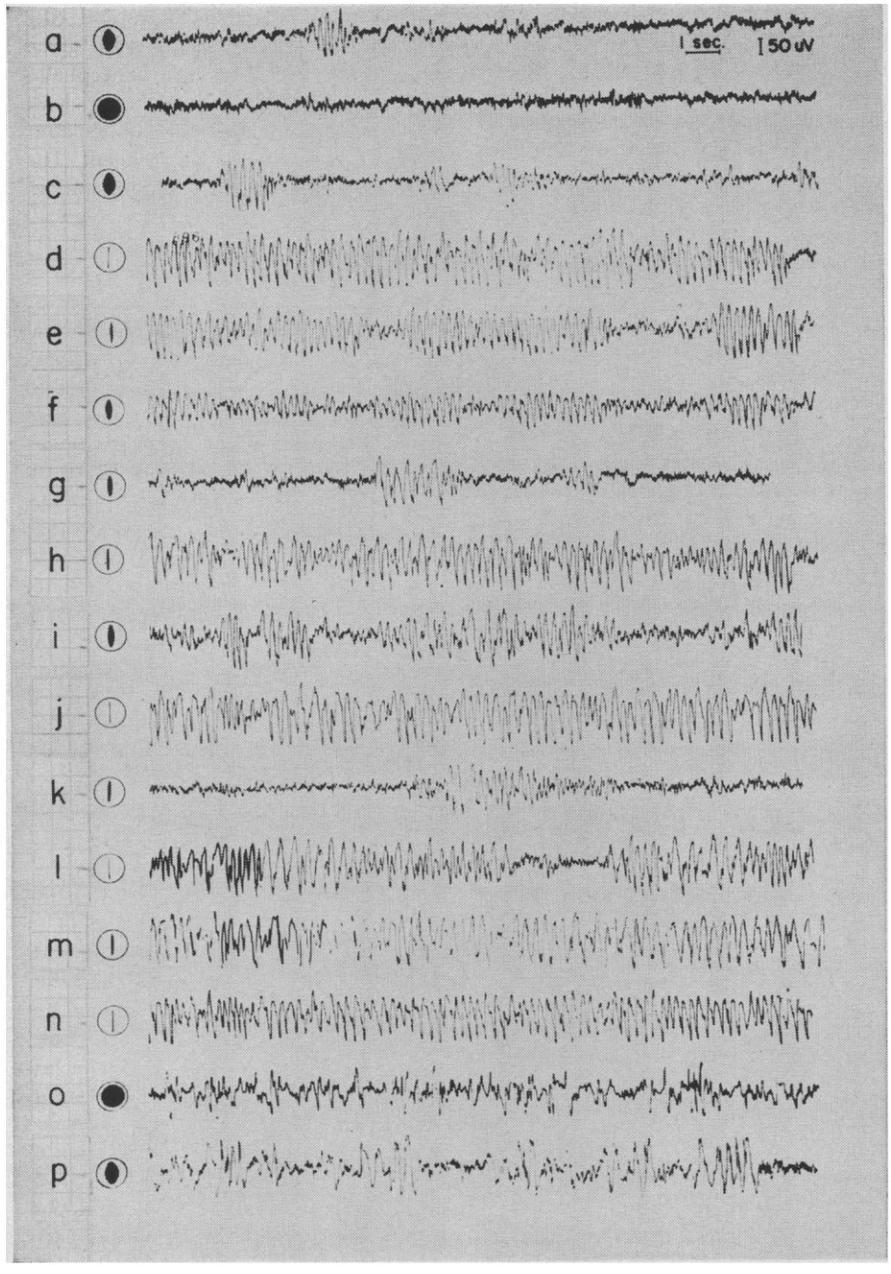

Fig. 1. Electhoencephalographic recordings from the left parietal cortex of epileptic cat No. 40. This cat was made epileptic about 4 years ago by placing aluminum hydroxide on the left sensorimotor cortex. The original focus was subsequently removed about 2 years ago. The size of the pupils at the time of recording is shown at left. *a*, Control resting state; relaxed. *b*, DOPA (25 mg/kg) plus vitamin B_6 (50 mg/kg), at 1 hour; very alert, attentive. *c*, Same as above, at 4 hours; relaxed. *d*, 5-HTP (25 mg/kg) plus vitamin B_6 (50 mg/kg), at 1 hour; very frightened, yowling, hissing when approached. *e*, Same as above, at 4 hours; no yowling or hissing, accepts caressing. *f*, Same as above, at 8 hours; under slight sedation. *g*, DOPA (25 mg/kg), 5-HTP (25 mg/kg), plus vitamin B_6 (100 mg/kg), at 1 hour; very alert but scared, hissing when approached. *h*, Same as above, at 4 hours; less alert, quiet and friendly. *i*, Marsilid (100 mg/kg), at 17 hours; very quiet. *j*, 5-HTP (10 mg/kg) plus vitamin B_6 [50 mg/kg, 17 hours after Marsilid (100 mg/kg)], at 1 hour; frightened, salivating, yowling, hissing when approached, diarrhea. *k*, Reserpine (0.1 mg/kg), at 4 hours; relaxed. *l*, Same as above, at 24 hours; markedly sedated, diarrhea. *m*, Reserpine [0.1 mg/kg, 17 hours after Marsilid (100 mg/kg)], at 4 hours; relaxed. *n*, Same as above, at 24 hours; markedly sedated, diarrhea. *o*, Atropine [1.0 mg/kg, 3 hours after reserpine (0.1 mg/kg), which was given 17 hours after Marsilid (100 mg/kg)], at 1 hour; relaxed and quiet. *p*, Same as above, at 21 hours (24 hours after reserpine, 41 hours after Marsilid); relaxed but not sedated.

normalities in the same animals (Fig. 1, *b* and *c*). When DOPA and 5-HTP were given simultaneously, together with an adequate amount of vitamin B_6, no immediate effect was observed until about 4 hours after administration, when an effect predominantly that of 5-HTP appeared (Fig. 1, *g* and *h*).

Three out of five epileptic animals given 15 to 50 mg of 5-HTP per kilogram developed an overt behavioral disturbance characterized by an attitude of extreme fright, with yowling, hissing, intermittent visual searching, and abrupt retreat. Such behavior was never observed in normal animals given up to 50 mg of 5-HTP per kilogram.

These results suggest that there may be a correlation between the epileptogenic activation noted and elevation of brain serotonin levels. Since barbiturates have been reported to increase brain serotonin levels (*10*), the well-known phenomenon of barbiturate activation of paroxysmal epileptogenic activity may involve the same biochemical process. The relative inertness of normal animals in response to the agents used suggests that epileptogenic cerebral structures may be hypersensitive to serotonin.

Behavioral and electrographic studies of modified levels of brain neurohumoral agents in cats and monkeys (*11*) have led to the belief that a change in the level of any single neurohumoral agent may be less important than production of a disequilibrium among the various endogenous agents. In this connection my co-workers and I have found that the paroxysmal abnormalities induced by administration of 5-HTP and Marsilid in conjunction with reserpine are at least partially eliminated by the administration of atropine (Fig. 1, *o* and *p*). This finding is difficult to explain, but the possibility that the cholinergic mechanism is involved in such activation is now being investigated. A recent report that high levels of serotonin markedly reduced cholinesterase activity (*12*) seems to support such a possibility.

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Effect of Dietary Nitrate on Thyroid Function

Abstract. Experimental results indicate that 0.31 and 0.92 percent dietary nitrate, when consumed by rats and sheep respectively, can affect the normal iodine metabolism of the thyroid gland. The dietary level of iodine appears to be highly important when nitrate is present in the diet.

It has been shown by Wyngaarden *et al.* (*1*) that several monovalent anions, including nitrate, when injected into the animal, can interfere with normal iodine metabolism of the thyroid gland. The effects of nitrate in natural feeds has concerned animal scientists in recent years (*2*). This report attempts to assess the effect of dietary nitrate on the normal functioning of the thyroid gland.

The method of Premachandra and Turner (*3*) was used to determine the goitrogenic effect of nitrate both qualitatively and quantitatively. Adult female rats weighing 200 to 300 g were kept under conditions of uniform temperature of 75°F. The animals were fed a finely ground corn-soybean oil meal diet calculated to contain 135 ppm iodine, for 1 week. Each rat was then injected with 3 μc of carrier-free I^{131}. A 24-hour external thyroid count was made on ether-anesthetized animals placed on a lead plate with the thyroid gland over a scintillation probe. Measurements were made with a scintillation counter connected to a rate meter. Nitrate was added to the ration as KNO_3 at concentrations of 0.5, 1.0, and 2.5 percent.

Table 1. Effect of dietary nitrate on serum total I^{131}, serum-protein-bound I^{131}, and blood NO_3 of sheep.

Activity (count/min per 100 ml serum)		Av. blood NO_3 (mg/100 ml)
Av. total serum I^{131}	Av. PBI^{131}	
Control (five sheep)		
93,170	83,510*	0.80
Treated with 1.5 percent KNO_3 (six sheep)		
59,580	53,950*	1.93

* Significantly different ($p < .01$).

The results, shown in Fig. 1, indicate that dietary KNO_3 at a level of 0.5 percent adversely affected the iodine uptake of the thyroid gland of the rat. This level of nitrate is not uncommon in some hays and ensilages (*4*). The higher levels of nitrate are more commonly found in hay, pasture, and ensilage that are grown either under conditions of unbalanced fertility or the onset of drought or other adverse conditions for plant growth.

Since the above-mentioned feeds are normally given to ruminants, sheep were fed a ration containing 1.5 percent KNO_3. This ration was composed of 58 percent corn cobs, 24 percent corn, 8 percent soybean oil meal, 7 percent molasses, 2 percent minerals, and 1 percent urea. The concentration of serum I^{131} fixed as protein-bound iodine 6 days after 100 μc I^{131} had been injected was used as the criterion (*5*) for normal thyroid function. Methemoglobin was determined and no difference was found between the control and experimental groups. The results in Table 1 indicate that some nitrate passed through the rumen unchanged and that this nitrate interfered with normal thyroid function.

Muhrer *et al.* (*2*) found symptoms of vitamin A deficiency in cattle that consumed nitrate. Garner *et al.* (*6*) showed increased depletion of vitamin A in rats fed nitrate. The function of the thyroid in the conversion of carotene to vitamin A is still debated. However, Johnson and Bauman (*7*) showed that a functioning thyroid gland is necessary for this conversion. In view of these facts, it is conceivable that the vitamin A deficiency is an indirect result of abnormal thyroid function induced by the nitrate.

From a practical view, it is fortunate that the interference of certain monovalent anions with normal iodine metabolism of the thyroid gland can be reversed by increasing the iodine concentration of the diet (*1*). This may help explain the extreme variability in nitrate effect reported by investigators located in different geographic areas. The dietary level of iodine is apparently important for evaluating a feed suspected of containing nitrate.

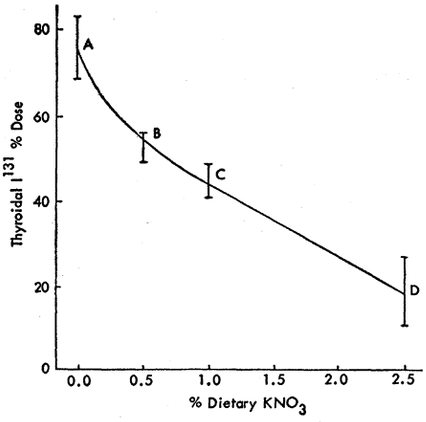

Fig. 1. The effect of dietary KNO_3 on thyroidal uptake of I^{131}. Vertical bars represent standard error of the mean. There were eight, six, six, and four rats in treatments *A*, *B*, *C*, and *D*, respectively. All treatments were significantly different from control *A*: *B*, $p < .05$; *C*, $p < .01$; *D*, $p < .01$.

This research indicates that dietary nitrate can adversely affect the normal functioning of the thyroid gland and suggests that some of the symptoms encountered in the field may be a result of impaired thyroid function of animals. On the basis of this work, we strongly recommend that animal rations suspected of containing nitrates be supplemented with adequate amounts of iodine and vitamin A (*8*).

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