Introduction. The discoveries of isolated clusters of cases of amyotrophic lateral sclerosis (ALS) and other degenerative diseases of the central nervous system (CNS) in the western Pacific Ocean area\(^1\)\(^-\)\(^4\) have centered attention on the variety of their possible causes. Current intensive efforts to determine the etiology of such diseases are directed towards exogenous rather than endogenous or hereditary factors. Compatible with the direction of these studies are the findings of epidemiologic investigations in the Kii Peninsula of Japan and in Guam which show a definite decrease over the past ten years or more in the frequency of ALS.

Since the successful transmission of the disease kuru from man to primate by Gajdusek et al.\(^5\) extensive viral studies on ALS were undertaken but attempts to transmit ALS to primates and other mammals have to date been unsuccessful.\(^6\)

Environmental studies to determine the cause of ALS have been conducted for the past ten years in areas where cases of ALS occur in clusters in the Kii Peninsula\(^7\) and in Guam\(^8\) with the finding of a high content of manganese in soils and waters and a low or lack of calcium and magnesium content in water.

A review of the neuropathology found in sporadic and clustered cases from Kii and Guam indicates that the basic changes occurring in these cases must be considered as part of an aging process in certain areas of the CNS and are seen with or without presenile or senile changes such as Alzheimer's neurofibrillary tangle, granulovacuolar degeneration, or the senile plaque.\(^9\)\(^-\)\(^11\)

From the results of these studies the trace metal content, in particular calcium and manganese, in CNS tissue of cases of ALS and their controls was undertaken using the neutron activation method of analysis.

\(^*)\) Division of Neurological Diseases, Wakayama Medical College.
\(^**\) Department of Neuropsychiatry Wakayama Medical College Hospital.
\(^***\) Research Reactor Institute, Kyoto University.
In this paper the results obtained in genetico-epidemiologic, clinico-neuropathologic, environmental, and CNS tissue analytical studies are presented and the etiopathogenetic process of ALS is discussed.

Results. Genetics and epidemiology. Epidemiologic survey has confirmed a high frequency of ALS in at least two areas in the Kui Peninsula.\textsuperscript{7) Genetic study in the two foci indicates that the disease occurs as the result of an irregular autosomal dominant trait with extremely low penetrance. In the second Kui focus (Hobara) there is a high familial occurrence (over 70\% of cases) of ALS. There are also cases presenting a Parkinsonian picture with mental retardation which seem to be clinically similar to the Guam Parkinsonism-dementia (PD)-complex cases. Table I gives a statistical comparison of the two Kii foci.

A recent study which reviewed Kii ALS cases for the past ten years disclosed a decrease in the mortality rate from 2–3 per 100,000 population to less than 1.0.\textsuperscript{12) Ex-manganese-miner survey in Guam. In Guam, the possible involvement of manganese became of interest because between December, 1941 and July, 1944 manganese deposits were mined in at least two and perhaps three locations. No other mining operations were conducted prior to or after occupation of the island by the Japanese who forced native laborers to mine the deposits.

|                  | Kozagawa          | Hobara           |
|------------------|-------------------|------------------|
| Location         | Mountainous       | Mountainous      |
| Population       | 6191              | 2059             |
| Occupation       | Farming           | Farming          |
| Motor Neuron Disease: Mortality per 100,000 | 13.1             | 45.0             |
| Incidence        | 14.4              | 55.3             |
| Prevalence       | 96.9 (73.9)*      | 192 (152.17)*    |
| Consanguinity %  | 4.3               | 10.0             |
| (First cousin)   |                   |                  |
| M/F              | 2.5/1             | 1.8/1            |
| Age at onset (yr.) | 53.5±7.3      | 50.6±2.3        |
| Age at death     | 57.5±5.7          | 53.8±2.3        |
| Duration of illness (yr.) | 3.2±1.7     | 2.6±1.5         |
| Initial symptom  | Upper             | Lower            |
| (over half the cases) | extremity      | extremity        |
| Familial occurrence | Uncertain      | Over 70\%     |

* Age-adjusted to the total population of Japan, 1969
In general, patients spending more time in the mine were more exposed to manganese than the controls. These data suggest that the Guam manganese miners with a higher degree of exposure were more likely to develop ALS and/or PD, all other factors being equal, than miners with a lesser degree of exposure.\(^8\)

**Analysis of calcium and manganese in environmental samples.** The content of calcium, iron, magnesium, and manganese was determined in soil and water samples from focus areas and their control areas in Guam and the Kii Peninsula. The content of manganese was considerably high in the soil samples from both Kii and Guam, and also in the river and drinking water from Guam. The Kii focus water samples showed a low content or lack of calcium and/or magnesium which is of particular interest (Guam samples were not checked for calcium content).

Geological study of the Kii\(^{13}\) and Guam\(^{14}\) areas points out that they are areas high in limestone deposits.

**Analysis of manganese content in hair of cattle.** Manganese intake in animals in one Kii focus and the control area was evaluated by neutron activation analysis of a biological specimen. Cows in Group A were living within the boundaries of the Kozagawa focus; Group B cows were from a nearby control area. An increase in manganese content in hair collected from the cows during May was demonstrated in the sample from Kozagawa. This manganese had been absorbed by ingestion of field grass, both fresh and dried. A statistically significant difference between the two groups is seen with a risk less than 0.05.

**Analysis of calcium and manganese in CNS tissue of ALS cases.** The quantitative analysis of calcium and manganese content in CNS tissue from 4 ALS cases and 3 controls was determined by the non-destructive method of neutron activation. There was a significant difference in the average calcium content between the cases of ALS (419±108 ppm) and the control cases (252±86 ppm) (\(p<0.001\)) (Table II). In the ALS cases this significantly higher content of calcium was found in the precentral, insula, and hippocampal areas, and in the thalamus, caudate nucleus, internal capsule, medulla, and spinal cord; and it is these areas which are areas of degenerative change in ALS. These results may indicate a soft tissue calcification process in the CNS of the ALS cases.

Also, the contents of calcium and manganese which were determined in CNS tissue of an ALS case and control show a difference in their distribution pattern.\(^{15}\) It is of particular interest that a definite correlation exists between the content of calcium and the content of manganese in the ALS case but not in the control case (Table III).
Discussion. Together with the importance of an environmental factor is the late age of the patient at onset of ALS which suggests that the aging process may contribute to the manifestation of the disease. During aging the disturbance in calcium metabolism is usually seen by a decalcification of most body organs except for the CNS and the skeletal muscles.\textsuperscript{16,17}

A significantly higher content of calcium in samples of CNS tissue from an ALS case deposited in brain sites which correspond to the areas of degeneration in ALS was confirmed by neutron activation analysis. With the high measurement of calcium in certain CNS area in the ALS case and their relevance as areas of neuropathological change in ALS it is therefore assumed that these degenerative changes may be soft tissue calcification with interaction of other diva-
lent cations such as manganese, etc. This assumption is reinforced by the fact of the significant correlation between calcium and manganese content in the ALS case but not in the control case. Manganese, however, cannot be considered as the only possible trigger which accelerates the process of calcification and other cations must be appraised, as a pilot study on content of aluminum in CNS tissue of ALS cases indicates an even higher correlation with calcium.18)

As these above mentioned cations compete as inhibitors of calcium-binding activity they may replace in part the calcium ions, and their action may be reversed by increasing the calcium concentration

| Area                      | ALS Ca | Mn | Control Ca | Mn |
|---------------------------|--------|----|------------|----|
| Precentralis              | 371    | 1.1| 216        | 1.7|
| Postcentralis             | 236    | 1.1| 236        | 1.4|
| Frontal                   | 452    | 1.7| 406        | —  |
| Parietal                  | 416    | 1.2| 407        | 1.7|
| Insula                    | 379    | 1.4| 249        | 1.3|
| Occipital                 | 387    | 1.8| 343        | 1.4|
| Temporal                  | 412    | 1.4| 395        | 1.4|
| Hippocampus               | 506    | 1.2| 233        | 1.8|
| Thalamus                  | 373    | 1.3| 176        | 1.7|
| N. Caudatus               | 406    | 1.4| 189        | 2.7|
| Capsula Interna           | 342    | 1.3| 152        | 1.7|
| Globus Pallidus           | 366    | 1.3| 201        | 1.8|
| Putamen                   | 289    | 1.3| 191        | 1.8|
| Crus Cerebri              | 501    | 1.7| 244        | —  |
| Substantia Nigra          | 352    | 1.1| 105        | —  |
| N. Ruber                  | 462    | —  | 200        | —  |
| Pons                      | 319    | 1.0| 288        | 1.6|
| N. Olivaris               | 634    | 2.2| —          | —  |
| Medulla                   | 538    | 1.5| 247        | 1.8|
| Spinal Cord               | 729    | 1.7| 314        | 2.3|
| Cerebellum (Gray)         | 379    | 1.7| 332        | 2.9|
| Cerebellum (White)        | 293    | 1.1| 170        | 1.3|
| Locus Ceruleus            | 492    | —  | —          | —  |
| N                         | 23     | 21 | 21         | 17 |
| \( \bar{x} \)            | 419    | 1.4| 252        | 1.8|
| SD                        | 108    | 0.3| 86         | 0.4|
| r                         | 0.6703 |    | 0.0297     |    |
| t                         | 3.7413 |    | 0.1151     |    |
| p                         | p<0.01 |    | 0.1<p      |    |
in the external medium. Thus, the potent manganese-binding protein may trigger calcification in the CNS to be replaced later by calcium ions under a secondary hypercalcemic state. It is suggested that low calcium intake over a period of time induces a compensatory hyperparathyroidism leading to calcium resorption from bone which may accelerate the soft tissue calcification in CNS in individuals living in the disease foci or in individuals in specific physical conditions. Many individuals, however, who live in the foci and are without symptoms of neurological disease must be considered in any hypothesis about susceptibility. The neuropathology of ALS demonstrates the existence of multinuclear nerve cells and/or a dislocation of the neuron, and this finding may well be one such disposing factor in the individual.

This research was supported in part by a USPHS NIH Grant (NS05441) and by Japanese Health and Welfare Ministry (1973) and Education Ministry (1973) Grants.

References

1) Arnold, A., Edgren, D., and Palladino, U.: J. Nerv. Dis., 117, 135-139 (1953).
2) Kurland, L., and Mulder, D.: Neurology, 4, 355-378 and 438-448 (1954).
3) ———: Neurology, 5, 182-196 and 249-268 (1955).
4) Kimura, K., Yase, Y., and Higashi, Y. et al.: Dis. Nerv. Sys., 24, 1-5 (1963).
5) Gajdusek, D., Gibbs, Jr., C., and Alpers, M.: Nature, 209, 794-796 (1966).
6) Gibbs, Jr., C., Gajdusek, D.: Motor Neuron Disease. Grune & Stratton, New York, pp. 269-279 (1969).
7) Handa, Y., and Yase, Y.: Igaku no Ayumi, 73, 478-484 (1970).
8) Yase, Y.: Lancet, 2, 282-296 (1972).
9) Shiraki, H.: Motor Neuron Disease. Grune & Stratton, New York, pp. 80-84 (1969).
10) Yase, Y., Matsumoto, N., and Azuma, K. et al.: Arch. Neurol., 27, 118-128 (1972).
11) Shiraki, H., and Yase, Y.: Handbook of Clinical Neurology. No. Holland Publ. Co., Amsterdam (in press).
12) Uebayashi, Y., Matsumoto, N., and Yase, Y.: XV Meeting of the Japan Neurological Association (1974) (unpublished).
13) Kimura, K., Yase, Y., and Higashi, Y. et al.: Psychiat. et Neurol. Jap., 65, 31-38 (1968).
14) Carano, P., and Sanchez, P.: The Island and Its People. A Complete History of Guam. Chas. Tuttle & Co., Rutland, Vt. pp. 1-30 (1964).
15) Yoshimasu, F.: J. Wakayama Med. Assoc., 20, 31-50 (1969).
16) Forbes, R., Cooper, A., and Mitchell, H.: J. Biol. Chem., 203, 359-366 (1953).
17) Forbes, R., Mitchell, H., and Cooper, A.: J. Biol. Chem., 233, 969-975 (1956).
18) Yase, Y.: unpublished.