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### SUBJECT OF INVESTIGATION

PHYSICO-CHEMICAL STUDIES  
ON  
THE MICROSOMAL RIBONUCLEOPROTEIN  
PARTICLES

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PHYSICO-CHEMICAL STUDIES  
ON  
THE MICROSOMAL RIBONUCLEOPROTEIN PARTICLES  
The Third Quarterly Progress  
Report in the Second Year

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I. THE SCHEME OF THE INVESTIGATION IN THE THIRD QUARTER.

1. To clarify the biochemical and physiological characteristics of brain microsomal fraction, the distribution of some neuro-physiologically active substances in the brain subcellular particles was examined by ultracentrifugation in the density gradient of sucrose and electronmicroscopical observation.
2. Isolation of brain nuclear ribosomes was attempted.

## II. RESULTS OBTAINED TO DATE.

### 1. Subcellular Distribution of Serotonine (5HT), Noradrenaline, Substance P (SP) and Acetylcholine (ACh).

Activity of these four neurophysiologically and neuropharmacologically active substances was found in so-called mitochondrial and microsomal fractions of brain homogenates. Further detailed studies by means of density gradient ultracentrifugation revealed that ACh and SP were chiefly found in the subfraction of 1.0 M sucrose of mitochondrial suspension, while 5HT and Nor in 1.2 - 1.4 M fraction. Succinic dehydrogenase (SDH), a typical mitochondrial enzyme, showed its highest activity in the 1.2 M fraction. In 1.0 M fraction, numerous lightly stained nerve-ending-like structures were observed electronmicroscopically, the greater the sucrose concentration of subfraction, the less they appear. On the other hand, nerve-ending-like structures stained more heavily with  $\text{OsO}_4$  were observed in the fraction of greater density (1.0 - 1.4 M)<sup>4</sup>. The counting of both lightly and heavily stained structures in the electronmicrographs showed that the number of the former in the each subfraction runs parallel with its content of ACh or SP, while that of the latter one with 5HT and Nor.

These nerve-terminal-like structures seem, in their electronmicroscopical appearance, to enclose small vesicular particles, a fact suggesting that the activity of these substances recovered in the so-called microsomal fraction is attributable to this microvesicular component which is liberated from the nerve endings by their disintegration during homogenation process. If so, the microsomal RNP, ribosome, would not be related with these physiologically active substances, their formation or their storage.

### 2. Nuclear Ribosomes of Rabbit Brain.

Applying the method of density gradient centrifugation, the nuclei of about 99 % purity was isolated (1), from which ribosomes were tried to isolate by the conventional method. But its yield was too small to examine its characteristics. Repeated trials failed to isolate enough amount of pure nuclear ribosome. It seems necessary for the detailed studies on the nuclear ribosome, therefore, to re-examine its isolation method thoroughly and to improve it.

### III. RESEARCH PLAN AT THE NEXT QUARTER.

1. Studies on the subcellular distribution of 5HT, SP, Nor and ACh.

Observations on the subcellular distribution of 5HT and other substances stated above will be repeated. If possible, the systematic subcellular fractionation method with density gradient ultracentrifugation will be studied.

2. Observations on the nuclear ribosomes.

As stated above, very pure nuclei could be obtained, but we failed to isolate the nuclear ribosome enough to study its physico-chemical properties. Therefore, we will repeat the experiments on them.

IV. LIST OF REFERENCES.

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