Abstract—The effects of thiamine (T) on diabetes mellitus (DM) and the T levels in the brain, heart, liver, kidneys, pancreas, muscle, adipose tissue and blood were measured. For the experimental DM model, alloxan (170 mg/kg, i.v.) was injected into male ddY mice and insulin was also administered for 5 days to prevent death by hyperglycemia (DM group). After 14 days, blood glucose level increased to 455 mg/dl, compared to 166 mg/dl in the normal control group (NC group). In the DM mice, the T level in the liver decreased to 7.71 μg/g, compared to 16.29 μg/g in the NC group. The T levels in the heart, pancreas, muscle and adipose tissue increased to 18.63 μg/g, 3.99 μg/g, 2.53 μg/g and 5.07 μg/g in the DM group, compared to 14.99 μg/g, 3.27 μg/g, 1.98 μg/g and 4.04 μg/g in the NC group, respectively. The T levels in the brain and kidney were 2.38 μg/g and 14.00 μg/g in the DM group, compared to 2.34 μg/g and 13.72 μg/g in the NC group, respectively. But, in the heart, an active form of a T co-enzyme decreased to 27%, compared to 95% for the NC group. These results indicate a T deficiency or an endogenous T deficiency in the DM group. All DM mice without insulin treatment died within 7 days but about 40% of the mice survived up to 14 days with the administration of T.

Thiamine (T) plays an important role in glucose metabolism as a co-enzyme and a deficiency of T causes a decrease in glycolysis. A decrease or increase in glycolysis may affect the T level in the blood (1). We have described the relationship between blood glucose and T levels in diabetes mellitus (DM) (2). In this paper, the effect of T on DM and T levels on the brain, heart, liver, kidneys, pancreas, muscle, adipose tissue and blood in the DM mice and the four types of T (free T, T monophosphate: TMP, T diphosphate: TDP and T triphosphate: TTP) were studied.

Materials and Methods
Male ddY mice weighing about 22 g and fed a regular diet (Oriental Kobo Co. Ltd., 350.6 Cal/100 g) were used as the normal control group (NC group). Alloxan (170 mg/kg, i.v.) was injected to make an experimental DM model and insulin was also administered for 5 days (1 IU for 3 days and 0.5 IU for 2 days) to prevent death by hyperglycemia (DM group). T (30 mg/10 g/day, p.o.) was administered to the DM mice and these were termed the DMT group. Mice in the DMT group which were fed for 7 days without T administration were named the DMT-7 group. Mice were sacrificed on the 14th day (NC, DM and DMT groups) and the 21st day (DM-7 group) after fasting for 18 hr. The T levels in some tissues were assayed by a biological method using Lactobacillus viridescens (3). The four types of T were separated by paper chromatography, developed in pyridine: n-butanol: water=3:4: 7(Tokyo filter paper, No. 51).
Results

Mice in the NC group took a diet of about 12.5% and drank water of about 9.2% respectively of their body weight. Mice in the DM group took a diet about three times greater and drank about eight times more than the NC group.

Body weight in the DM and DMT groups decreased to 62% of the NC group. Brain and kidney weight did not change in the DM and DMT groups. Heart and liver weight decreased in the DM and DMT groups. Blood glucose levels increased in the DM and DMT groups, but there was no significant difference among them (Table 1).

The T levels in the brain were about 2.81 μg/g in the NC group and they did not change in the DM, DMT and DMT-7 groups. The T levels in the heart were about 14.99 μg/g in the NC group and did not change in the DM group. But these levels increased to 18.61 μg/g in the DMT and DMT-7 groups. The T levels in the liver were about 16.29 μg/g in the NC group and did not change in the DMT and DMT-7 groups. But, in the DM group, these levels decreased to 7.71 μg/g. The T levels in the kidney were about 13.72 μg/g in the NC group and they did not change in the DM and DMT-7 groups. But, these levels increased to 34.79 μg/g in the DMT group. The T level in the pancreas was about 3.27 μg/g in the NC group and did not change in the DM and DMT-7 groups. But this level in the DM and DMT groups increased to 3.99 μg/g and 15.82 μg/g, respectively. The T levels in the muscle were about 1.98 μg/g in the NC group and did not change in the DMT-7 group. But these levels in the DM and DMT groups increased to 2.53 μg/g and 8.19 μg/g, respectively. The T levels in the adipose tissue were about 4.04 μg/g in the NC group and increased to 5.07 μg/g in the DMT-7 group. The T levels in the blood were about 0.35 μg/ml in the NC group and did not change in the DM and DMT-7 groups. But these levels increased to 1.18 μg/ml in the DMT group (Fig. 1).

The four types of T, i.e. free T, TMP, TDP and TTP, in the liver and heart were separated by paper chromatography. The Rf values of free T, TMP, TDP and TTP were about 0.66, 0.37, 0.57 and 0.55, respectively. Percentage values were indicated for the total T levels in the heart. In the NC group, 95% of all T levels were in the 0.33-0.54 range and 5% were about 0.65, and these seem to correspond to TDP plus TTP and free T, respectively. In the DM group, 67% of all T levels were in the 0.18-0.36 range, 27% were in the 0.41-0.59 range and 6% were in the 0.64-0.72 range, and these seem to correspond to TMP, TDP plus TTP and free T, respectively. In the DMT group, 20% of all T levels were in the 0.14-0.27 range, 66% were in the 0.36-0.58 range and 14% were in the 0.65-0.70 range, and these seem to correspond to TMP, TDP plus TTP and free T, respectively (Fig. 2). In the liver, 71% of all T levels were TDP plus TTP in the NC group, and these...

| Table 1. Body weight and brain, heart, liver and kidney weights and blood glucose levels. |
|---------------------------------------------------------------|
| **Body weight (g)** | **Brain weight (g)** | **Heart weight (g)** | **Liver weight (g)** | **Kidney weight (g)** | **Glucose levels (mg/dl)** |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------------|
| NC                  | 29.5                | 0.438               | 0.121               | 1.626               | 0.427                     | 166                       |
| DM                  | 18.7**              | 0.413               | 0.081**             | 1.025*              | 0.429                     | 455***                    |
| DMT                 | 18.4***             | 0.408               | 0.088**             | 1.038*              | 0.448                     | 517***                    |

NC: normal control group, DM: diabetes mellitus group with insulin treatment for 5 days, DMT: thiamine (30 mg/10 g/day, p.o.) administered to diabetes mellitus group. Mice were sacrificed after 14 days following 18 hr of fasting. *P<0.02, **P<0.01, ***P<0.001 (n=5)
levels did not change in the DM and DMT groups.

Without insulin treatment, all DM mice died within 7 days by hyperglycemia. But 40% survived until 14 days with the administration of T (Fig. 3).

Discussion

Blood T levels in some animals and humans have been reported. These were about 55 ng/ml in humans (2), 212 ng/ml in rabbits (2), 275 ng/ml (4) to 417 ng/ml (5) in rats and 350 ng/ml in mice. Comparing these results, small animals had higher blood T levels than large ones, and basal metabolism in small animals was higher than that of large ones. Mice took about 440 Cal/kg/day which was about ten times as much as humans. That is,
blood T levels and basal metabolism seem to be correlated with each other.

Administration of large doses of T didn’t affect blood glucose levels in the DM mice. The T levels in the brain, heart, liver, kidneys, pancreas, muscle, adipose tissue and blood were assayed, and the results were classified into the following four types: 1) no change in the DM, DMT and DMT-7 groups (brain), 2) increase in the DM or DMT-7 groups and DMT group (heart, pancreas, muscle and adipose tissue), 3) decrease in the DM group (liver), 4) increase in the DMT group (kidneys and blood). Among these types, 2) and 3) gave very interesting results. In the DM group, the T levels in the liver decreased to about 47% of the NC group and this decrease in the T levels was not seen in other tissues. As the liver weight was about 5.5% of their body weight and the T level in the liver was relatively high, the DM mice seemed to be in a T deficient state. The T levels in the heart, pancreas, muscle and adipose tissue increased in the DM group, but the T in these tissues seemed to be supplied by the T released from the liver.

On the other hand, glucose uptake by the heart, muscle and adipose tissue was accelerated by insulin. Glucose and T were adsorbed by active transportation and a sodium-dependent process from the small intestine (6, 7), and they were transported to the red blood cells by facilitated diffusion without expenditure of metabolic energy (8). That is, the biodynamics of glucose and that of T seem to be similar. The administration of insulin with vitamin B was more effective than insulin alone (9). The T deficient rats proved to have glucose intolerance, which is attributable to the inhibition of insulin release from the pancreas (10). There are interesting correlations among the biodynamics of glucose, T and insulin in the body.

In the DM group, an active form of T, i.e. TDP, decreased in the heart. Though the total T levels in the heart increased in the DM group, the DM mice were in an endogenous T deficient state. Sato et al. reported that the incorporation of $^{35}$S-T into the heart was about one-fourth of that into the liver and kidneys (11). But the T levels in the heart were the same as those in the liver and kidneys. T in the heart may be secondarily supplied from the liver after phosphorylation.

Considering these results, the DM mice must have been in a T deficient or an endogenous T deficient state.

Recently heart diseases from T deficiency were reported (12–14). We experienced sudden death of DM mice without any symptoms. The incidence of cardiovascular disease among diabetic men and women was twice and three times higher, respectively, than that of non-diabetics (15). Attention to the endogenous T deficiency in the heart must be given in the DM group.

Without insulin treatment, all DM mice died within 7 days by hyperglycemia, and large doses of T administration affected the survival days of these mice. But the mice in the DM group survived for about 60 days (15–95 days) and in the DMT group about 58 days (15–95 days) and no sig-
significant differences were seen between them.

We intend to study the effect of TDP on DM and of T treatment with insulin on DM.

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