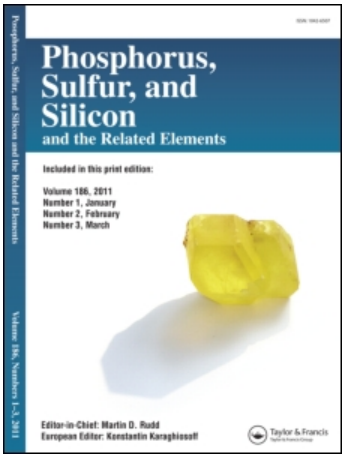

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Suppression Of Ages Formation In Spontaneous Diabetic OLETF Rat By Organic Germanium Compound [Poly-trans-(2-carboxyethyl) Germasesquioxane] (Ge-132)

KUNIE NAKAMURA[a], YOSHIKUNI FUJITA[b], MITUO AKIBA[c], TOMOYOSHI HOSOKAWA[c], NORIHIRO KAKIMOTO[c] and TOSHIHIKO OSAWA[d]

[a]*Molecular Biology Laboratory,* [b]*Department of Internal Medicine, Kitasato University School of Medicine, 1–15–1 Kitasato, Sagamihara, Kanagawa, 228–8555 Japan,* [c]*Biremo Science Co., 1–1–1 Manpukuji, Asoh-ku, Kawasaki, 206–0004 Japan and* [d]*Laboratory of Food Dynamics, Nagoya University Graduate School of Agriculture, Furocho, Chikusa-ku, Nagoya, 446–5678, Japan*

The model rat for the type 2 diabetes mellitus (NIDDM) in human were observed for 72 weeks after birth, administering an organic germanium compound [Bis(2-Carboxyethyl) germasesquioxane, Ge-132] perorally 100 mg/kg/day since 24 weeks old. Clinical examinations were followed throughout the observation period. Blood and urinary glucose in positive control OLETF rats tended to be higher than those treated with Ge-132. At the end of the 72nd week, animals were sacriificed to examine the pathological changes, specifically in pancreas, kidney and brain. Anti-AGE antibody stained proximal and distal tubles and basement membrane of glomerules in kidney, and accumulated AGE masses in cortex, hippocampus and cerebellum of OLETF rat's brain. Amyloid stains by basic congo red on kidney and brain revealed that the deposits of amyloid in kidney mesangium and in cortex, hippocampus and cerebellum were observable in OLETF rats. Ge-132 suppressed the deposition of amyloid tangles in kidneys and brains. Anti rat complement C'3 antibody reacted with AGE and amyloid tangles that were sensitive to anti-AGE antibody. AGE generated *in vitro* by incubating human serum, human gammaglobulin (HGG), or bovine serum albumin (BSA) with glucose activated complements, showing the consumption of complements in the hemolysis of hemolysin-coated sheep red blood cells. A novel device *Quantum Resonance Spectrometer (QRS)* could read the subtle bio-magnetism memorized in serum samples, demonstrating quantitative values reflecting the patho-physiology of OLETF rats.

Keywords: OLETF rat; diabetes mellitus (DM); amyloid deposit; advanced glycation end products (AGE); complement activation; quantum resonance spectrometer (QRS); subtle bio-magnetism

INTRODUCTION

Otsuka Long Evance Tokushima Fatty (OLETF) rat is accepted to be an appropriate model animal for type 2 diabetes mellitus (NIDDM) in human, showing highper glycemia and accompanied complications characteristic in NIDDM (1). It has been reported that an organic germanium compound [poly-trans(2-Carboxyethyl)germasesquioxane] (Ge-132) suppresses the formation of advanced glycation endproducts (AGEs) in streptozotocine-DM rats and senescence accelerated mouse (SAM) (2-4), and in galactose-induced cataract in rat (5).

In the present experiments, the effects of Ge-132 on the suppression of AGE formation in OLETF rats were examined, observing for 72 weeks after birth. Immuno-pathological observations revealed that characteristic accumulation of AGE and amyloid fibriles in kidney and brain of OLETF rats was remarkable, and that was suppressed by the effects of Ge-132 via the oral administration. Furthermore, AGE and amyloid masses could be stained by anti-rat-C'3 antibody, showing the site specific deposits of AGE or amyloid, leading to the tissue damages through the classical pathway of complement activation. In this study, a newly developed device *Quantum Resonance Spectrometer* (QRS) was applied to quantitate patho-physiology of DM in OLETF rats, to confirm the correlation between the morphological findings and numerable values obtained by measuring subtle bio-magnetism generated in OLETF rat. This technology may contribute in the evaluation of patho-physiological changes with numerable values in the analysis of every bio-medical field.

[MATERIALS AND METHODS]

Animals. Twenty normal control rats, Long Evance Tokushima Otsuka (LETO) and 60 spontaneous diabetic rats, Otsuka Long Evance Tokushima Fatty (OLETF) were gifted by Otsuka Pharmaceutical Research Institute (Tokushima-shi, Tokushima-ken, Japan) at the age of 4 to 5 weeks (1). Rats were sent to the animal facility of The New Drugs Developing Institute in Eniwa-shi, Hokkaido. Animals were allowed to access to food and tap water *ad ribitum* in an airconditioned clean room.

[poly-trans-(2-Carboxyethyl)germasesquioxane] (Ge-132). This drug was mixed into rat chow at the concentration of 0.22% (W/W) during 25 to 31 weeks, and 0.17% (W/W) since 32 weeks old (Nippon Crea Co., Tokyo). This concentration corresponds to an approximate uptake of 100 mg/kg body weight per day according to the rat's growth curve and food intake amounts. At the age of 25 weeks old, rat chow mixed with Ge-132 was fed and free access was allowed. Ge-132 were supplied from Biremo Science Co. 1-1-1 Manpukuji, Asoh-ku, Kawasaki-City, Kanagawa Prefecture, 215 Japan.

Clinical examinations. During the observation period, body weight, food intake, water intake, urinary volume, urinary protein and glucose (glucose kinase method by the kit of Glucomesser Direct, Sinotest Co., Tokyo), plasma and urinary creatinine were quantified by the creatinine iminohydrolase method with a commercially available kit (Reflotron, Bohringer-Yamanouchi, Tokyo), β-D-N-acetylglucosami-nidase (NAG)(6), insulin by ELISA (7), fructosamine (8), glycated albumin (9) were examined every 4 to 8 week until the end of 72nd week. Urinary MRX (Maillard Reaction-product X)(15) was developed as a novel parameter for DM, and applied to compare OLETF and those treated with Ge-132 (15).

Morphological Observations. Monoclonal anti AGE antibody (Wako Pure Chemical Co., Osaka)(10), and a coloration kit (Vectastain, Vector Laboratories Inc., Burlingame, CA), anti rat complement C'3 antibody labeled with FITC (Nordic Immunology, Tiburg, The Netherlamds)

were perchased. Organs fixed by the fixation cocktail consisted from 95% ethanol, 1% acetic acid and 4% distilled water at 4°C for 3 days, were dehydrated by alcohol, and passed through xylene 3 times, and embedded in paraffine with a lower melting point of 52°C. Thin sections at 4 μm were made by a microtome, and these specimens were deparaffined through xylene and chronological ethanol bathes. Anti AGE stain by peroxidase-lebeled antibody was visualized by the reaction with diaminobenzidine and hydrogen peroxide (13) Amyroid tangles in thin sectioned specimens were stained by 1% basic Congo red solution disolved in 10 mM carbonate buffer, pH10.2. Specimens were observed under a photo-microscope equipted with a set of polarizing filters (type BH-POL, Olympus Optical Co., Tokyo). Specimens stained by FITC-labeled anti rat-C3' antibody were mounted in 90% glycerol and 10% of 100 mM carbonate buffer pH10.2, and observed under a fluorescent microscope type FL-II, Olympus Optical Co., Tokyo. Co-localization of AGE and amyroid tangles were examined by serial sections being observed under a conventional photo-microscope (FHT, Olympus Optical Co.).

AGE Formation in vitro, and Complement Consumption Test. Heparinized human serum 1 ml, 10 mg of human gammaglobulin, or 10 mg of bovine serum albumin (BSA) SIGMA Chem., Co. St. Louis, Mo) in 1 ml PBS were incubated with 200 mg glucose at 37°C for various periods. An aliquot of each specimens were separated in a small tube, and stored at -80°C until use. Consumption of complements by these samples were tested by the following method; well-rinsed sheep red blood cells (SRBC) 10^8 were suspended in 1 ml PBS, and rabbit serum containing anti-SRBC antibody (SIGMA Chem., Co.) diluted in PBS to 200 times were equally mixed and incubated at 37°C for 1 hour. SRBC were rinsed by PBS 3 times by centrifugation at 1500 x g for 10 min at 4°C, and then resuspended in 1 ml of PBS. Fresh guinea pig serum diluted in PBS in series 100 μl and the same volume of SRBC suspension were mixed in tubes, and incubated at 37°C for one hour in a shaking water bath. Tubes were centrifuged at 1500 x g for 10 min, and the supernates were measured by a spectro-photometer at 541 nm (UV-160A, Shimadzu, Kyoto) to quantify hemoglobin residing in supernates.

The twice optimal concentration of guinea pig serum and AGE samples harvested at various days were mixed and incubated at 37°C for 2 hours, and then transfered to the other tubes containing 10^8 SRBC coated with anti-SRBC antibody. Tubes were incubated at 37°C for 1 hour, centrifuged at 1500 x g for 10 min, and the supernates were measured by a spectrophotometer at 541 nm to quantify the %-suppression of SRBC-lysis.

Quantitative Assessment of Patho-Physiology of OLETF Rats by the Quantum Resonance Spectrometer (QRS). QRS was developed by the Quantum Science Research Institute, Co., 4-24-12 Sendagaya, Shibuyaku,Tokyo Japan. Principle and mechamisms of QRS manipulation: Details wqere already written in the previoous paper (11, 12). Briefly, A special circuit for detecting less than 50 miligauss is integrated in QRS. Subtle magnetism produced from bio-materials are trapped and sent through a transmitter and condencer, followed by elimination of silent electric capacity and electric resistance toward the analytical circuit. Then the waves were arranged in the order according to frequencies and heights, and the objective wave shape is hocked up by the predetermined standard wave shape, and the integrated computer calculates the degree of aberration occuring in atoms. When wave aberration is found, the computer indicates its wave-shape and degree by an unresonant sound. When there is no change, a resonant clear sound indicates. The Fourier's theory for collecting the definitive informations is applied in this technique.

Method for analysing subtle bio-magnetism by QRS: It is necessary to predetermine many codes for each wave shape from elementary particles, atoms, molecules, cells and organs. To detect subtle bio-magnetism by QRS, an appropriate code number corresponding to the objective to be tested must be input into the device. A counter magnetic wave is produced by the device and subtle bio-magnetism and the standard wave are compared and calculated for their differences or similarities. When QRS finds aberration in the bio-magnetism, it indicates its degree in a relative range from -21 to +21. By combining

simultaneously two codes, as a disease foci and causative factors, whether they correlate or not can be determined. Bio-magnetic waves consist of a great many components derived from many atoms, molecules, cells, and organs. A whole wave from an individual person appears as one synthetic wave of all component-waves. From this synthetic complicated waves, it is absolutely necessary to pick up one objective wave, eliminating all other contaminants. For this purpose, many predetermined probes or codes to define each wave shape must be prepared. Using each code as the standard information, the Fourier's procedure is applied to get the definitive analysis. As is clear from the principle, contaminated magnetisms can be completely eliminated and only the desired one is selectively analyzed by reffering to the standard code corresponding to the objective. For the precise analysis, many predetermined accurate codes should be prepared.

RESULTS

Clinical Examinations.

Among many clinical data performed throughout the observation

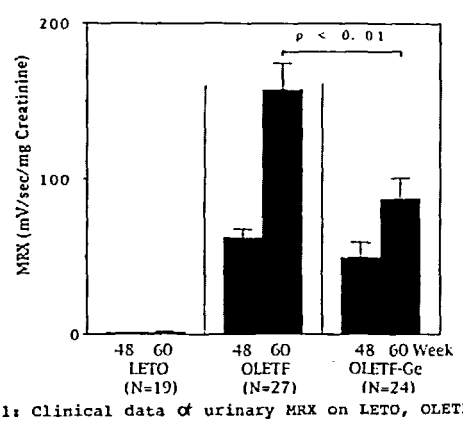


Figure 1: Clinical data of urinary MRX on LETO, OLETF, and OLETF rats treated by Ge-132 100 mg/kg/day were followed for 72 weeks were illustrated. Significant differences were calculated by Student t-test, and determined at the level $p<0.05$. Urinaly glucose seemed to be significantly different.

period, urinary MRX was remarkable in judging the effect of organic germanium compound (Fig. I). Urinary glucose in Ge-132 administered OLETF rats was significantly lower than the control OLETF rats. Many other examinations showed slightly lower results in Ge-132 treated OLETF rats than untreated positive controls without a

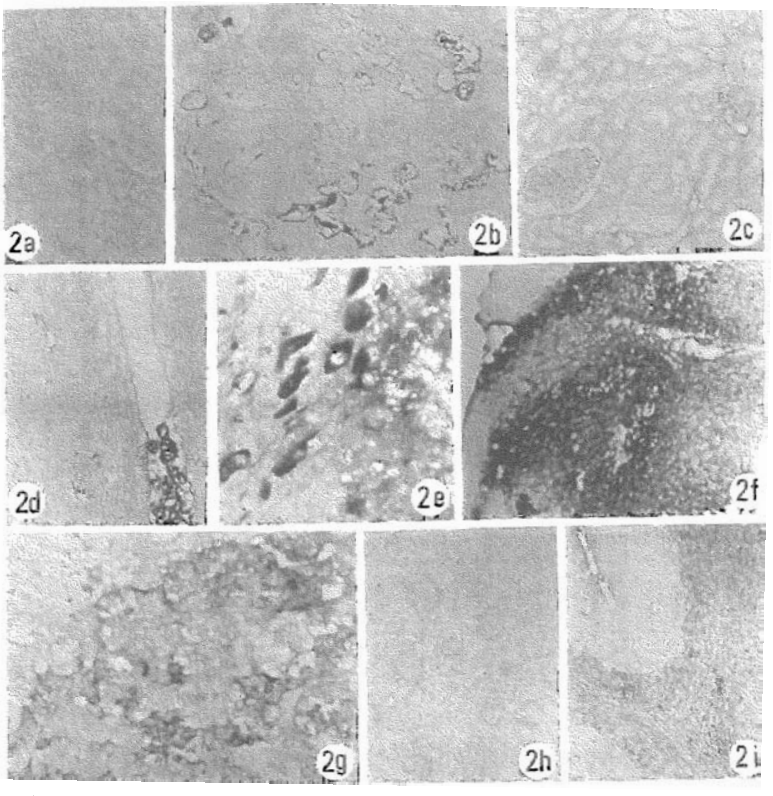


Figure 2: Immuno pathological stains by anti-AGE antibody for kidnies and brains of LETO and OLETF rat at the age of 72 weeks old.

a: LETO rat kidney (negative control) x 100.

b: deposition of amyloid in mesangium of OLETF rat kidney x 100.

c: OLETF rat kidney treated with Ge-132 100 mg/kg/day for 49 weeks x100.

d-g: brain sections of OLETF rats (d & f:x 100), (e & g: x 200).

h-i:brain section of OLETF rat treated with Ge-132, at hippocampus (h) and cerebellum (i) (x100). Nuclear ounter stain by methyl green. See Color Plate III at the back of this issue.

significant difference (data not shown).

Morphological Observations

(1) Anti-AGE Antibody Stain. Pancreas, kidney and brain sections made from 72 weeks-old animals were stained by anti-AGE antibody labeled with peroxidase, after coloration by diaminobentizine tetrachloride. As shown in Figure 2, enlarged parts of proximal and distal tubules and glomelurar basement membranes were stained in OLETF kidney. Comparing with this positive controls, the same organs of OLETF rat treated with Ge-132 showed a weaker degeneration and accumulation of AGE on proximal and distal tubules and glomelurus. Specifically, glomelular lysis in Ge-132 treated rats was weaker than the positive controls.

In panceas of OLETF rats, clear accumulation of AGE on acinar cells and Langerhans'cells could not be observed (data not shown). Hematoxylin-Eosine (HE) stains revealed that there are many infiltrating lymphocytes around acinal cells, implicating the existence of inflammational processes in the tissue damage of pancreas (Fig. 6). In negative control LETO rats, such positive stain could not be observed.

In brain, typical masses of AGE could be stained in OLETF rats. In cortex, hypocampus, cerebellum and aqua-ductus cerebri, the accumulation of AGE were remarkable as shown in Fig. 2. In Ge-132 treated animals, anti-AGE stain appeared to be a weaker than OLETF rats. In normal control LETO, the accumulation of AGE in brain could not be observed at all (Fig. 2).

(2) Deposition of Amyloid in Tissues.

The stains by congo red and observation under a photo-microscope equiped with the polarizing filter set revealed the accumulation of amyroid around arteries in both LETO and OLETF rats. The kidneys and brains of OLETF rats showed characteristic depositions of amyloid in kidney-mesangium and in brain cortex, hypocampus, and cerebellum. In Ge-132 treated OLETF rats, amyloid stains in mesangium and brain became weaker as shown in Figure 3h and 3i. Amyloid in pancreas could not be observed, due to the severe degeneration of the tissue (data not shown).

In brain of OLETF rats, the foci with AGE and amyloid positive

stains co-localized with figures demonstrating like sunflower petals, or masses of glanular deposits (Fig. 2d. 2G, 2h, 3d, 3f, 3g).

(3) Anti-Complement C'3 Antibody Stain.

It was demonstrated that β-amyroid fibrils activate complements via

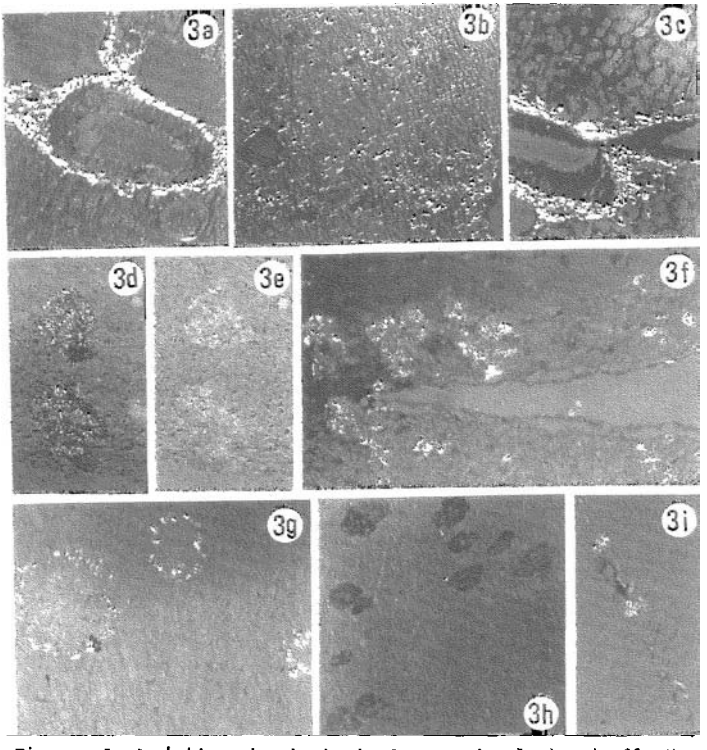


Figure 3: Amyloid stains by basic Congo red solution in 50 mM carbonate buffer, pH10.2 were observed under a microscope through polarizing filters.
a:LETO kidney. b: mesangium in OLETF kidney. C:OLETF rat kidney treated with Ge132 100 mg/kg/day for 49 weeks since 25th weeks old. d:deposition of amyloid in the striatum of OLETF rat, when observed by polarizing filters, and e : just the same field without apolarizing filter. f: foci of amyloid deposition at cortex of OLETF-brain. G : ring-form deposition of amyloid at hippocampus (X 100). h & i: brain derived from OLETF rat treated with Ge-132 100 mg/kg/day for 49 weeks. h or small deposition of amyloid were observed by means of polarizing filters (x 100).
See Color Plate IV at the back of this issue.

Table I

Appearance of Amyloid in Brain

Rat strain	frequency	Significance by χ^2 test
LETO (Control)	0/18	P<0.01
OLETF	11/27	

the classical pathway and conduct cell death in brain tissue (14). Anti-rat C'3 antibody labelled with FITC clearly stained proximal and distal tubular cells in OLETF rats (Fig. 4b, 4c). By the treatment with Ge-132, the brightness of antibody stain become weaker in kidney (Fig. 4d), and specifically in brain (Fig. 5e, 5f). At the same locus stained by anti-AGE and congo red in brains, very strong depositions of anti-C'3

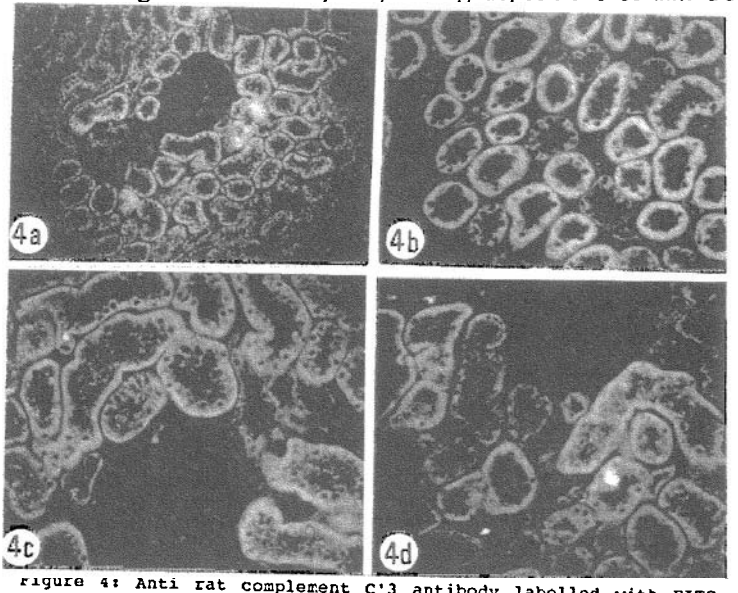
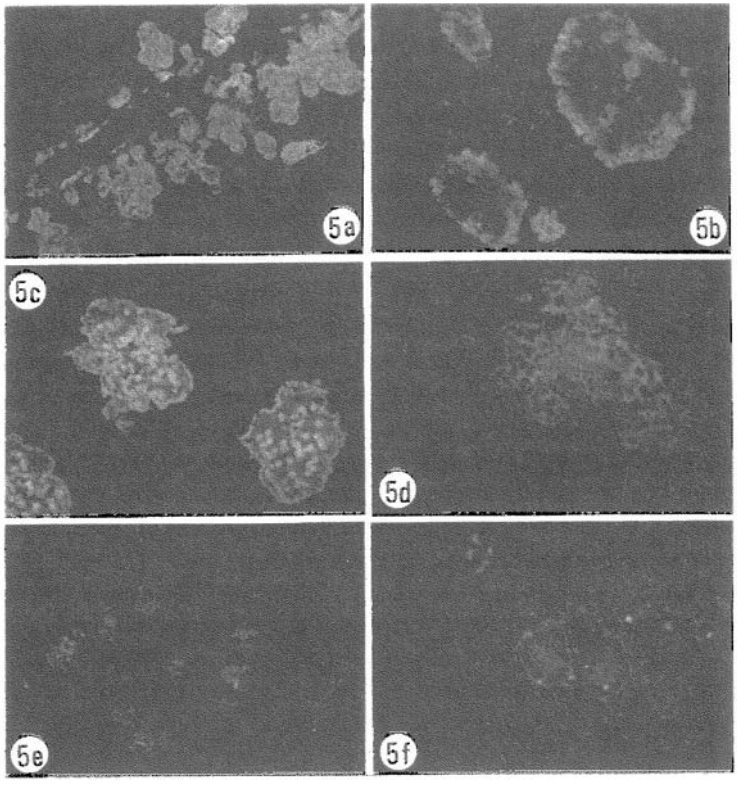


Figure 4: Anti rat complement C'3 antibody labelled with FITC staining for kidney sections.
A: LETO rat (x 100). b:distal portion of tubulus of OLETF rat kidney (x 200). c:glomelus and proximal tubulus of OLETF rat. d:OLETF rat treated with Ge-132 100 mg/kg/day for 49 weeks, showing a slightly white-yellowish green in proximal tubules (x 200). See Color Plate V at the back of this issue.



Figture 5: Brain sections were stained by anti rat complement C'3 antibody labelled with FITC. At the fissure of a OLETF rat, large masses of C'3-reactive structure could be observed (x 100). b-d:different types of structure reacting with C'3, using 4 um sections derived from different rats.(x 200). e-f: OLETF rat treated with Ge-132 100 mg/kg/day for 49 weeks (e: x100, f: x 200). See Color Plate VI at the back of this issue.

antibody were observed in different three types as shown in Figure 5a-5d. Specifically, fluorescence in Ge-132-treated OLETF kidney changed to white-yellowish green, indicating small deposition of C'3 on tubular cells.

In OLETF brain treated with Ge-132, weak monotonous fluorescence with a small number of bright grains were observed, showing the deterrence of amyroid depositins in and around the area where AGEs were accumulated. (Fig. 5e-f).

In pancreas, also very strong fluorescence was observed in OLETF rats on acinal cells. A very few intact Langerhans' islet cells could be observed in positive control OLETF (Figure 6c, d), provably due to a issue lysis. The infiltration of lymphocytes and decrease in acinus cells was observable in OLETF rats by HE stain (Figure 6c), meaning the degradation of Langerhans' islet and ascinus cells due to the inflammatory attack by auto-reactive lymphocytes.

The adhesiveness of complements on these locations implicates that the damages in pancreas can be induced by the activation of complements through the classical pathway of complement activation due to antibody-C' interaction in diabetic rat-pancreas.

Activation of Complements by AGE.

Consumption of complements by AGE was examined *in vitro* by using human serum (HS), human immunoglobulin (HGG), and bovine serum albumin (BSA) incubated with 200 mg glucose/ml for various periods. As shown in Figure 7, HGG without glucose did not consume complements and rather stimulated SRBC-hemolysis. However, HGG incubated with glucose consumed complement up to 63% in the

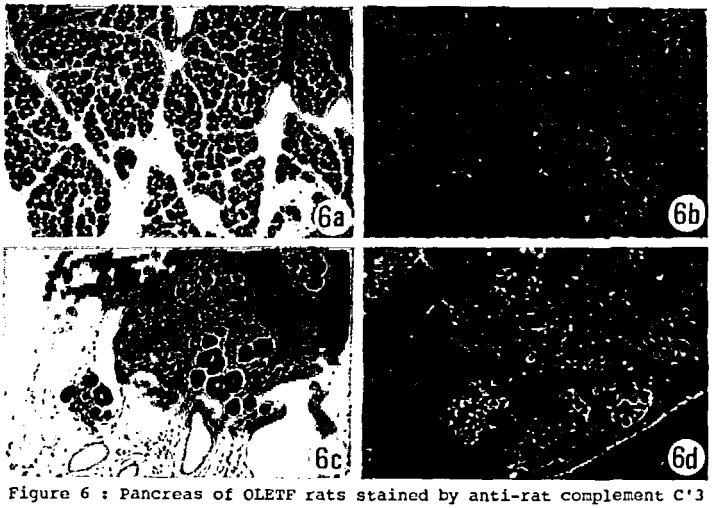


Figure 6 : Pancreas of OLETF rats stained by anti-rat complement C'3 antibody labelled with FITC. 1a, 1c, 1d:stained by Hematoxilin and Eosine. a pancreas section from LETO rat, b: LETO rats pancreas stained by anti-C'3 antibody labelled with FITC. c & d : positive control OLETF rat (c:HE, d: anti-C'3 Ab).
See Color Plate VII at the back of this issue.

hemolytic reaction using antibody-cooated SRBC. Human serum incubated with glucose activated a half amount of complements (Fig. 7). AGE composed of BSA actibated less amount of complements as same degree as BSA without glucose. These results indicate that serum components such as immunoglobulin converts to AGE, and consequently induces the consumption of complements, leading to cytolysis or tissue damages through the classical pathway of complement activation.

Analysis of Patho-Physiology of DM in OLETF Rat by QRS.

A subtle bio-magnetic energy were assessed by a newly developed device entitled as *Quantum Resonance Spectrometer (QRS)*. As shown in Table I, the score is ranging approximately between +21 and -21. This one count corresponds to approximately 1 mili-gauss of magnetism discharged from body-constitutive substances.

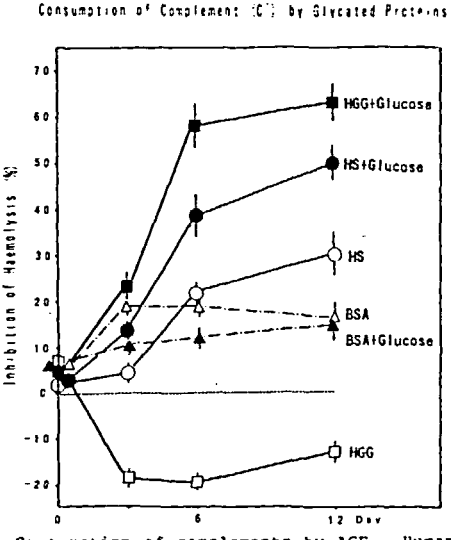


Figure 7 : Consumption of complements by AGE. Human serum (HS), human gamma globulin (HGG) 10 mg/ml, or bovine serum albumin (BSA) 10 mg/ml in PBS were incubated at 37C for 12 days with /without glucose 500 mg/dl. Antibody-coated SRBC were mixed with the same volume of the mixture consisting of above protein-solutions and fresh human serum after incubating for 60 min, comparing with the 100% hemolytic titer of human fresh serum. Each point consisted from quintplicated tubes with standard deviation of the mean.

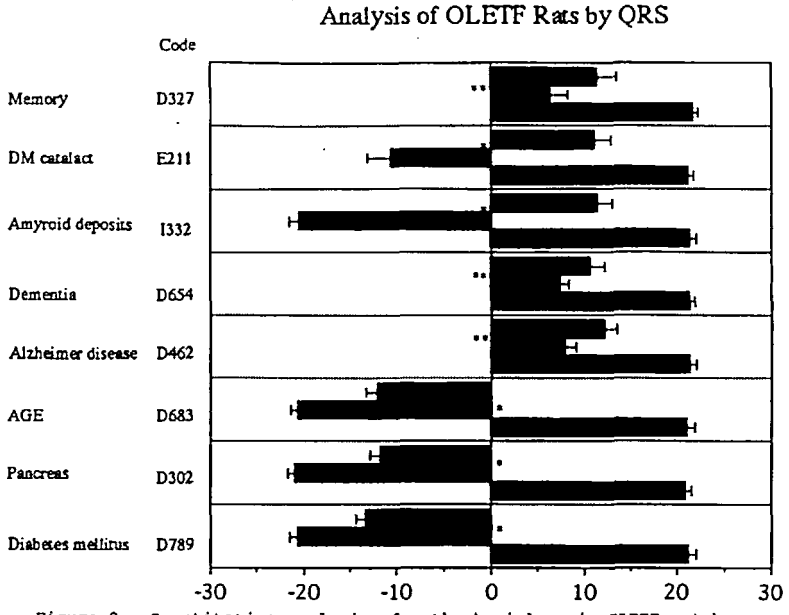


Figure 8 : Quantitative analysis of pathophysiology in OLETF rat by Quantum Resonance Spectrometer (QRS) was performed. Results were demonstarated by the scores between -21 to +21. Minus scores mean the functional defect in the items, and pulus score show descrease in function in those items. A sterics shows a significant difference between OLETF and those treated with Ge-132 with the risk $p<0.05$, two asterics mean $p<0.01$ by Student t-test.

Figure 8 demonstrates that many items show the significant differences in Ge-132 treated OLETF rats, comparing with positive control OLETF. Negative control LETO rats showed no disease or tissue damage when read by QRS. These results strongly suggest that the morphological observations reflect on the quantitative assessment of patho-physiology in DM rats by a novel analytical machine QRS.

DISCUSSION

Spontaneous type 2 DM model in OLETF rats demonstrated the remarkable changes in kidney, pancreas and brain. Organic germanium compound Ge-132 prevented the progress of AGE accumulation, especially in a clinical examination such as MRX. This results

implicates that the Ge-132 tended to suppress the progress of kidney damages as usually expressed as a main DM-complication.

By the morphological observations, many important findings were noted in this study. AGE deposited in kidney and brain in the particular fashion. The enlarged proximal and distal tubular cells and glomerular basement membrane in kidney were main target of anti-AGE antibody, meaning AGE could adhere to these portions, that caused diabetic nephropathy. Anti complement C'3 antibody stained the same portions, indicating the cell-lysis through the complement activation via the classical passway. In brain , the reactivity of anti-AGE antibody, anti-C'3 antibody and congo red was remarkable, demonstrating the deposition of AGE and amyroid tangles to the same defined portions, making great masses of these products. In DM patients, usually dementia cannot be clearly recognized throughout the clinical observation. However, even not so strong dementia, DM patients sometimes complaint the detelioration in memory. In human brain, we usually use only less than 10% in daily life. By the reason, it is very difficult to define the appearance of dementia in human patients. Comparing the disease longevity in human and OLETF rat, the animals establish such strong accumulations of AGE and amyroid tangles during only 72 weeks. This factor is important to compare the patho-physiology of both mammals. When we examine the human brains suffered from DM for long term, provably the same phenomenon may be observed by immuno-stains.

This study showed the activation of complements by AGE and amyloid fibrills. It is demonstrated in the recent paper that β-amyloid protein has the receptor sequence to complements, and this leads to cell-damages and developes dementia in Alzheimer diseases (14). The observations described in this paper can support such description, and further suggest the correlation between amyloid accumulation and protein glycation. Both AGE and amyloid consisted the masses in brain, indicating the strict correlation in making such protein compounds therough the amino-carbonyl reaction known as the Maillard reaction. Not only β-amyloid tangles, but also AGE can activated complements in various tissues. This could be confirmed by making AGE-products *in vitro*, in the SRBC hemolytic reaction. Causative proteins in tissue

damages may be focused to immunoglobulin that reacted with glucose, changing through the Amadori rearrangement to the final product AGE. However, BSA incubated with glucose activated about 20% of complements. This results indicate that every protein can transform to AGE, and thus reacts with complements, resulting in tissue damages in DM animals.

In this study, a new device entitled QRS was applied for the analysis of patho-physiology of DM in OLETF rats. In the previous study, the author used this device in the analysis of DM induced by streptozotocine. This macine can read the subtle bio-magnetisms discharged from every substance, specifically from electrons and elementary particles which fulfill the circumustances around electron. The later energy is nomenclatured as Fundam, because of playing "the fundamental roles" in constructing substances. QRS can collect such minute energies discharged from every atome, using the Fourier's transformational method. The results described in this paper is indicative to show the complications of DM in the numerical values. Even though we can catch the morphological changes through the microscopic observations, it is impossible to evaluate the real differences in numerical fashions. QRS is really a convenient device to assess patho-physiological processes in animals and human. Further wide applications of QRS to medical fields may give us the paradigm shift of medicine in the next century

References

[1] Kawano K., Hirashima T., Mori S., Kurosumi M., and Natori T.; Spontaneous longterm hyperglycemic rat with diabetic complications in Otsuka Long Evance Tokushima Fatty (OLETF) strain, *Diabetes* **41**:1422–1428, 1992.

[2] Nakamura K., Nomoto K., Kariya K., Nakamjima Y., Nishimoto H., Uga S., Miyata M., Osawa T., Kakimoto N.:Prevention and reversible solubilization of advanced glycation end products AGE) by organic germanium compounds as derivatives of amino acids. *Amino Acids* **1**:263–278, 1992.

[3] Nakamura K., Osawa T., Kawakishi S., Kakimoto N.: Reguration of the Maillard reaction by organic germanium compounds. Molecular mechanism on the prevention and reversible solubilization of advanced glycation endproducts (AGE). *Main Group Metal Chemistry* 19(5), 301–306, 1996.

[4] Nakamura K., Uga S., Miyata M., Kakimoto N.: Prevention of the Maillard reaction and reversible destruction of advanced glycation end products (AGE) by novel organic germanium compounds. *Maillard Reaction in Food Processing, Human Nutrition and Physiology.* Berkhauser Verlag, Basel. pp481–488, 1990.

[5] Unakar N. J., Johnson M., Tsui J., Cherian M., Abraham E. C Effect of Germanium-132 on galactose cataracts and glycation in rats. *Exp. Eye Res.* **61**:155–164, 1995.
[6] Weissman B., Cashman D. C., Santiago R. Concerted action of beta-glucuronidase and beta-acetylglucosaminidase onhyalurono -dextrins. *Connective issue Research.* 3(1):7–15, 1975.
[7] Folling I.: Insulin-anti-insulin complexes. *Acta Endocrinologica Supplementum.* 205:199–209, 1976.
[8] Johnson R. N., Meetcalf P. A., Baker J. R.: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. *Clinica Chemica Acta.* **127**(1):87–95, 1983.
[9] Yatscoff R. W., Tevaarwerk G. J., MacDonald J. C. : Quantification of nonenzymatically glycated albumin and total serum protein by affinity chromatography. *Clinical Chemistry.* 30(3): 446–449, 1984.
[10] Horiuchi S., Araki N., Morino Y.:Immunochemical approach to characterize advanced glycation end products of the Maillard reaction; Evidence for the Presence of a common structure, *J. Biol. Chem.* 266: 7329–7332, 1991.
[11] Nakamura K., Miyata M., Shimojo N., Naka K., Kakimoto N.: Analysis of subtle bio-Magnetism Magnetism of diabetes mellitus in rat by the Quantum Resonance Spectrometer (QRS). *J. Subtle Energy Res.* 1(1):43–50, 1996.
[12] Nakamura K.:The dawn of quantum medicine. *J. Subtle E Nergy nergy Res.* 1(2): 23–28, 1996.
[13] Nakane P. K., Pierce G. B. Jr.: Enztme labeled antibodies for he light and electron microscopic localization of tissue antigens. *J. Cell Biol.* 133:307–312, 1967.
[14] Velazques P., Cribbs D. H., Poulos T., Tenner A. J.: Aspartate residue 7 in amyloid B-protein is critical for classical complemen pathway activation: Implications for Alzheimer's disease pathogenesis. *Nature Mdicine* 3(1): 77–79, 1997.
[15] Ohya T., Kumon H., Kobayashi H., Akiba M., Hosokawa †., Kakimoto N., Nakamura K., Osawa T.: A novel biomarker for hyperglycemia, MRX isolated from hydrolysate of glycated protein. *Biochem. Biophys Reseach Comm.* 246:267- 271, 1998.