— Original —

Vascular Damage in Diabetes Mellitus*

By

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Recent advancement of modern medicine especially therapeutic medicine brought a significant prolongation of life expectancy and at the same time brought in the lime-light the problem of arteriosclerosis which is in the highest incidence among geriatric diseases. This tendency is particularly significant in diabetes mellitus on account of invention of insulin and antibiotics. Namely, in diabetes mellitus, vascular damage, i. e., arteriosclerosis could be seen in its incidence and its mortality as high as six times that of the non-diabetics, and also arteriosclerosis occurs ten years earlier in the diabetics than in the non-diabetics. However, our present knowledge on arteriosclerosis is not sufficient and the treatment or measures are still within a territory of "try and failure".

Although I am not a specialist in diabetes mellitus, I become interested in diabetes mellitus which belongs to a disease of metabolic disturbance in relation with my research on hypertension and arteriosclerosis in which I have observed metabolic disturbances of the arterial walls especially disturbance of ATP-ATPase system in the vessels. In this connection I will proceed the presentation of our research work.

The etiology and pathogenesis of arteriosclerosis still remain obscure, but it is generally agreed that arteriosclerosis can be classified into the following three types: (Table 1)

1. "Atherosclerosis": This occurs in the elastic region of the medium-sized and large arteries.

TABLE I. THREE TYPES OF ARTERIOSCLEROSIS IN WIDE SENSE

Type	Arteries involved	Histological Aspects
Atherosclerosis	Large & medium-sized -elastic type	Atheromatous plaques in intima
Medial Sclerosis (Mönckeberg Type)	Large & medium-sized -muscular type	Fibrosis, area of focal necrosis, calcareous deposits in media
Hypertensive Arteriopathy (Hyperplastic Type)	Small arteries & arterioles, sometimes large	Hypertrophy & thickening of media

N.B.; In diabetics atheromatous type of lesion is outstanding and this is true even in the muscular arteries, either alone or superimposed upon medial sclerosis.

It is characterized by the presence of yellowish plaque-like deposit in the intima.

2. "Medial arteriosclerosis": This occurs in the muscular region of the large and medium-sized arteries. It is characterized primarily by fibrosis, areas of focal necrosis, calcium deposits, and sometimes ossification in the medial coat. This medial sclerosis was described by Mönckeberg.

3. "Hypertensive(hyperplastic) arteriopathy": This is characterized by marked hypertrophy and thickening of the medial coat of the arterioles, small arteries and sometimes in the aorta, in ossification with arterial hypertension.

I have experimentally produced this type of lesion, as I reported the assignment research on "Hypertension, Its Causal Aspects" at the 17th Annual Meeting of the Japanese Circulation Society.

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TABLE II. DISEASES CAUSED BY DIABETIC ARTERIOSCLEROSIS

1. <i>Diabetic Gangrene</i>
2. <i>Coronary Artery Disease</i>
3. <i>Hypertensive Cardiovascular Disease</i>
4. <i>Diabetic Nephropathy</i> —Kimmelstiel-Wilson's Syndrome
5. <i>Ocular Changes</i>
6. <i>Diabetic Neuropathies</i>

In the cases of diabetes mellitus (Table 2) atheromatous sclerosis is popular and conspicuous, affecting not only elastic type of arteries, but also muscular ones. Medial arteriosclerosis may exist, resulting in characteristic arteriosclerosis obliterans.

This arteriosclerosis obliterans causes anoxia in various tissues and organs. From the point of the internal medicine, coronary artery disease, hypertensive cardiovascular disease and diabetic nephropathy are included in this category. I will mention not only these diseases, but also spontaneous gangrene which has some relation with the diseases mentioned above, and then refer to the gangrene experimentally produced by us.

TABLE III. PATHOPHYSIOLOGY OF DIABETIC ARTERIOSCLEROSIS

1. <i>Disturbance in Carbohydrate and Protein Metabolism</i>
2. <i>Partial Destruction of Basement Membrane and Aneurysma Formation of Capillaries</i>
3. <i>High Serum Lipoprotein</i>

Though the etiology of arteriosclerosis (Table 3) is still obscure, the following explanation may be most appropriate as a theory on the etiology of arteriosclerosis in the case of diabetes mellitus. A disturbance of carbohydrate- and proteinmetabolism leads to depolymerization of cement in the capillary basement membrane followed by partial destruction, and to the formation of "Kimmelstiel nodules". These nodules are found not only in the retina and glomerular capillary tuft, but also in the capillaries of other parts of the body. The rise in the blood lipoprotein level also promotes the formation of atherosclerosis.

Here I want to report our clinical and critical experimental results with special reference to ATPase in the blood and io acid phosphatase in the urine of diabetic patients.

TABLE IV.

Total number of hospitalized patients: 1933-1952 (20 years)		Cases	%
I	<i>Hypertensive</i>	734	6.8
II	<i>Diabetic</i>	173	1.6
	<i>Both combined</i>	52	0.5
III	<i>1 Diabetes precedes hypertension</i>	23	44
	<i>2 Hypertension precedes diabetes</i>	8	15
	<i>3 Unknown</i>	20	39
	<i>4 Cushing's disease</i>	1	2

Remarks

* Percentage with reference to I., i.e., 5.9 fold in %

** Percentage with reference to II., i.e., 4.7 fold in %

1) *Diabetes Mellitus and Hypertension*

(Table 4)

Our statistical study on total of 10,798 patients admitted to our clinic during the past 20 years (1933-1952) showed that hypertension was found in 734 cases (6.8%), diabetes mellitus in 173 cases (1.6%), and a combination of hypertension and diabetes mellitus in 52 cases (0.5%), and that hypertension occurred in diabetics 4.7 times as often as in non-diabetics and diabetes occurred in hypertensive patients 5.9 times as often as in non-hypertensive patients. This finding is generally consistent with the reports from abroad.

2) *Changes in the Pulmonary and Cardiovascular Complications in Diabetes Mellitus with the Lapse of the Times* (Table 5)

In order to know the changes in the pulmonary and cardiovascular complications in diabetes mellitus with the lapse of the times, I have made a statistic on 77 patients who had been admitted to my clinic from 1946 to the present time. The cases were divided into two groups: One is a group before streptomycin Era and the other after streptomycin Era. And the complications in each group were studied.

Before streptomycin Era (1946-1954) pulmonary tuberculosis occupied 24.4% and vascular disorder 9.8% of all complications. After streptomycin Era (1955-1958), on the contrary, pulmonary tuberculosis occupied only 5.5% and vascular disorder 41.6%.

It is noted from this finding even on a small number of cases that before streptomycin Era pulmonary tuberculosis was decidedly a frequent complication of diabetes, but after streptomycin Era

TABLE V. INCIDENCE OF COMPLICATIONS AMONG 77 DIABETICS IN MAEKAWA CLINIC (1946-1958)

Year	Complications	Without Complications	Vascular Lesions	Tuberculosis	Others
	Cases				
1946-1954	41	20 (48.8%)	4 (9.8%)	10 (24.4%)	7 (17.0%)
1955-1958	36	13 (36.1%)	15 (41.6%)	2 (5.5%)	6 (16.7)%

Pre S. M. Era (1946-1954)

48.8

9.8

24.4

17.0

Post S. M. Era (1955-1958)

36.1

41.6

5.5

15.7

D.M.

V.L.

T.B.C.

Others

Note; D.M.: Diabetes Mellitus V.L.: Vascular Lesion T.B.C.: Tuberculosis

Photo. 1. Examples of E.C.G. in Diabetic Patient without Hypertension.

hypertension took the place of pulmonary tuberculosis as the most frequent complication of diabetes.

3) *Diabetes Mellitus and the Electrocardiographic Findings* (Table 6)

TABLE VI.

	No. of Cases	Percent	Referring to			
			Blood Pressure		Abnormal Eye Ground	Abnormal Tendon Reflex
			Normotension	Hypertension		
Normal	29	59.2	24 (70.6)	5 (36.7)	6 (40.0)	7 (43.8)
T Lowering	7	14.3	5	1	1	1
T Inversion	1	2.0	1	0	0	1
ST Depression	3	6.1	0	2 (63.3)	2 (60.0)	2 (56.2)
ST Depression with T Lowering	4	8.2	1	4	3	3
Conduction Disturbance	1	2.0	0	1	1	0
Premature Contraction	4	8.2	3	1	2	2
sum	49	100.0	34 (100.0)	14 (100.0)	15 (100.0)	16 (100.0)

Note; (): Percent. Hypertension: Above 150 mmHg in max. and/or 90 mmHg in min.

Photo. 2. Examples of E.C.G. in Diabetic patient with Hypertension.

The electrocardiographic studies were done on 49 out of the 77 diabetic patients previously described. In about a half of them (40.8%) there were observed abnormal electrocardiographic patterns and in such high percentages as 63.3% in the group with abnormal tendon reflexes. The electrocardiographic changes in diabetic patients in this series were mainly consisted of the patterns of coronary insufficiency which are seen in the group with vascular sclerosis. Therefore, it may be considered that the electrocardiographic changes in diabetic patients were caused by coronary sclerosis. (Photo. 1, 2)

4) Renal Functions in Diabetes Mellitus

On 62 out of the 77 diabetic patients renal functions were investigated. Renal clearance tests (CPAH and CS_2O_3) were performed 43 times in total, on 34 patients. Tm_G 17 times on 14 patients.

The patients were classified into 2 groups according to the presence or absence of hypertension as a complication. Each of them was further classified by patient's age, duration of diabetes and other conditions, and then observed statistically.

1. Average values of renal clearance tests in diabetes mellitus as a whole were within normal limits. Proteinuria was detected in 21.7% of the patients, in accordance with the former report by the author.

In the group of diabetes combined with hypertension, both GFR and eff. RPE were decreased to show mean values of 77.6 and 334 ml/min. per 1.48 m^2 of body surface area, respectively.

These values are slightly lower than those of

132 patients with essential hypertension. But FF was maintained within normal limits, i. e., 0.247 in the former, while it was 0.271 in the latter group.

2. Age (Table 7. Fig. 1, 2)

Patients were divided into 3 groups according to the age at the time of hospitalization, namely, before 29, from 30 to 49, and above 50 years.

In the third group (sixth decade or later) GFR and eff. RPF were decreased (the gradient is marked)

Fig. 1. Renal Clearance of Diabetics classified by Age and Complication with Hypertension, compared with Essential Hypertensives.

TABLE VII. RENAL FUNCTIONS OF DIABETICS CLASSIFIED WITH AGE AND COMPLICATION OF HYPERTENSION

RELATIONSHIP BETWEEN RENAL FUNCTION WITH AGE AND COMPLICATION OF HYPERTENSION													
Age	Cases		Compli.	Renal Function Test					Tm _G			PSP	Pro-teinuria
				Cases	GFR	RPF	RBF	FF	Cases	Tm _G mg/min.	GFR Tm _G	% at 15'	
29	9	9	Total	5	151	685	1148	0.228	5	405	0.394	36.8	0
			D.M.	5	151	685	1148	0.228	5	405	0.394	36.8	0
			D.M.+H.	0	/	/	/	/	/	/	/	/	/
			E.H.	6	149	601	1099	0.255	/	/	/	/	/
30-49	21	18	Total	7	121	543	998	0.248	3	458	0.431	25.3	9.52
			D.M.	5	141	589	1092	0.253	3	458	0.431	25.3	5.5
			D.M.+H.	2	100	429	764	0.236	0	/	/	/	33.3
			E.H.	26	92.5	371	615	0.277	/	/	/	/	/
50-81	32	20	Total	22	88.0	371	641	0.244	6	271	0.469	19.1	35.5
			D.M.	12	100	418	721	0.240	5	287	0.490	24.5	26.3
			D.M.+H.	10	73.1	315	549	0.249	1	189	0.360	14.2	50.0
			E.H.	100	89.4	345	579	0.270	/	/	/	/	/
Total	62	47	Total	34	106	453	792	0.242	14	359	0.434	23.3	21.7
			D.M.	22	121	517	906	0.240	13	372	0.440	27.0	13.3
			D.M.+H.	12	77.6	334	585	0.247	1	189	0.360	14.2	46.7
			E.H.	132	93.4	361	609	0.271	/	/	/	/	/

Fig. 2. Age Incidence of Proteinuria in Diabetics.

to the mean values of 88.0 and 371 ml/min. per 1.48 m², respectively. The Tm_G too had the same tendency, and GFR/Tm_G inclined to increase.

The appearance of proteinuria was as high as 35.5% in the third group above 50 years of age. This tendency becomes significant when the patients are divided by whether combining with hypertension or not. That is, the values of GFR and eff. RPF in the hypertensive group of diabetes were considerably lower than those in the non-hypertensive group. The appearance of proteinuria also

was increased by 2 to 3 times. It is interesting to see that, in diabetes mellitus a marked impairment of renal functions takes place for the first time in the 6th decade or later, while in essential hypertension a marked impairment takes place in the 4th to 5th decade.

This evidence may speak of differences in the pathogenesis of renal damage in the two diseases.

3. Duration (Table 8)

According to the duration of diabetes mellitus, patients were divided into 3 groups; less than 5 years, 5 to 10, and more than 10 years, respectively. There is a marked difference in the renal functions between the "less than 5 years" group and the "more than 5 years" group. Taking the hypertensive and non-hypertensive groups into account, the difference in the renal functions between the "less than" and the "more than 5 years" groups becomes more distinct in the hypertensive group. It is interesting to find the evidence that in some cases whom hypertension preceded diabetes, the renal functions were maintained almost normal in spite of long standing diabetes of more than 10 years. Since the number of the cases was not large enough in this series, I would avoid to make a definitive conclusion about this matter, but it seems true because we see many of such cases among outpatients.

4. Which occurred first, Diabetes or Hypertension? (Table 9)

Patients with both diabetes and hypertension

TABLE VIII. RENAL FUNCTIONS OF DIABETICS CLASSIFIED WITH DURATION AND HYPERTENSION-COMPLICATION

Duration	Complication	Cases	Renal Clearance Test						PSP Cases		Urinalysis H&A. Sulf.	
			Cases	Age	GFR	eff. RPF	eff. RBF	FF				
—4	Total	42	23	50	119	514	906	0.242	24	26	6/42	9/42
	D.	34	17	45	130	548	957	0.251	20	28	4/34	6/34
	H.+D.	3	3	56	98	496	890	0.199	2	14	0/3	0/3
	H. Unknown	5	3	70	79.3	339	633	0.232	2	17.5	2/5	3/5
	D.+H.	0	/	/	/	/	/	/	/	/	/	/
5—9	Total	2	2	62	60.3	347	636	0.158	1	14	1/1	1/1
	D.	1	1	57	80.9	417	695	0.172	1	14	/	/
	H.+D.	1	1	67	39.6	277	577	0.143	/	/	1/1	1/1
	D.+H.	0	0	/	/	/	/	/	/	/	/	/
	Total	12	8	57	69.1	292	444	0.253	5	13	6/12	7/12
10—	D.	5*	2	58	77.0	419	770	0.180	/	/	1/5	1/5
	H.+D.	1	1	88	135	504	861	0.267	1	28	1/1	1/1
	D.+H.	6	5	56	52.8	199	217	0.280	4	9	4/6	5/6
	Total	12	8	57	69.1	292	444	0.253	5	13	6/12	7/12
	D.	5*	2	58	77.0	419	770	0.180	/	/	1/5	1/5

* One of which shows Good Function.

S.I. Diabetes, about 30 years.

without Hypertension.

Angina Pectoris.

R.R. 100/80-70 mm Hg.

109 573 1049 0.190 / (—) (—)

TABLE IX. WHICH IS ANTECEDENT, DIABETES MELLITUS OR HYPERTENSION?

	Cases	Renal Clearance Test								PSP		Urinalysis		
		Cases	Age	Ave. yrs. from onset		GFR	eff. RPF	eff. RBF	FF	Cases	% in 15'	H.&A. test	Ery.	Cyl.
				H.	D.									
D.+H.	6	5	56 y 5 m	ca. 4 y	19 y 7 m (10 m~ 37 y)	52.8	119	318	0.280	4	9.3	5/6	3/6	2/6
H.+D.	5	5	58 y 7 m	10 y (1 m~ 23 y)	3 y 11 m (1 m~ 13 y)	73.7	454	822	0.201	3	19	2/5	1/5	0
Unknown	5	3	70 y 4 m	?	ca. 2 y (6 m~ 5 y)	79.3	339	633	0.232	2	18	2/5	2/4	0

were divided into 3 group, i. e., diabetes preceded hypertension, hypertension preceded diabetes, and unknown. In the diabetes preceded-group, in comparison with the hypertension preceded-group, the renal clearance values were decreased significantly. Proteinuria and others showed the similar tendency.

5. Classification by the Ophthalmological Findings (Table 10)

Findings of the eye-ground were classified according to Keith-Wagener, and the patients were divided into 3 groups which were K. W. O, I-II, and III respectively, for the statistics.

In the K. W. O group, renal functions were normal. In the K. W. I-II group, both GFR and eff. RPF showed approximately 60% of the mean normals, and in the K. W. III group, renal clearance values were about 50% of the mean normals. That is to say, there is a distinct difference in renal clearances, between the K.W. O group and the K.W. I-II group. PSP test showed parallelism to renal clearances in connection with eye-ground changes.

The presence of proteinuria in these groups was 12.9, 28.6, and 100 per cent respectively, showing a distinct difference between the K. W. I-II group and the K. W. III group.

6. Body Weight (Table 11)

Classified into 3 groups as follows,

- (B.L. cm-100) kg-B.W. kg ≤ 0 A (obese)
 0 < (B.L. cm-100) kg-B.W. kg < 10 B (ordinary)
 (B.L. cm-100) kg-B.W. kg > 10 C (slender)

Throughout each age, generally, the values of GFR, eff. RPF seemed to be highest in A group and lowest in C group (A > B > C).

Summary

Summarizing the above, renal functions in diabetes mellitus take a down-hill course, as the age progresses, and in and after the 6th decade their considerable fall is noted. The decrease is more remarkable in such patients whom diabetes lasted more than 5 years and, in addition to this, hypertension was combined. The grade of decrease in renal function has an almost parallel correlation with the grade of sclerosis in other organs. But the cases in whom hypertension preceded to diabetes are exceptional, because in most of such cases renal functions may be well maintained for many years.

Although the cases whom diabetes was combined with the pre-existing hypertension seem to be prevalent in our country, we have not had much

TABLE X. RENAL FUNCTIONS OF DIABETICS CLASSIFIED WITH OCULAR FINDINGS

	No. of cases examin.	Renal Clearance Test						TmG			PSP		Urinalysis		
		No. of cases examin.	GFR	eff. RPF	eff. RBF	FF	No. of cases examin.	TmG	GFR TmG	RPF TmG	No. of cases examin.	% at 15 min.	Protein-uria in %	Haemat-uria in %	Cylind-uria in %
K.W. O	31	17	124	536	944	0.242	10	379	0.467	1.772	20	27.8	12.9	25.0	10.7
I-II	7	5	83.1	338	621	0.264	1	277	0.415	1.646	4	16.5	28.6	28.6	14.3
III	7	6	68.0	299	512	0.229	1	189	0.360	1.709	5	13.4	100.0	42.9	14.3
Retinitis diabetica	4	3	54.1	245	373	0.211	0	/	/	/	2	11.0	75.0	50.0	50.0
Cala-racta	Diabet.	3	2	53.0	212	358	0.279	0	/	/	3	21.7	33.3	33.3	0
	Senil	4	2	61.5	367	624	0.168	0	/	/	3	21.3	50.0	75.0	50.0
	sum	7	4	57.2	290	491	0.224	0	/	/	6	21.5	42.9	57.1	28.6

TABLE XI. RENAL FUNCTIONS CLASSIFIED WITH AGE AND BODY WEIGHT

Age Group	Type	Renal Clearance Test					PSP % 15 min.	Proteinuria H. & A. Test
		Cases	GFR	eff. RPF	eff. RBF	FF		
29	A	0	/	/	/	/	/	/
	B	1	162	743	1281	0.219	45	0/3
	C	4	148	671	1115	0.230	24	0/3
30	A	4	134	602	1137	0.235	26	0/7
	B	2	136	507	941	0.271	15	0/5
	C	1	97.2	382	558	0.254	33	1/3
50	A	4	108	445	763	0.258	21	2/6
	B	12	87.1	371	668	0.234	19	4/15
	C	6	76.8	316	525	0.254	21	3/7

Note; A: {Body Length (cm) - 100 - Body Weight (kg)} ≤ 0
 B: 10 > " > 0
 C: " > 10

opportunity to observe such kind of diabetes, for such cases usually take a slow and long standing course with slight symptoms and do not come to the university hospital.

5) Serum Protein of the Diabetic Patients

1. Generally speaking, there was no remarkable change observed in the electrophoretic patterns of the serum protein of the diabetics. But when they were classified into groups by the existence of complication, it was revealed that the amount of the total serum protein was within normal limits in most of them, and decreased or increased in a few cases. In the diabetics who combine with renal diseases, the total protein was markedly decreased and so was the albumin. The α , β , γ -globulins were slightly increased in general, and this tendency was noticeable in the cases with renal complications.

2. Serum Protein in Connection to Ages (Fig. 3)

The 20 diabetic patients without complication were classified into three groups according to their age, (namely, A group under 29 years, B group between 30-49 years and C group above 50 years old) and decrease of albumin was observed more frequently in the aged (C group) than in the younger patients (A group). α , β , γ -globulins, particularly β -globulin were often increased in the aged patients (C group). In comparison with the same age groups of essential hypertension without complication increase of β -globulin was observed more often in the diabetic patients than in the hypertensives.

3. Serum Protein in Connection with Duration of Diabetes Mellitus (Fig. 4)

By classifying the cases into two groups according to the duration; the one within a year and the other more than a year from the onset of the disease, it was revealed that there was not much difference between the two groups except β -globulin being slightly increased in the latter group (of more

Fig. 3. Serum Protein Fractions of Diabetic & Hypertensive Patient in Reference to Age.

Fig. 4. Serum Protein Fractions of Diabetics in Reference to Onset.

than 2 years in duration) when there was no complication. However, when diabetes was accompanied by complications, there were a decrease in albumin and an increase in α and β , especially β -globulin in the latter group.

4. In Connection with Body Weight (Fig. 5)

An increase of α - and β -globulins was observed in most patients over weighed.

As mentioned above, our findings correspond to the already known evidences, and it is not uncommon to find an increase of β -lipoprotein in the sera of the diabetics when diabetes was accompanied by its complications especially renal complication.

Fig. 5. Serum Protein Fractions of Diabetics in Reference to Body Weight.

6) Diabetes Mellitus and Serum Cholesterol (Fig. 6)

The normal values of serum cholesterol among Japanese are from 150 to 220 mg/dl. In our study too, this value was more increased in the diabetics than in the hypertensives of the same age group, and was markedly increased particularly in the elderly diabetic patients. However, there were some exceptional cases so that a sweeping statement could not be made.

Fig. 6. Serum Cholesterol in Patients with Diabetes Mellitus and Uncomplicated Hypertension.

7) Histological Changes of the Arteries in Diabetic Patients

Table 12. illustrates the summary of the micro-

TABLE XII. RELATIONSHIP BETWEEN DIABETES MELLITUS AND ARTERIOSCLEROSIS

Casa	Age	Sex	Clinical Diagnosis	Arteriosclerosis								
				Aorta	Heart	Liver	Spleen	Kidney	Pancreas	Lung	Brain	Mesenterium
R. A.	66	F	D. M. & Gangrene of the right foot	+++	++	+	++	+	+	-	-	-
?	8	M	?	-	-	±	-	-	-	-	-	-
Y. H.	22	M	D. M. & Splenomegaly Peritonitis	±	-	-	-	±	-	-	-	-
M. N.	44	F	Acromegaly with Glycosuria	++	++	+	-	+++ ICGS*	++	-	-	++
B. I.	62	M	D. M.	++	+	+	++	+	±	-	-	-
S. Y.	43	F	D. M. & Psychic Reaction	+	-	-	+	-	+	-	-	-
T. I.	48	M	D. M. & Pulm. Tuberc.	-	±	+	±	±	-	+	-	±

* Intercapillary Glomerulosclerosis

scopic findings of 7 cases among the 12 diabetics who were autopsied at the Department of Pathology, Kyoto University, during the period between 1901 to 1957. **One of these patients, a 44 year old woman, died of Kimmelstiel-Wilson's Syndrome. (Fig. 7) The patient was admitted when she was

Fig. 7. M. N. 44 y o. f.

26 years of age, and its clinical course had been followed up in detail until her death. Hypertension had combined since 40 years of age. General dropsy, increase of serum NPN content, retinal bleeding and so forth were observed in the terminal stage. Her clinical course is illustrated in Photo. 3. (through the courtesy of Dr. Miyoshi, The First Division of Internal Medicine, Kyoto University Hospital.)

Some histological findings of the same patient are shown in Photo. 3, 4, 5 and 6.

As a conclusion of histological findings of the arteries in the diabetic patients, it may be stated that the intimal proliferation and degeneration were

Photo. 3. Diabetic capillary glomerulosclerosis.

Photo. 4. Atherosclerosis of the aorta.

most characteristic, that is, as already known, typical atherosclerosis, though there may be a few exceptions. And it should be noticed that, even when hypertension was combined, the histological examination of the diabetics revealed no hyperplastic medial sclerosis in the small arteries especially in those of the kidneys.

** It was reported in Japanese Archives of Internal Medicine: Vol. 2, No. 5 (1955) under the title of "A case of Acromegaly Associated with Glycosuria under Observation over a Prolonged Period" by Dr. A. Miyoshi et al.

Photo. 5. Coronary sclerosis.
(anterior descending artery)

Photo 6. Sclerotic artery of the pancreas.

8) *Significance of Adenosinetriphosphatase and Glycerophosphatase in Diabetes Mellitus*

1. ATPase and GPase in Serum (Table 13)

The activity of ATPase was found to be increased at pH 7.4, 5.2 and 9.2 in the sera of diabetic

patients as compared to normal individuals. Especially acid ATPase activity which is hardly found in normal individuals could be found in diabetic patients. However this acid ATPase did not have the same characteristic as that of the acid ATPase in hypertensive patient, i. e., this acid ATPase was not activated by Na ion or suppressed by F ion. It is also interesting to note the disappearance of the activity of acid ATPase in the sera of the diabetics when hypertension was combined with diabetes. This evidence shows that the hypertension in diabetes mellitus is different from the hypertension in other origins. Acid GPase (at pH 5.2) was increased in the sera of some diabetic patients, but not in every case. The activity of alkaline GPase also was slightly increased in some cases but not so much increased as in hepatic diseases or hyperthyroidism.

2. ATPase and GPase Activities in Urine

11 determinations were done on 8 diabetic patients. Except one case, in 10 determinations on 7 patients there was markedly increased activities of acid ATPase and especially acid GPase. (pH 5.2) (Table 14)

In the control group, 15 gamma units were the maximal activities. (Activity is expressed in a term of gamma P at 37°C, 30 minutes incubation.) (Table 15)

Besides diabetes mellitus, 10 out of 12 cases of nephritis and nephrotic syndrom had activities of more than 20 units. Although it has been known that acid GPase activity was found considerably

TABLE XIII. ATP- AND GP-DEPHOSPHORYLATING ACTIVITY OF SERA IN DIABETICS
WITHOUT AND WITH HYPERTENSION

NAME	AGE	SEX	BLOOD PRESSURE	pH 5.2				pH 9.2			
				ATPase			GPase	ATPase			GPase
				Na	Mg	Non.		Na	Mg	Non.	
S. N.	48	M		8.4	7.9	10.0	0.0	7.9	7.9	10.4	3.6
T. F.	36	F		1.8	/	2.2	2.0	/	/	0.0	6.2
I. M.	25	F		2.7	1.3	2.2	1.8	/	3.2	3.3	2.5
T. A.	47	F		1.4	1.4	4.0	1.3	6.7	3.2	3.1	0.9
S. G.	64	M		3.1	0.5	1.1	/	1.1	1.1	0.9	4.3
M. I.	52	M		2.3	1.8	2.3	0.0	2.5	3.2	3.8	2.2
H. T.	62	M		/	/	1.4	0.0	/	/	3.9	3.0
K. O.	50	F		1.4	0.4	0.0	0.0	3.8	0.0	1.6	4.7
S. T.	49	M		/	/	1.4	0.0	/	/	4.0	6.1
K. S.	20	M		1.4	2.7	2.5	2.0	0.9	3.4	2.2	6.6
O. T.	40	M		2.3	2.0	2.5	0.0	6.7	6.7	8.3	2.5
U. S.	58	M	210/115	0.0	0.0	0.0	0.0	3.4	0.5	1.4	3.1
T. H.	63	M	190/90-86	1.1	2.9	2.9	0.0	7.4	6.1	6.1	6.5
Y. S.	67	M	184/96-90	0.4	0.0	0.0	0.0	2.0	2.2	2.2	2.9
H. H.	62	M	162/98-94	0.0	/	0.0	1.1	0.5	4.9	2.3	7.7
K. M.	58	M	160/90-86	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4

Activity Unit : Gamma P per ml (5 min. at 37°C)

TABLE XIV. ATP- AND GP-DEPHOSPHORYLATING ACTIVITY OF THE DIALYZED URINE IN DIABETIC AND NEPHRITIC PATIENTS

	NAME	AGE	SEX	pH 5.2		pH 9.2	
				ATPase	GPase	ATPase	GPase
Diabetes Mellitus	S. N.	42	M	8.4	48.6	25.4	0.9
	"	"	"	10.9	56.6	6.0	1.0
	S. T.	49	M	5.2	34.0	0.9	1.5
	I. H.	43	M	12.6	30.0	1.6	0.0
	H. T.	62	M	10.5	60.3	6.7	2.1
	"	"	"	9.6	150.0	0.6	0.6
	K. S.	20	M	0.0	26.4	1.8	3.9
	"	"	"	6.5	19.4	4.5	1.9
	K. O.	50	F	17.9	39.0	1.5	3.6
	T. O.	40	M	21.6	60.0	0.0	0.8
Nephritis	T. A.	47	F	8.4	4.9	0.9	1.0
	N. I.	26	M	4.3	93.3	2.4	3.4
	Y. K.	25	F	5.2	28.4	0.8	0.9
	K. N.	59	F	25.4	10.5	26.5	10.0
	A. M.	19	M	7.4	31.9	6.3	5.7
	I. H.	46	M	1.5	41.3	2.8	5.7
	M. N.	37	F	1.6	3.4	32.6	36.6
	T. I.	28	M	3.5	35.6	0.8	0.3
	A. O.	28	F	2.4	50.6	1.6	8.8
	C. H.	30	F	6.0	28.2	0.0	1.0
	K. S.	71	M	50.8	137.9	7.0	5.3
	K. W.	52	M	4.4	23.3	4.3	3.0
	N. S.	30	M	2.2	26.1	11.9	15.6

Activity Unit: Gamma P per ml. (30 min. at 37°C)
Showing increased acid GPase activity over 10 units in most cases.

TABLE XV. ATP- AND GP-DEPHOSPHORYLATING ACTIVITY OF THE DIALYZED URINE

	NAME	AGE	SEX	pH 5.2		pH 9.2	
				ATPase	GPase	ATPase	GPase
Contrast Group	S. A.	31	M	6.0	1.6	2.4	1.0
	U. K.	56	M	0.0	10.6	0.0	0.9
	K. S.	30	M	3.7	2.4	0.7	2.7
	T. S.	41	M	3.3	2.0	7.6	5.6
	J. N.	29	M	0.0	10.1	0.0	0.0
	M. M.	61	F	0.0	0.0	1.9	0.9
	S. G.	64	M	0.0	3.6	0.0	0.4
	K. N.	55	F	2.6	4.4	1.9	0.9
	K. T.	17	M	7.0	15.8	/	13.1
	S. O.	73	M	0.0	1.8	0.9	0.9
	M. N.	66	F	1.4	2.3	3.5	4.7
	S. T.	51	M	0.7	7.2	0.0	0.7
	T. T.	25	F	0.0	0.0	1.0	3.0
	M. N.	26	M	3.9	7.4	0.7	0.5
	H. S.	49	F	1.8	7.0	0.7	0.2
	U. Y.	41	F	0.0	2.7	0.0	0.2
	S. M.	11	M	0.5	12.3	0.0	0.0
	Y. W.	54	M	9.4	13.9	0.4	0.0
	K. Y.	21	M	0.0	1.0	0.5	5.8
	S. K.	72	M	2.3	6.7	3.5	2.8

Activity Unit: Gamma P per ml. (30 min. at 37°C)

high in the prostate secretion, in the female patients too with such diseases as diabetes, nephritis or nephrosis, acid GPase activity was increased. Therefore, at least part of acid GPase activity seems to be derived from the upper urinary tract, presumably the kidney. In this connection, urine specimens were obtained by the following method;

nothing by mouth after midnight. At 6 a. m. the bladder was made empty by urination and 2 hours later the urine specimen was obtained by urination.

3. Behaviour of Acid GPase in Urine upon Glucose Tolerance Test

This GPase is conceivably same as the dephosphorylating enzyme of Glucose-6-Phosphate. To verify this, glucose tolerance test was done on 5 diabetic patients and a patient with Friedreich's ataxia, and acid GPase activity in the urine was quantitatively measured simultaneously with blood sugar and urine sugar.

Method: In a fasting condition, the bladder was washed through a catheter with 1000 ml of distilled water or normal saline solution and the catheter was left open until the rinsing solution was completely voided, and then the first urine specimen in 30 min. duration was obtained as a control specimen.

Glucose solution was injected intravenously in 15 minutes with a dosis of glucose 0.5 gm per kg of body weight. Urine specimens were collected every 30 minutes for 3 hours. Blood specimens for blood sugar were also collected every 30 minutes for 3 hours. The urine specimens were dialysed against redistilled water in a refrigerator for 24 hours and then used for the determination of the dephosphorylating activity.

(Fig. 8) K.O. age 52, female, Friedreich's ataxia

At 30 min. from the start of GTT, blood sugar level was 234 mg/dl and glycosuria was appeared.

Fig. 8. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note; U.V.: Urine Volume U.S.: Urine Sugar

The slight fluctuation of the total acid GPase activity in each urine specimen seems to have a correlation with the amount of urine sugar.

(Fig. 9) T. O. age 40, male, Diabetes mellitus

Blood sugar content was 238 mg/dl and glycosuria was present at 30 min.. There was significant fluctuation of acid GPase activity in each urine specimen corresponding to the fluctuation of blood sugar levels and the severity of glycosuria.

Fig. 9. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

(Fig. 10) H. T. age 62, male, Diabetes mellitus
Same as the above.

Fig. 10. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

(Fig. 11) I. M. age 26, male, Juvenile Diabetes mellitus.

Acid GPase activity in each urine specimen showed reverse correlation with blood sugar curve.

Fig. 11. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

(Fig. 12) K. I. age 53, female, Diabetes mellitus

Fluctuation of acid GPase activity in each specimen was in parallel correlation with the blood sugar curve with slight delay.

Fig. 12. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

(Fig. 13) T.A. age 47, female, Diabetes mellitus
Same as the above.

Fig. 13. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Glucose Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

(Fig. 14) T. O. age 40, male, Diabetes mellitus
Insuline tolerance test revealed the total acid GPase activity in each urine specimen at 30 min. interval was in the parallel correlation with the change of blood sugar level with slight delay.

Fig. 14. Relationship between Acid GPase of Urine and Blood Sugar on the Intravenous Insulin Tolerance Test.

Note: U.V.: Urine Volume U.S.: Urine Sugar

Summary

Upon GTT and ITT, it was revealed that acid GPase activity in the urine of the above mentioned cases except one case fluctuated in accordance with the changes of blood sugar level and the changes in the severity of glycosuria, but it was not an absolutely parallel correlation.

4. Enzymological Characteristic of Acid Phosphatase in Urine

a. The characteristic of the acid phosphatase

appeared in the urine of diabetic and nephritic patients was that it was not activated by Mg ion but inhibited by F ion, i. e. similar to that of a prostatic origin. In order to verify the origin of acid GPase, urine specimens were obtained before and after washing the urinary bladder through the catheter. The acid GPase activities in both urine specimens were measured and it was found that in men the acid GPase activity was not always lesser in the latter urine specimen than in the former specimen, on the other hand in women the activity was sometimes lesser in the latter specimen than in the former specimen. (Table 16) This result is

TABLE XVI. GP-DEPHOSPHORYLATING ACTIVITY OF THE CATHETERIZED URINE BEFORE AND AFTER BLADDER IRRIGATION

NAME	AGE	SEX	DIAGNOSIS	GPase activity at pH 5.2	
				before	after
T. O.	40	M	Diabetes Mellitus	33.6	4.2
C. H.	30	F	Nephritis	1.8	0.7
M. K.	29	M	Nephritis	23.3	57.4
				24.5 (Mg)	58.7 (Mg)
				1.8 (F)	1.8 (F)
A. O.	28	F	Nephritis	7.4	4.2
Y. K.	25	F	Nephrose	/	4.7

probably due to the dilution of urine by the bladder washing. Of course, there was no influence on the enzymological characteristic by the bladder washing.

b. Concentration

According to the method shown in Table 17, the urine specimens of the diabetics and nephrotics were concentrated, and upon the study of the characteristic of the acid ATPase and acid GPase in the concentrated material, it was found that both acid ATPase and acid GPase were not activated by Mg ion but markedly inhibited by F ion. (Table 18)

5. Physiological Effects of Acid Phosphatase in Urine

(Fig. 15, 16) As mentioned above, the urine which had been concentrated and dialyzed against redistilled water was injected into the tail vein of albinorats under no anesthesia, and then blood specimen was obtained from the rats before the injection and 15 minutes and 30 minutes after the injection. The blood sugar level rose approx. 50-100% above the initial level. The reactions to the injection such as hematuria & hemoptysis were observed.

6. Change in Blood Sugar upon Injection of W-Fraction of Kidney ATPase (Fig. 17)

The physiological effects of acid GPase in urine are very similar to the effects of W-fraction of the

TABLE XVII. METHODE OF CONCENTRATION OF URINE ACID GPASE

TABLE XIIX. ATP- AND GP-DEPHOSPHORYLATING ACTIVITY OF CONCENTRATED URINE AT pH 5.2

NAME	AGE	SEX	DIAGNOSIS	ATPase			GPase		
				Non.	F	Mg	Non.	F	Mg
K. S.	70	M	Diabetes Mellitus	26.5	0.0	26.5	107.1	2.3	107.1
I. M.	26	M	Diabetes Mellitus				154.0		
K. I.	53	F	Diabetes Mellitus	27.7	7.7	28.8	94.0	3.2	83.0
Y. K.	25	F	Nephrose	59.4			182.9	94

NAME	M.K.	K.N.
AGE	29	29
SEX	Male	Male
DIAGNOSIS	Chronic Nephritis	Normal
Activity unit/0.1Kg (Rat Weight)	24g	21g
* Ratio: max./init.	1.99	1.79

Fig. 16. Effect of GPase Fraction of Urine on Blood Sugar in Rat.

*; Maximal Blood Sugar Level/Initial Blood Sugar Level. HEMATURIA was observed in every case.

Fig. 15. Effect of GPase Fraction of Urine on Blood Sugar in Rat.

*; Maximal Blood Sugar Level/Initial Blood Sugar Level. HEMATURIA and HEMOPTYSIS were observed in every case.

Fig. 17. Effect of Crude Kidney (Weber's) Fraction.

pressor kidney ATPase which caused in dogs a rise of blood sugar level to 180% of the initial level, hematuria and bloody sputa. The further investigation is under the way at present.

Summary

It was found that in the sera of diabetic patients, the activities of acid ATPase and acid GPase were increased but when hypertension was combined with diabetes mellitus those activities were not detected. In the urine of diabetic patients, the activity of acid GPase was markedly increased and ATPase slightly or moderately increased. The acid GPase activity was also increased in the urine of nephritic patients. This acid phosphatase is enzymologically similar that of prostatic origin, that is, not activated by Mg ion but inhibited F ion. It, however, decomposes ATP on the contrary to the acid GPase of prostatic origin. The reason for it may be sought in the impurity of the enzyme.

Furthermore, that there is the acid GPase in urine of non-prostatic origin can be understood by the fact that its activity was increased in the urine of female diabetic and nephritic patients, and also upon GTT and ITT the activity fluctuated corresponding to the changes of blood sugar or urine sugar levels. The acid phosphatase in urine has the physiological effects such as a rise in blood sugar and capillary hemorrhages in albino rats which were given intravenous injections of acid phosphatase fraction of urine.

In this respect, the acid phosphatase in urine has similar physiological effects as those of the non-purified kidney ATPase, i. e., W-fraction which we obtained previously.

The origin and the meaning of this substance on diabetes mellitus or nephritis are still obscure and it should be discussed upon the further investigation, it, however, may be presumed that the acid

GPase plays an important role on the increment of blood sugar content and excretion and reabsorption of sugar in the kidneys.

It is my feeling that destruction of the capillaries could be ensued when this substance abnormally increased in the blood stream. Namely, hematuria and hemoptysis could be resulted when it increased acutely, and Kimmelstiel nodules or a similar vascular damage could be resulted when it increased chronically.

9) Diabetes Mellitus and Gangrene

Gangrene is not an exclusively characteristic complication of diabetes mellitus. Among 11,958 patients entering the Department of Surgery of Kyoto University Hospital between 1950- to 1955, there were 64 cases (0.53%) of gangrene of the extremities. (Table 19) Among them fifty nine cases (97%) had Buerger's disease and only two cases had diabetic gangrene.

TABLE XIX. NUMBER OF PATIENTS WITH THROMBOANGIITIS AND ARTERIOSCLEROSIS OBLITERANS IN RELATION TO AGE AND SEX

(DEPARTMENT OF SURGERY, KYOTO UNIVERSITY HOSPITAL. 1950-1956.)

Decade	Sex	Thromboangiitis obliterans	Arteriosclerosis obliterans	Total
2	M	1	0	
	F	0	0	1
3	M	13	0	
	F	2	0	15
4	M	20	0	
	F	2	0	22
5	M	13	0	
	F	3	1**	17
6	M	5	0	
	F	0	0	5
7	M	0	0	
	F	0	2*	2
8	M	0	1*	
	F	0	1**	2
Total		59	5	64

* Complication with Myocardial Infarction

** Complication with Diabetes Mellitus

Now I would show some results of our experimental spontaneous gangrene. Following the procedures shown in Table 20, gangrene was produced in the tails of mice. (Photo. 7) All of these experimental procedures were assumed to cause some metabolic disturbance of ATP in the tissue. The lesion of gangrene consisted of degeneration with capillary bleeding, but other cellular reactive changes were hardly seen. (Photo. 8) Photo. 9 illustrates degenerative changes of the arterial wall

TABLE XX. INCIDENCE OF THE EXPERIMENTAL GANGRENE, CLASSIFYING WITH CAUSATIVE AGENTS

Administred Materials	Administ-ration Method	Experi-mental Animals	Cases with Gangrene
2-4 Dinitrophenol	i.p.	Rat	3
2-4 Dinitrophenol	i.v.	Rabbit	1
Butylic Acid	i.p.	Rat	3
Phlorhizin + Butyric Acid	i.p.	Rat	1
Phlorhizin	i.p.	Rat	5
Salt	per os & i.p.	Rat	12
Dog Kidney-ATPase	i.p.	Rat	6
Rat Kidney-ATPase	i.p.	Rat	3

Conclusion of Histologic Findings :

In the proximal part of necrotic tail, capillary bleeding and stasis of blood in arteries and veins were seen, but significant changes were hardly observed in the arterial wall.

In the cases of butylic acid, however, arteries showed the tendency to intimal hyperplasia.

Photo. 7.

Photo. 8.

in the tail stained by Masson's method. No occlusion is present.

From these studies, I would speculate as follows: spontaneous gangrene is usually caused by obstructive arterial diseases resulting in anoxia in the distal part of the tissue. In our experiments mentioned above, no arterial occlusion could be

Photo. 9.

seen in the presence of gangrene, so it is evident that there was no anoxia in the tail. In this event, however, some disturbance of ATP metabolism might occur in the tissue, just corresponding to the metabolic disturbance due to anoxia. It seems likely that deficiency of ATP pooling in the tissue may be the direct cause of spontaneous gangrene.

By the way, regarding the action of phosphatase, it suppresses glucose consumption in the peripheral tissue and lessens ATP pooling, thus leading to a rise of blood sugar. In acute experiment, it might cause capillary bleeding and in chronic experiment, it might cause degeneration of the capillary vessels.

CONCLUSION

Arteriosclerosis is one of the essential changes in diabetes mellitus and impairs the pooling of high energy phosphate bond through metabolism, and a lack of high energy phosphate pooling may be considered as a direct cause of degenerative diseases. In diabetes the disturbance of acid phosphatase is considered as a most possible cause of the lack of high energy phosphate pooling, namely, the disturbance of acid phosphatase causes hyperglycemia and renal glycosuria.

1. Hyperglycemia leading to degeneration of β cell resulting lack of insulin, that is diabetes mellitus.

2. Renal glycosuria leading to Fanconi's Syndrom.

On the other hand, impairment of high energy phosphate pooling results in capillary degeneration as well as capillary aneurysm, and in combination with cholesterol deposition, results in atheromatus change and sclerosis of the arteries.

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