Combined Clinical and Pathologic Study of Diabetic and Nondiabetic Peripheral Arterial Disease

D. E. Strandness, Jr., M.D., R. E. Priest, M.D., and G. E. Gibbons, M.D., Seattle

SUMMARY

The results of a combined clinical and pathologic study of large and small arteries in the diabetic and nondiabetic patient show that the diabetic has the same incidence of occlusion in the femoral-popliteal system but a higher incidence below the knee. A specific lesion of small arteries and arterioles which distinguishes the diabetic from the nondiabetic was not observed in this study. The results of this investigation provide no support for the concept that a specific disease of small arteries and arterioles in diabetic patients contributes to the ischemic process. We believe the ischemic lesions found in the diabetic patient can adequately be explained on the basis of arteriosclerotic obstruction of the major arteries, and those ulcers occurring in the absence of major artery disease are attributable to peripheral neuropathy.

There have been many speculations in regard to the increased incidence of ischemic lesions in the lower extremities of the diabetic patient. Since the atherosclerotic process involving the cognate system of arteries does not appear to differ, at least qualitatively, from that of the nondiabetic, some investigators have attributed this increased incidence to specific abnormalities in the small unnamed arteries and arterioles.[1,2] Our interest in this problem stems from a preliminary study (carried out in 1959) of the arteriolar lesions in extremities amputated for complications of arteriosclerosis obliterans.[3] Although arteriolar changes were seen, there did not appear to be any appreciable difference between vascular lesions in the diabetic and nondiabetic patient. The extent and location of these changes were extremely variable and bore no direct relationship to the extent of the large artery involvement and site of tissue necrosis. The initial study was too small to warrant definite conclusions but has been continued and expanded in a prospective fashion. The findings of this study which are based on clinical evaluation, plethysmography and detailed study of amputated extremities both grossly and microscopically do not agree with a number of the observations reported in the literature.

From the Surgical and Pathology Services, Veterans Administration Hospital, and the Department of Surgery, University of Washington School of Medicine, Seattle, Washington.

METHODS AND MATERIALS

The forty-two diabetic and thirty-five nondiabetic patients considered in this study were referred to and studied in our vascular laboratory because of complaints involving the lower extremity, presumably secondary to occlusive disease of the arterial system. Of the thirty-five patients who were considered nondiabetic, seventeen had fasting blood glucose below 80 mg. per 100 ml., five between 80 to 90 mg. per 100 ml., and two between 90 to 100 mg. per 100 ml. The remaining eleven patients had a normal two-hour postprandial blood glucose.* The patients were divided into two groups: 1. Nonoperative group, thirty-seven diabetic and thirty-nine nondiabetic extremities. None of the patients in this group had an amputation during the period of this study. Each had complete clinical evaluation and plethysmography. 2. Amputation group, seventeen diabetic and nineteen nondiabetic extremities were evaluated.

THE STUDY PROGRAM

The study program was as follows:

1. Complete history with particular reference to lower extremity symptoms and signs.

2. If diabetic, the duration, type of therapy and adequacy of control were determined. Particular attention was directed to the presence or absence of some of the complications of the disease, namely retinopathy, renal disease, and peripheral neuropathy.

3. Physical examination: *a.* Pulses—the pulse categories suggested by Smithwick[4] were used throughout this study (table 2):

Group I—one or both pedal pulses palpable;
Group II—pedal pulses absent, popliteal pulses present;

*It is possible that some of these patients had latent diabetes not detected by these tests. What effect this might have on the validity of this study is uncertain but, as will be shown, there were significant differences between the diabetic and nondiabetic as we classified them.

Group III—femoral pulses present, none distal to this level;

Group IV—all pulses absent in the extremity.

b. Neurologic examination—status of deep tendon reflexes, superficial and deep sensation and position sense were noted.

c. Tissue integrity—if necrosis or gangrene were present, the site and extent were carefully noted.

4. Special studies. Twenty-seven nondiabetic and twenty-nine diabetic patients had further evaluation of the vascular system of the upper and lower extremities using the mercury strain gauge plethysmograph. Previously described technics[5] were utilized and, in brief, consisted of the following: *a.* Digit volume pulse and contour were recorded; obstruction in the arterial tree from the aorta to the tips of the digit can be diagnosed by changes in the contour of the pulse and its amplitude. The normal digit pulse shows a sharp systolic peak with a dicrotic wave on the downslope (figure 1A). With arterial obstruction, the high frequency components are lost and the sharp systolic peak with a dicrotic wave disappears (figure 1B).

b. Segmental leg pressures—by placing cuffs at the ankle, below the knee, above the knee, and at the upper thigh, the systolic blood pressure can be measured by noting the level of pressure in the cuff which obliterates the digit pulses. The gradient between any two successive points should not exceed 20 to 30 mm. Hg. If it is greater, arterial obstruction between these two points is present. It is possible to localize the level or levels of arterial obstruction by this method. We have previously tested the reliability of these measurements in predicting the site of occlusion and found it was accurate in 84 per cent of the cases studied.[5]

c. With the mercury strain gauge on the terminal digit, it is possible to evaluate vasomotor activity. Normally with an intact sympathetic nervous system there are rhythmic changes in vasomotor tone which are reflected in a change in the amplitude of the digit pulse and the toe volume (figure 2). With deep inspiration, there occurs a transient reflex vasoconstriction (figure 3). The rhythmic changes in vasomotor tone and the inspiratory reflex are lost in peripheral neuropathy and are very useful in the early diagnosis of this complication in the patient with diabetes.

Pathologic studies. Since all the amputations were either above or below the knee, except for one limited foot amputation, the procedure followed in each case was identical.

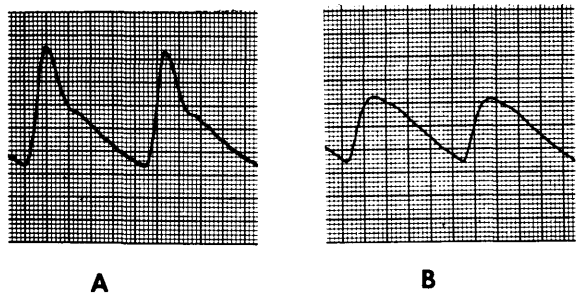

FIG. 1. (A) illustrates a normal digit pulse with a sharp systolic peak and a dicrotic wave on the downslope. In the presence of arterial obstruction (B), the curve loses its sharp systolic peak and the dicrotic wave disappears.

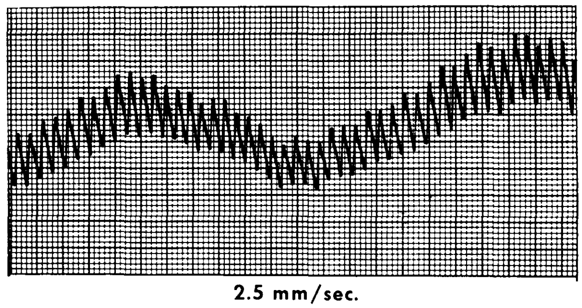

FIG. 2. Changes in the amplitude of the digit pulse reflect the normal rhythmic alterations in sympathetic activity. These are dependent upon an intact sympathetic nervous system.

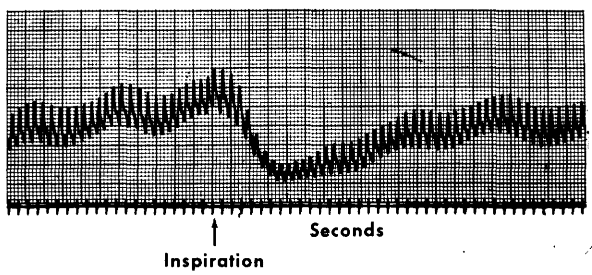

FIG. 3. The sharp decrease in digit volume and pulse height is the result of vasoconstriction after a deep inspiration. This is a sympathetic reflex.

1. The major arteries, veins and nerves were removed en bloc.

2. Multiple transverse sections were made at all levels, carefully mapping out sites of occlusion, stenosis and recanalization.

3. Multiple transverse incisions were made on the dorsal and ventral surfaces of the foot to evaluate the extent of involvement in the plantar and digit vessels but this was not completely satisfactory because of the small size of these vessels. An attempt was made to evaluate these smaller vessels by the histologic sections of the subcutaneous tissue and muscles of the foot. This still did not permit an accurate mapping of the involvement of all these vessels. A more satisfactory

approach to this area would be that used by Edwards[6] who used postamputation angiograms to outline these vessels. In a small series he found essentially no difference in the extent of involvement between the diabetic and nondiabetic patient.

4. Tissue removed for histologic study included (1) major vessels and nerves; (2) skin, subcutaneous tissue and muscle from the foot as well as from those areas supplied by the posterior tibial artery, anterior tibial artery, peroneal artery, and at the level of the amputation.

5. The tissues were embedded in paraffin and sections stained with hematoxylin and eosin, periodic acid-Schiff reagent and with colloidal iron for acid mucopolysaccharides. All slides were examined by a single investigator (R.E.P.) without prior knowledge of the clinical history or gross pathological findings. An attempt was made to separate the patients into two groups, diabetic and nondiabetic, based upon the criteria of Goldenberg and associates.[1] Particular attention was paid to the small arteries and arterioles. Arterial vessels of a diameter less than 100 microns were arbitrarily classified as arterioles and those with a diameter greater than 100 microns were classified as small arteries. The degree of hyalinization was scored on a subjective basis as: absent, mild, moderate or severe. The presence or absence of endothelial proliferation was noted. The presence or absence of intimal material staining with periodic acid-Schiff but not with colloidal iron was noted. Observations were recorded separately for small vessels of the skin, nerve, muscle and for the vasa vasorum of the major arteries. Any area demonstrating a significant inflammatory reaction was excluded.

RESULTS

In the nonamputation group, the average age of the patients with diabetes and occlusive disease was sixty years, as compared to sixty-three years in the nondiabetic. In the amputated group the average ages were identical at sixty-four years of age. The average duration of the diabetes was ten years in the amputation group as compared to sixteen years in the nonamputation group.

Consideration of the diabetics and nondiabetics in the nonamputated group revealed few differences (table 1). The pulse categories were nearly the same, and the plethysmographic studies indicated nearly the same pattern of occlusive involvement of the major arteries. One important difference was the presence of a peripheral neuropathy which occurred in 27 per cent of the diabetic but in none of the nondiabetic patients.

TABLE 1

Pulse categories* of diabetic and nondiabetic extremities with named artery occlusive disease

	Nonamputated†		Amputated	
	Diabetic	Nondiabetic	Diabetic	Nondiabetic
I	6	8	1	1
II	6	2	4	0
III	16	17	7	5
IV	9	7	4‡	13‡
Total	37	34	16	19

*Group I—one or both pedal pulses palpable; Group II—pedal pulse absent, popliteal pulse present; Group III—femoral pulse present, none distal to this level; Group IV—all pulses absent.
†—x^2—3.68, $p > .20$, corrected for continuity.
‡—x^2—4.93, $p < .05$, corrected for continuity.

The pulse categories in the amputation group differ in the incidence of type II and IV pulses but, as shown by the p values (table 1), these differences are significant for category IV only. Since consideration of pulse categories alone gives only the most proximal extent of the occlusive involvement, reference to the detailed dissections is necessary (tables 2, 3 and 4). The diabetic patients have a significantly higher incidence of involvement in the anterior tibial, posterior tibial and peroneal arteries than do the nondiabetics.

In the entire series there were two patients only who had amputations which were not necessitated by occlusive involvement of the cognate arteries. Both of these patients were in the diabetic group and are of sufficient interest and importance to present in some detail.

CASE REPORTS

Patient No. 1 (*No. 1, table 2*) is a thirty-five-year-old white male with a fourteen-year history of diabetes mellitus. He had had two previous admissions for ulcers of both feet. Peripheral pulses were normal. There was a deep 2 x 2 cm. painless ulcer over the base of the fifth toe on the left. The patient had a marked peripheral neuropathy bilaterally. Plethysmography revealed normal digital pulses without evidence of vasomotor activity (figure 4), indicating that the patient had an "autosympathectomy." A below-the-knee amputation was performed. Dissection of the amputated extremity revealed no occlusive disease of the major vessels.

Patient No. 2 (*No. 17, table 2*) is a forty-three-year-old white male with diabetes mellitus for eighteen years. He had a painless plantar ulcer of the right foot which had been present for three years and failed to heal. The pedal pulses were present and normal. Plethysmography revealed normal digit pulses and ankle pressure and complete sympathetic denervation (figure 5). The autosympathectomy was secondary to the severe peripheral neuropathy. A below-the-knee am-

TABLE 2

Exact sites of occlusion in the diabetic patients with amputation

	Indication for amputation	Aorta-iliacs*	Femoral-popliteal	Anterior tibial	Posterior tibial	Peroneal	Dorsalis pedis
1.	Foot—ulcer	Patent	Patent	Patent	Patent	Patent	Patent
2.	Toe—gangrene	Patent	Patent	Occluded	Occluded	Absent	Patent
3.	Toe—gangrene	Patent	Patent	Occluded	Occluded	Stenosis	Patent
4.	Foot—rest pain	Patent	Occluded	Occluded	Occluded	Occluded	Patent
5.	Toes—gangrene	Patent	Occluded	Patent	Patent	Patent	Patent
6.	Toe—gangrene	Stenosis, iliac	Occluded	Patent	Occluded	Patent	Occluded
7.	Foot	Occluded					
	Lower leg—ulcer	Occluded	Occluded	Absent	Patent	Patent	Absent
8.	Toe—gangrene	Patent	Occluded	Stenosis	Occluded	Patent	Occluded
9.	Toe—gangrene	Patent	Occluded	Occluded	Stenosis	Occluded	Patent
10.	Ankle	Patent	Occluded	Occluded	Occluded	Stenosis	Patent
	Heel—ulcer						
11.	Foot—ulcer	Patent	Patent	Occluded	Occluded	Absent	Occluded
12.	Toe—gangrene	Stenosis, iliac	Stenosis	Stenosis	Occluded	Occluded	Patent
13.	Toes—gangrene	Patent	Occluded	Occluded	Occluded	Occluded	Patent
14.	Toes—gangrene	Patent	Occluded	Occluded	Occluded	Stenosis	Patent
15.	Foot—ulcer	Stenosis, iliac	Stenosis	Stenosis	Occluded	Occluded	Patent
16.	Foot—ulcer	Patent	Stenosis	Stenosis	Occluded	Stenosis	Patent
17.	Foot—ulcer	Patent	Patent	Patent	Patent	Patent	Occluded

*Based upon evaluation of femoral pulse.

TABLE 3

Exact sites of occlusion in the nondiabetic patients with amputation

	Indication for amputation	Aorta-iliacs*	Femoral-popliteal	Anterior tibial	Posterior tibial	Peroneal	Dorsalis pedis
1.	Toe—ulcer	Occluded	Occluded	Occluded	Patent	Patent	Patent
2.	Foot—rest pain	Patent	Occluded	Patent	Patent	Patent	Patent
3.	Foot—ulcer	Stenosis	Stenosis	Stenosis	Occluded	Occluded	Occluded
4.	Foot—gangrene	Stenosis	Stenosis	Stenosis	Occluded	Stenosis	Patent
5.	Foot—gangrene	Patent	Patent	Stenosis	Occluded	Patent	Patent
6.	Toe—gangrene	Patent	Occluded	Stenosis	Stenosis	Stenosis	Patent
7.	Toe—gangrene	Occluded	Occluded	Absent	Patent	Stenosis	Absent
8.	Ankle—ulcer	Stenosis	Occluded	Patent	Patent	Patent	Occluded
9.	Leg—gangrene	Occluded	Occluded	Patent	Patent	Occluded	Patent
10.	Foot—rest pain	Occluded	Occluded	Occluded	Patent	Patent	Patent
11.	Foot—ulcer	Occluded	Occluded	Patent	Stenosis	Patent	Patent
12.	Toes—gangrene	Occluded	Occluded	Stenosis	Occluded	Stenosis	Stenosis
13.	Toes—gangrene	Occluded	Occluded	Stenosis	Stenosis	Stenosis	Stenosis
14.	Toes—gangrene	Stenosis	Patent	Patent	Patent	Patent	Occluded†
15.	Foot—gangrene	Stenosis	Occluded	Stenosis	Patent	Patent	Patent
16.	Foot—rest pain	Occluded	Occluded	Occluded	Occluded	Occluded	Patent
17.	Foot—rest pain	Patent	Occluded	Occluded	Stenosis	Patent	Patent
18.	Foot—gangrene	Patent	Stenosis	Patent	Patent	Stenosis	Patent
19.	Foot—rest pain	Patent	Occluded	Patent	Stenosis	Patent	Patent

*Based upon evaluation of femoral pulse.
†Plantar and digital arteries.

putation was performed. The only occlusive involvement was a short segmental occlusion in the dorsalis pedis artery. This occlusion was not sufficient to impair digit blood flow and could not explain the ulcer on the plantar surface of the foot in the distribution of the posterior tibial artery.

Careful microscopic examination of skin, subcutaneous tissue and muscle from the dorsal and ventral surfaces of the foot, lower leg and amputation margin in these two patients failed to demonstrate either arteriolar or small artery lesions which could explain the ulcers. The only possible explanation of these lesions seems to be that they occurred secondary to the severe peripheral neuropathy. These patients had loss of deep pain sensation, with resultant inability to recognize minor trauma, blisters, etc., which often go on to the formation of painless nonhealing ulcers.

Detailed microscopic examination of the material from the extremities amputated for major artery oc-

TABLE 4

Comparison of extent of major artery involvement in patients undergoing amputation

Level of involvement	Diabetic (15 extremities)* Vessels involved Number	Per cent	χ^2†	p	Nondiabetic (19 extremities) Vessels involved Number	Per cent
Aorta-iliac	4	27	4.29	<.05	13	68
Femoral-popliteal	12	80	.05	not significant	17	89
Anterior tibial	12 1 absent				13 1 absent	
Posterior tibial	13	81	4.29	<.05	10	57
Peroneal	9 2 absent				9	

*Cases 1 and 17 (table 2) are not included since there was no major artery involvement.
†Corrected for continuity.

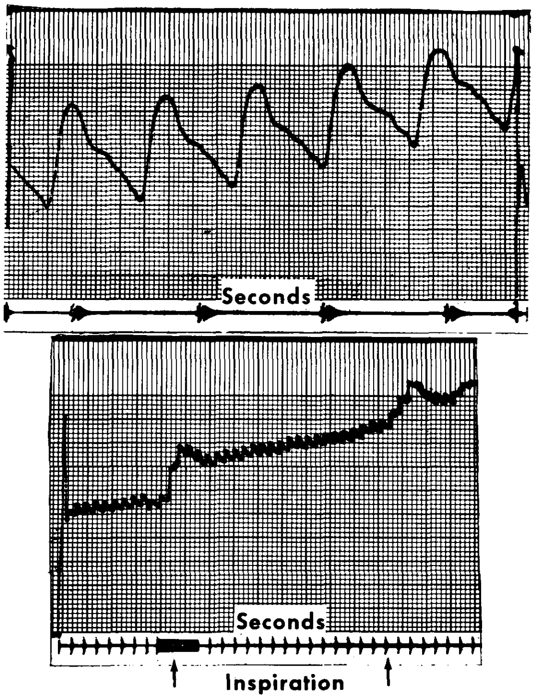

FIG. 4. Case 1, the digit pulses are normal in contour and volume. The inspiratory reflex is absent, indicating sympathetic denervation.

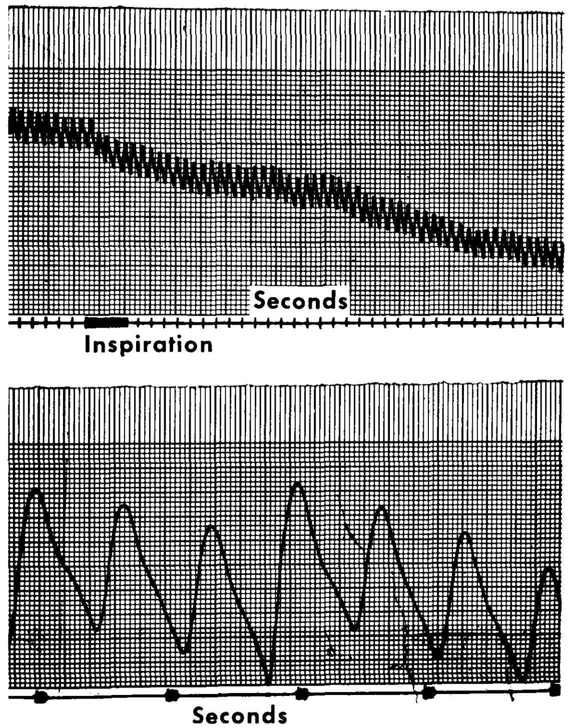

FIG. 5. Case 2, the tracings demonstrate the same findings as Case 1 in figure 4.

clusive disease revealed no significant or consistent difference between the diabetic and nondiabetic in the severity of the arteriosclerosis. Lesions in the small arteries or arterioles with the characteristics described by Goldenberg and associates[1] as specific in diabetic patients were rarely identified in these specimens. The specific lesion described by these authors consisted of endothelial proliferation and the deposition of PAS-positive material in a reticulated pattern which is colloidal iron negative.

They observed this lesion in 92.4 per cent of the patients with diabetes mellitus and in only 8 per cent of the nondiabetic patients with arteriosclerosis obliterans.

In our study, proliferative changes of the endothelium of sufficient degree to be easily identifiable were distinctly unusual. Deposition of material staining with periodic acid-Schiff but not with colloidal iron was difficult to distinguish as a process separate and distinct from other changes in the intima. Changes of this nature were not found with sufficient frequency to constitute criteria for dividing the specimens into the diabetic and nondiabetic groups. None of the lesions of this type was severe enough to encroach sufficiently upon the vascular lumen to impair blood flow.

DISCUSSION

In the nonamputation group of patients there was no significant difference between the diabetic and nondiabetic with respect to pulse categories. Although this suggests that the pattern of major artery occlusive involvement is the same, this may not be true since the pulse categories provide information only as to the most proximal level of occlusive involvement. The only certain method of determining the exact extent of involvement would be angiography, which was not performed in most of these patients. From a clinical and plethysmographic standpoint, the major observed difference is the presence of peripheral neuropathy in 27 per cent of the diabetics and in none of the nondiabetics.

Information as to the exact extent of the major artery disease is available in the amputation group. In this group of patients the pattern of occlusive involvement of the major arteries is distinctly different in the diabetic than in nondiabetic patients, the former having a higher incidence of involvement below the knee in the anterior tibial, posterior tibial and peroneal arteries. Thus, the diabetic quantitatively has more extensive major artery obliterative disease below the knee than does the nondiabetic. In physiologic terms it follows that the diabetic will have a greater reduction in peripheral blood flow and more ischemia than does the nondiabetic.

The level and extent of occlusive involvement is critical with respect to limb survival. Halsted stated in 1912, "There is abundant evidence in support of the view that, in a general way, the larger the artery or nearer it is to the heart, the less the impairment of the circulation attending its ligation."[7] Although Halsted refers to the effects of acute ligation, this rule is still generally true in the chronic occlusive state which is being considered in this study. Thus, the diabetic extremity is subject to double jeopardy since it has essentially the same incidence of involvement in the femoral-popliteal as the nondiabetic but has more extensive involvement below the knee. The extensive involvement below the knee forces the blood through smaller, higher resistance pathways which are much less effective than either the geniculate vessels about the knee or the profunda femoris artery in the groin. In the diabetic group there was either stenosis or occlusion of 81 per cent of the three major vessels below the knee as compared to 57 per cent of these vessels in the nondiabetics.

This observation alone seems sufficient to explain the increased incidence of gangrene in the diabetic patient.

An additional factor of importance is the occurrence of peripheral neuropathy in diabetics. The sensory modalities are more frequently affected,[8] and the sensory deficit can have disastrous effects. As illustrated in our case reports, nonrecognized, indolent ulcers often occur which necessitate amputation of the limb.

According to Goldenberg et al.,[1] diabetic vascular lesions have been found in the vasa vasorum and periadventitial vessels of cognate system arteries as well as in the small arteries and arterioles of nerves and skin. Using the same histologic technics we found it impossible to distinguish between the diabetic and nondiabetic. Dolman,[7] in a necropsy study of diabetic neuropathy, found nearly identical changes in the arterioles of the hypertensive nondiabetic and in the diabetic and suggested that the state of the small vessels does not play a primary role in the etiology of the peripheral neuropathy. If these histologic changes had been extensive enough to result in or contribute materially to the genesis of tissue necrosis in the diabetic, we should certainly have been able to distinguish them.

Thickening of the capillary basement membranes has been observed in the skin and muscle of diabetic patients.[9,10] It is certainly conceivable that such lesions might impair nutrient exchange between tissues and the microcirculation. Technics to evaluate such a phenomenon were not provided for in the investigation reported here, nor has any evaluation of the matter been found in the literature. The possibility of such restriction of nutrient exchange, therefore, cannot be excluded at this time. However, the clinical and pathologic findings do not demand that such a restriction be postulated in order to explain the increased incidence of tissue necrosis in the diabetic patient.

SUMMARIO IN INTERLINGUA

Un Combinate Studio Clinic e Pathologic de Diabetic e Non-Diabetic Morbo de Arteria Peripheric

Le resultatos de un combinate studio clinic e pathologic de alterationes del grande e micre arterias in le patiente diabetic e non-diabetic indica que le diabetico ha le mesme incidentia como le non-diabetico in occlusiones in le systema femoro-popliteal sed un plus alte incidentia infra le genu. Nulle lesion specific del micre arterias e del arteriolas que distinguerea le diabetico ab le non-diabetico esseva observate in le presente studio. Le resultatos del investigation provide nulle evidentia in supporto del conception que un specific morbo del micre arterias e del arteriolas contribue in

le diabetico al processo ischemic. Nos opina que le lesiones ischemic trovate in le diabetico pote esser explicate adequatemente a base del obstruction arteriosclerotic in le major arterias e que le ulceres que occure in le absentia de morbo de arteria major es attribuibile e neuropathia peripheric.

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Beta-glucuronidase and Atherosclerosis

Recently, S. Kayahan (Lancet II:667, 1960) reported that the serum beta-glucuronidase activity was low in a group of animals suitable for producing experimental atherosclerosis and high in a group of animals unsuitable for this purpose. Kayahan investigated the relation of beta-glucuronidase to the production of atherosclerosis in chickens.

He had three groups of ten chickens; the first group was fed commercial chicken mash, the second and third groups were given the mash plus 2 per cent cholesterol and 5 per cent cottonseed oil. In addition, the third group received an injection of 500 units of beta-glucuronidase per day. These studies were carried out for ten weeks.

A fourth group of chickens was given the diet that would induce atherosclerosis and, in addition, received 800 units of beta-glucuronidase per day for fourteen weeks. The animals receiving the diet without the cholesterol had no atherosclerosis, while those receiving the cholesterol had atherosclerosis graded at 0.7 to 2.8 degrees. The two groups of animals receiving cholesterol and the beta-glucuronidase averaged 0.1-0.5 degree atherosclerosis. The average serum total lipid level expressed as mg. per 100 ml. was 306 for the control group and 4,854 for the animals on the atherosclerotogenic diet. The lipid levels for the third and fourth groups of chickens receiving the beta-glucuronidase were 824 and 1,862 mg. per 100 ml. respectively. The serum cholesterol increased about tenfold in the animals on the cholesterol-containing diet and was a half to three quarters of this level in those receiving the beta-glucuronidase.

Thus, it appears that the animals receiving the beta-glucuronidase had lower levels of both cholesterol and total serum lipids. The serum beta-glucuronidase of the chickens fell when they were developing atherosclerosis. The chickens receiving the injections had levels approximately 1.2 times that of the control animals. It would be interesting if these investigators had injected inactivated beta-glucuronidase to see whether or not the protein that the chickens were receiving along with the enzyme activity might have some influence on the blood lipid levels.

In the study of beta-glucuronidase activity of the aortic intima in twenty individuals who did not have atherosclerosis and twenty patients with atherosclerosis it was found that the beta-glucuronidase activity in the aortic intima of patients with atherosclerosis varied from 0 to 5.7 units per gram of tissue with an average of 2.3, while those of the nonatherosclerotic individuals varied from 12.9 to 16.6 with an average of 14.2. The serum beta-glucuronidase activity in the atherosclerotic patients was also below the non-atherosclerotic patients, the two averages being 6.20 and 9.8. Ten individuals who had atherosclerosis were given beta-glucuronidase intramuscularly at the rate of 25,000 units per day for fifteen days, then given weekly injections for two or more weeks.

Comparing the pretreatment level of total lipids and total cholesterol in ten individuals, this investigator finds significant decreases in both of these lipids in all ten individuals who received the beta-glucuronidase. Also, all ten patients improved clinically. The question arises whether or not there is a definite cause- and effect-relationship between the beta-glucuronidase and lipid metabolism or whether the changes noted were the result of some secondary reaction as administration of a foreign protein to these individuals.

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