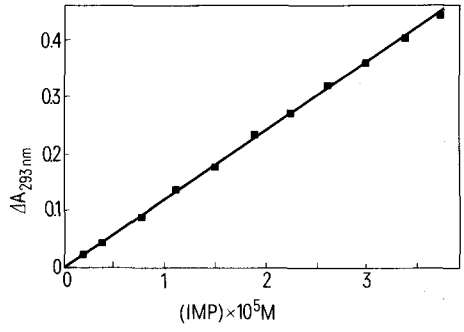
absorbance. HGPRT (1.5×10^{-4} IU) was then added to 1 ml of the mixture and the reaction was followed to its completion at 293 nm. The reaction went to completion after 2 h at 37 °C. The reaction mixture without IMP was used as the blank.

Results and discussion. The calibration curve for the IMP assay obtained by applying the procedure described above on IMP solutions of known concentration is reported in the figure. The relationship between IMP concentration and the optical density variation at 293 nm is linear in the range studied. From the same series of experiments a SD of ± 0.005 OD was obtained for the IMP assay.



Calibration curve for the enzymatic assay of IMP. The experimental conditions were as described in the text.

It is well established that HGPRT catalyzes IMP pyrophosphorolysis[7,9] and that the reaction can be followed spectrophotometrically at 293 nm in the presence of XOD activity[7]. When, at the end of the reaction, 0.04 IU of uricase were added to 1 ml of the incubation mixture, a decrease in optical density at 293 nm to the initial absorbance value was observed confirming that uric acid was the chief end product absorbing at this wavelength.

Human HGPRT has been studied extensively with regard to affinity for substrates[10]. The enzyme binds 6-oxo and 6-thiopurines but not 6-amino compounds. HGPRT, in the presence of magnesium ions, also catalyzes the reaction between GMP and PP_i to form guanine and phosphoribosylpyrophosphate. Since guanine is not a substrate for XOD, uric acid is not formed in the presence of GMP. AMP is not a substrate for HGPRT. In the presence of xanthine and/or hypoxanthine, the IMP assay can be carried out by adding HGPRT only after the purine bases have been completely oxidized to uric acid by XOD.

1 W.N. Kelley and J.B. Wyngaarden, in: Advances in Enzymology, vol. 41, p. 1. Ed. A. Meister. John Wiley, New York 1974.
2 P. Mandel, in: Progress in Nucleic Acid Research and Molecular Biology, vol. 3, p. 299. Ed. J.N. Davidson and W.N. Cohn. Academic Press, New York 1964.
3 M.S. Hershfield and J.E. Seegmiller, J. biol. Chem. *251*, 7348 (1976).
4 T. Fujita and Y. Hashimoto, Nippon Suisan Gakkaishi *26*, 907 (1960).
5 R.B. Hurlbert, H. Schmitz, A.F. Brumm and V.R. Potter, J. biol. Chem. *209*, 23 (1954).
6 I.C. Caldwell, J. Chromatogr. *44*, 331 (1969).
7 A. Giacomello and C. Salerno, J. biol. Chem. *253*, 6038 (1978).
8 E. Gerlach, R.H. Dreisbach and B. Deuticke, J. Chromatogr. *18*, 81 (1965).
9 C. Salerno and A. Giacomello, J. biol. Chem. *254*, 10232 (1979).
10 T.A. Krenitsky, R. Papaioannou and G.B. Elion, J. biol. Chem. *244*, 1263 (1969).

Sequential changes in serum glucose, triglycerides and cholesterol in aging of normal and alloxan-diabetic rats[1]

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Summary. 16-week-old Wistar, alloxan diabetic rats exhibited progressive elevations in levels of serum glucose, total triglycerides, cholesterol and creatinine over a period of 8 weeks; hyperglycemia preceeded hyperlipidemia and hypercreatininemia and hypertriglyceridemia preceeded hypercholesteremia. Age-matched control rats failed to develop any signs of hyperglycemia or hypercreatininemia, but did develop both hypercholesterolemia and hypertriglyceridemia at 24 weeks of age. This suggests that the progressive cardiovascular derangements (e.g., atherosclerosis, hypertension) noted in experimental diabetes mellitus and in the normal aging (and maturation) process may be brought about by distinctly different biochemical processes.

There is considerable evidence, both experimental and clinical, that the high incidence of cardiovascular complications of aging and diabetes mellitus are related to derangements in carbohydrate and lipid metabolism[2-8]. It is not certain whether progressive alterations in the serum content of glucose, cholesterol and total triglycerides are causally interrelated to the progression of the cardiovascular derangements noted in aging and diabetes mellitus. The mechanism(s) responsible for the increased incidence of hypertension and atherosclerosis seen in animals and human subjects in diabetes mellitus, and on aging, are incompletely understood. There is clinical evidence to suggest that the former may be related to a progressive hyperlipidemia preceded by a hyperglycemia[8,9].

With these points in mind, the present study was undertaken to determine if, under controlled experimental conditions, progressive changes in these serum parameters could be observed in 16-week-old rats made diabetic with alloxan as well as in age-matched (17, 20 and 24 weeks old) rats. A restricted time interval of 8 weeks was chosen in the hope that subtle biochemical alterations would be observed in early aging (i.e., maturing adult) versus diabetic animals. In addition, recent in vivo and in vitro experiments reveal that such normal aging and diabetic rats exhibit progressive alterations in blood pressure, and arterial and arteriolar reactivity, at these time intervals[6,10,11].

Methods. Male Wistar strain rats, initially 16 weeks of age (i.e., 300-335 g), bred and housed in our laboratory were

Comparison of body weights and serum biochemical parameters in normal aging and alloxan-diabetic rats

Time after treatment	N	Body weight (g)	Serum levels (mg/100 ml) Glucose	Cholesterol	Total triglycerides	Creatinine
1 Week						
Saline	12	419.8±33.5	122.7± 10.5	54.1±5.4	619.7±130	0.30±0.09
Alloxan	12	417.0± 8.4	454.6±81.7[a]	55.7±5.5	754.7±173	0.37±0.06
4 Weeks						
Saline	10	468.1±23.1	123.9± 6.02	64.5±3.5	786.6±117	0.25±0.05
Alloxan	10	426.4±24.2	704.8±80.3[a,b]	74.5±8.0	1417 ±285[a]	0.47±0.05[a]
8 Weeks						
Saline	11	495.4±24.7[d]	120.0±8.13	80.6±5.0[c]	1728 ±211[c]	0.34±0.05
Alloxan	11	412.5±27.7	677.8±34.5[a,b]	100.8±8.8[a]	3080 ±587[a,e]	0.54±0.05[a]

[a] Significantly different from saline controls ($p < 0.05$); [b] significantly different from 1-week alloxan diabetics ($p < 0.05$); [c] significantly different from 1- and 4-week saline controls ($p < 0.02$); [d] significantly different from 1-, 4- and 8-week alloxan diabetics ($p < 0.01$) [e] significantly different from 1- and 4-week diabetics ($p < 0.02$).

utilized. All animals were given standard rat chow and tap water ad libitum throughout the study. A group of age-matched (16 weeks) animals were injected with alloxan monohydrate (Sigma Chemical Co.), 150 mg/kg, in 0.3–0.4 ml of isotonic sterile saline, i.p. Another group of age-matched rats were injected with the saline vehicle (0.3–0.4 ml), i.p. 1, 4 and 8 weeks after the alloxan and saline injections, the animals were decapitated and mixed venous blood samples were obtained for analysis of serum glucose, cholesterol, total triglycerides (free glycerol plus glycerol released from triglycerides), and creatinine.

Serum glucose, cholesterol, total triglycerides, and creatinine were determined on a centrifugal analyzer (Centrific Chem Model 400, Union Carbide, Rye, N.Y.). Serum glucose was determined by the glucose oxidase method[12]. Cholesterol and total triglycerides were determined by enzymatic methods[13,14], while serum creatinine was determined with an automated reaction-rate method[15]. Where appropriate, means (± SE) were determined and compared for statistical significance by means of a Student's t-test.

Results. The control rats continued to gain weight, in a progressive manner, over the 8 week study period (table). In contrast to this, the diabetic rats didn't increase in weight, progressively, over the 8 week study period. Although the control rats did not exhibit any changes in serum glucose levels, the diabetic rats exhibited almost a 4-fold elevation in serum glucose 1 week after the administration of alloxan, and almost a 6-fold elevation 4 and 8 weeks after alloxan. The cholesterol levels rose 60% and 100% in the age-matched control and 8-week alloxan-treated rats, respectively. The serum total triglyceride level was elevated almost 300% in the 24-week-old control animals and approximately 500% in the age-matched alloxan-treated rats. It should be noted that the serum total triglyceride level was elevated almost 230% 4 weeks after the administration of alloxan. Although the serum level of creatinine did not change in the control animals, it became elevated progressively after the induction of diabetes.

Discussion. While the present results clearly show that a serum hyperglycemia precedes hyperlipidemia in alloxan-induced experimental diabetes mellitus, a similar series of metabolic events does not seem to take place in the early aging (or maturation) process, at least in male Wistar rats. Normal, male Wistar rats appear to develop the first signs of a hypercholesterolemia and hyperlipidemia at about the same time (i.e., at 24 weeks of age), but do so in the absence of any change in blood glucose or creatinine levels. A hypertriglyceridemia develops (4 weeks after alloxan) prior to a hypercholesterolemia (8 weeks after alloxan) in the diabetic rats. With such findings, it seems doubtful that one could link the elevation of blood pressure or development of atherosclerosis, seen in rats early in aging[6,16] or made alloxan diabetic[10,17], to a similar series of metabolic events. It would also appear from our findings that the atherogenesis that is observed in alloxan-diabetic rats[18,19] may be more dependent upon the blood total triglyceride level than the blood cholesterol level, since the former becomes elevated prior to the latter.

Since serum glucose is elevated early after alloxan, as one would expect from the literature[20,21], and prior to an alteration in the assayed blood lipid components, such data would support the hypothesis promulgated previously by numerous workers[5,8,9] that the hyperglycemia somehow acts as a trigger to set in motion the other metabolic and cardiovascular derangements observed in both clinical and experimental forms of diabetes mellitus.

The findings of a progressive rise in the serum creatinine after alloxan administration, but no such change in the control age-matched rats, is suggestive of a developing nephropathy in the advancing alloxan-diabetes mellitus syndrome. Such data might indicate that pathological alterations in renal function may play an important role in the alloxan-induced hypertension found in the diabetic rat. In this context, it has been reported that the renin-angiotensin system is altered in alloxan diabetic rats[4]; activation of the latter system could play an important role in both the elevation of blood pressure and the progressive atherosclerosis found in the syndrome.

Using non-enzymatic methods, Carlson et al.[22] reported, previously, that the amount of cholesterol and triglycerides in the plasma of male Sprague-Dawley rats rose sharply sometime between 4 and 9 months of age; no further elevation was noted in these parameters in rats 18 months of age. If similar results were to be found for other strains of rats (e.g., Wistar's used here), such data could indicate that the early changes seen in plasma cholesterol and triglycerides, noted here and elsewhere[22], are related to a maturation phenomenon rather than an aging phenomenon. In any event, the dissociation of serum biochemical parameters in the aging, matured adult rats versus diabetic rats reported herein could prove useful in the elucidation of the sequential biochemical processes which give rise to the cardiovascular alterations found in normal aging and diabetes mellitus. In view of these findings, it would be of interest to determine if similar serum biochemical changes are associated with the developing, as yet unexplained, chronic hypertension recently shown to be induced by the diabetogenic agent, streptozotocin, in rats[23].

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2 P. White, Diabetes *5*, 445 (1956).
3 K.W. Walton, in; The Biological Basis of Medicine, vol. 6, p. 193. Ed. E.E. Bittar and E. Bittar. Academic Press, New York 1969.
4 A.R. Christlieb, Am. J. Cardiol. *32*, 596 (1973).
5 P.C. Scott, Am. Heart J. *90*, 283 (1975).
6 B.M. Altura and B.T. Altura, in: Factors Influencing Vascular Reactivity, p. 169. Ed. O. Carrier, Jr, and S. Shibata. Igaku-Shoin Ltd, Tokyo 1977.
7 D.E. McMillan, Am. Heart J. *96*, 401 (1978).
8 K.M. West, Epidemiology of Diabetes and Its Vascular Lesions. Elsevier, New York 1978.
9 P. Petrides, L. Weiss, G. Löffler and O.H. Wieland, Diabetes Mellitus: Theory and Management, Urban & Schwarzenberg, Baltimore 1978.
10 T.A. Cavaliere and D.G. Taylor, Fedn Proc. *37*, 490 (1978).
11 P.D.M.V. Turlapaty, G. Lum and B.M. Altura, Fedn Proc. *39*, part 2, 635 (1980).
12 P. Trinder, Ann. clin. Biochem. *6*, 24 (1969).
13 C.C. Allain, L.S. Poon, C.S.G. Chan, N. Richmond and P.C. Fu, Clin. Chem. *20*, 470 (1974).
14 G. Buccolo and H. David, Clin. Chem. *19*, 476 (1973).
15 D.L. Fabiny and G. Ertingshausen, Clin. Chem. *17*, 696 (1971).
16 B. Wexler, Atherosclerosis *34*, 277 (1979).
17 Y. Hashimoto, Jap. Circulation J. *33*, 1315 (1969).
18 N. Kalant, J.L. Teitelbaum, A.A. Cooperberg and W.A. Harland, J. Lab. clin. Med. *63*, 147 (1964).
19 S.G. Lams and B.C. Wexler, Br. J. exp. Path. *58*, 177 (1977).
20 F.D.W. Lukens, Physiol. Rev. *28*, 304 (1948).
21 W.E. Dulin and M.G. Soret, in; The Diabetic Pancreas, p. 434. Ed. B.W. Volk and K.F. Wellmann. Plenum Press, New York 1977.
22 L.A. Carlson, S.O. Fröberg and E.R. Nye, Gerontologia *14*, 65 (1968).
23 H. Kawashima, T. Igarashi, Y. Nakajma, V. Akiyama, K. Usuki and S. Ohtake, Naunyn-Schmiedebergs Arch. Pharmak. *305*, 123 (1978).

Proteolytic capacity in mouse cardiac muscle following strenuous exercise[1]

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Summary. Proteolytic capacity in mouse cardiac muscle was analyzed 1, 3, and 6 days after exhaustive intermittent or submaximal prolonged running. No significant changes were recorded in the activities of acid or alkaline proteases, β-glucuronidase or trypsin inhibitor. Similarly, no changes were found in the rates of acid or neutral autolysis.

Cell edema, mitochondrial changes and disintegration of myofibrillar structure are among the indications of ischemic myocardial or skeletal muscle injuries[2-4]. Similar ultrastructural phenomena are found in both muscle types after exhaustive exercise[5-7]. Ischemic damage may be reversible, or irreversible leading to cell necrosis. Some skeletal muscle fibres are necrotized both by exhaustive intermittent[8] and by submaximal prolonged running[9], but physical stress does not cause lethal lesions in the cardiac muscle of healthy animals[5,10].

A common sign of sublethal cell injuries are alterations in the lysosomal system, e.g. increased autophagic degradation[11]. In skeletal muscle, the post-exercise[8,12] or post-ischemia[13] lysosomal acid hydrolytic capacity of surviving fibres is strongly increased 3-7 days after the exercise, suggesting sublethal cell injuries. The aim of the present study was to determine whether similar changes in the cellular digestive mechanisms also occur in cardiac muscle.

Methods. 4-month-old NMRI mice were made to run to exhaustion on a motordriven treadmill by the intermittent running schedule described earlier[8]. In the submaximal prolonged running the mice ran for 9 h on the treadmill with 6° uphill tracks at a speed of 13.5 m/min. The mice were killed 1, 3, and 6 days after the exercise. The activities of acid protease[12], alkaline protease[14], β-glucuronidase[12], and trypsin inhibitor[15] and the rates of acid[16] and neutral[17] autolysis were analyzed.

Results and discussion. Acid hydrolase activities increase in reversibly injured skeletal muscle fibres after exhaustive exercise[8] or ischemia[13]. It has been suggested that this response reflects subcellular regenerative processes in muscle fibres[12]. However, in mouse cardiac muscle strenuous exercise did not affect the estimates of acid hydrolytic capacity (table). Only a small ($p < 0.05$) increase in acid protease activity in cardiac muscle was recorded after exhaustive running. However, the acid hydrolytic capacity

Estimates of proteolytic capacity in mouse cardiac muscle 1, 3 and 6 days after intermittent exhaustive or submaximal prolonged running

Variables		Intermittent exhaustive exercise			Submaximal prolonged exercise		
	Controls (n = 14)	1 day (n = 9)	3 days (n = 10)	6 days (n = 9)	1 day (n = 8)	3 days (n = 11)	6 days (n = 9)
Acid autolysis	148 ± 5	150 ± 6	148 ± 6	138 ± 4	154 ± 7	151 ± 5	144 ± 6
Acid protease	1110 ± 35	1180 ± 60	1240 ± 35*	1110 ± 30	1160 ± 64	1180 ± 39	1080 ± 40
β-Glucuronidase	11.9 ± 0.3	12.8 ± 1.7	11.4 ± 0.3	12.0 ± 0.6	11.7 ± 0.7	12.9 ± 0.7	13.1 ± 1.2
Neutral autolysis	5.8 ± 0.3	6.0 ± 0.3	6.0 ± 0.4	5.6 ± 0.3	5.8 ± 0.3	6.2 ± 0.2	5.4 ± 0.4
Alkaline protease	17.4 ± 0.7	15.6 ± 0.4	17.8 ± 0.8	16.6 ± 0.9	15.4 ± 0.6	17.2 ± 0.6	17.2 ± 0.8
Trypsin inhibitor	13.2 ± 0.9	12.9 ± 0.9	14.6 ± 0.9	13.0 ± 0.5	13.1 ± 0.9	14.2 ± 0.6	12.3 ± 0.8
Malate dehydrogenase	744 ± 15	742 ± 20	725 ± 19	742 ± 18	742 ± 18	753 ± 19	726 ± 24
Protein content	182 ± 2	182 ± 4	186 ± 2	183 ± 2	186 ± 2	187 ± 2	182 ± 3

Activities (means ± SE) are expressed as pmoles/min/mg fresh muscle, except malate dehydrogenase activity (nmoles/min/mg fresh muscle) and trypsin inhibitor activity ($\Delta E \times 10^3$/min/mg fraction protein). Protein content is given as μg/mg. * $p < 0.05$.