Br. J. Pharmac. (1976), **57**, 229–234

EFFECT OF EXPERIMENTAL DIABETES, FOOD DEPRIVATION AND GENETIC OBESITY ON THE SENSITIVITY OF PITHED RATS TO AUTONOMIC AGENTS

J.M. FOY & P.D. LUCAS
School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP

1 The sensitivities of alloxan and streptozotocin diabetic and hereditary obese pithed rats to acetylcholine, isoprenaline and noradrenaline were compared to those of controls.

2 Blood pressure and heart rate recordings made before dosing was started showed the streptozotocin-treated animals to have a significantly reduced heart rate and increased pulse pressure as compared with controls.

3 Both diabetic groups were found to have reduced sensitivities to the pressor effect of noradrenaline, the depressor effect of acetylcholine, the positive chronotropic and inotropic effect of isoprenaline and the reduction in diastolic pressure induced by isoprenaline. The reduction in sensitivity was generally much greater in the streptozotocin diabetic animals.

4 The genetically obese rats were found to have similar sensitivities to all three agents as did their non-obese litter mates.

5 When either diabetic group was deprived of food for 24 h preceding the tests the sensitivities were found to be raised significantly towards normal in almost all cases.

6 The results are contrasted with previous *in vitro* results and possible causative metabolic factors discussed. It is suggested that sensitivity changes are unevenly distributed within the cardiovascular system.

Introduction

Neurological and cardiovascular malfunctions are accepted as being much more common among diabetics than among the general population (Ellenberg, 1973; Wheeler & Watkins, 1973; Riff & Riff, 1974). Studies using experimentally diabetic animals have indicated changed sensitivities to autonomic agents, e.g. hyperresponsiveness to noradrenaline has been demonstrated in perfused rat hindquarters from alloxan diabetic animals (Brody & Dixon, 1964) and in isolated aortic strips from alloxan diabetic rabbits (Cseuz, Wenger, Kunos & Szentivany, 1973). However, a study using both alloxan and streptozotocin diabetic anaesthetized rats revealed no change in sensitivity to noradrenaline (Christlieb, 1974). This contrast may indicate that noradrenaline sensitivities vary in different parts of the cardiovascular system. Alternatively variations in the diabetogen used, its dose and the time allowed for the development of the diabetes may have resulted in animals with different patterns of metabolic and perhaps of consequent cardiovascular malfunctions being studied. Streptozotocin and alloxan have been reported to result in different forms of diabetes with high serum free fatty acid and ketone levels in alloxan-treated and more normal levels in streptozotocin-treated animals (Mansford & Opie, 1968). However, the pattern of metabolic changes depends also on the dose of diabetogen and the time allowed for its effects to develop (Schein, Alberti & Williamson, 1971; Bavli & Gordon, 1971).

A comparative study of the cardiovascular sensitivities to autonomic agents of streptozotocin and alloxan diabetic rats both fed and deprived of food and of genetically obese rats was carried out in order to try to link any changes in sensitivities with the diabetic state. The doses of the two diabetogens used and the periods allowed for development of diabetes had previously been found to result in similar levels of hyperglycaemia.

Methods

Noradrenaline, isoprenaline and acetylcholine sensitivities

All rats were pithed under ether and artificially respired, blood pressures recorded via carotid cannulae and drugs injected via a femoral vein. Heart rate was determined from the blood pressure

recordings. Prior to dosing, a 0.1 ml sample of blood was withdrawn from each animal via the venous cannula for blood glucose determination. Responses to noradrenaline (100 ng, 300 ng, 1 μg and 3 μg/kg), isoprenaline (10 ng, 30 ng, 100 ng and 300 ng/kg) and acetylcholine (100 ng, 300 ng, 1 μg and 3 μg/kg) were recorded, time being allowed for recovery between each dose.

Alloxan and streptozotocin diabetic rats

Male C.F.E. rats (250–350 g) received 1 ml/kg of a freshly prepared solution of alloxan (50 mg/ml saline), streptozotocin (60 mg/ml pH 4.5 citrate buffer) or saline (0.9% w/v NaCl solution) alone via a tail vein. The last group served as controls. All rats were then allowed free access to food and water for one week in the case of the alloxan-treated and their controls and two weeks in the case of the streptozotocin-treated and their controls. Groups of alloxan and streptozotocin diabetic and control rats were then denied food for a further 20–28 h before test; these groups are referred to as 'starved' rats. All groups were allowed water *ad lib.*

Obese rats

Zucker obese rats were used at 2–3 months of age at which time their metabolic abnormalities should be present (Bray & York, 1971). At test their non-obese litter mates served as controls.

Blood glucose and free fatty acid determinations

Blood sugar was determined in the 0.1 ml blood samples withdrawn from the test animals by a micro-

Table 1 Blood glucose and free fatty acid determinations

Group	n	Blood glucose (mg/100 ml)	n	Blood free fatty acids (nM)
Controls fed	20	131.8 ± 8.0	6	0.1315 ± 0.329
Controls starved	6	73.9 ± 10.1**	5	0.1632 ± 0.0146
† ADR fed	16	553.7 ± 48.6***	9	0.1613 ± 0.0338
† ADR starved	18	214.1 ± 37.7***	6	0.1073 ± 0.0108
‡ SDR fed	11	463.4 ± 42.2***	6	0.1206 ± 0.0149
‡ SDR starved	11	183.0 ± 40.1***	6	0.1346 ± 0.0361
Zucker obese	6	206.0 ± 21.4		—
§ L.M.	8	186.3 ± 29.6		—

†‡ Alloxan diabetic (ADR) and streptozotocin diabetic (SDR) rats respectively. § Non-obese litter mates of the Zucker obese rats.
* Significant differences between fed diabetic and fed controls and between starved and equivalent fed groups are indicated by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2 Mean blood pressure, heart rate and pulse pressure measurements of pithed rat groups before drug infusion

Group	n	+ Mean blood pressure (mmHg)	Heart rate	Pulse pressure (mmHg)
Controls fed	20	58.1 ± 2.1	271.4 ± 11.7	25.5 ± 1.6
Controls starved	15	59.8 ± 3.1	255.2 ± 14.9	23.7 ± 1.6
† ADR fed	12	52.9 ± 4.5	260.6 ± 20.1	25.8 ± 2.8
† ADR starved	10	55.1 ± 4.2	238.0 ± 13.6	20.5 ± 1.7
‡ SDR fed	13	55.7 ± 2.6	197.2 ± 19.9***	44.5 ± 3.0***
‡ SDR starved	18	52.7 ± 3.9	220.5 ± 20.1	31.1 ± 3.8
Zucker obese	9	62.8 ± 3.7	253.7 ± 22.2	29.6 ± 2.5
§ LM	8	57.4 ± 2.4	238.6 ± 35.6	33.3 ± 3.7

+ Mean blood pressure is taken as diastolic + ½ pulse pressure.
†‡ Alloxan diabetic (ADR) and streptozotocin diabetic (SDR) rats respectively. § Non-obese litter mates of the Zucker obese rats.
* Significant differences between fed diabetic and fed controls and between starved and equivalent fed groups are indicated by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

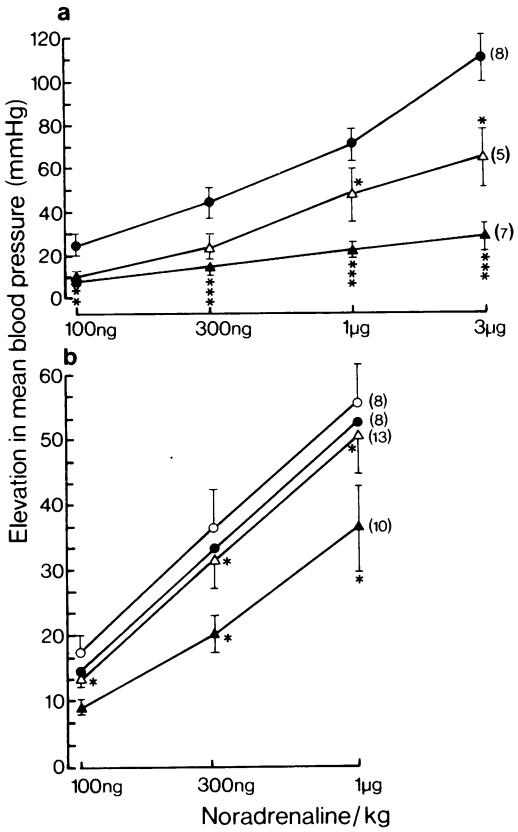

Figure 1 The effects of diabetes and starvation on the pressor response to noradrenaline. Each point represents the mean of *n* observations indicated in parentheses. Vertical lines show s.e. mean. (●) Fed controls; (○) starved controls; (▲) fed diabetic; (△) starved diabetic rats. (a) Effect of streptozotocin diabetes and starvation; (b) effect of alloxan diabetes and starvation. Significant differences between fed diabetic and fed controls and between starved and fed diabetic are indicated by: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

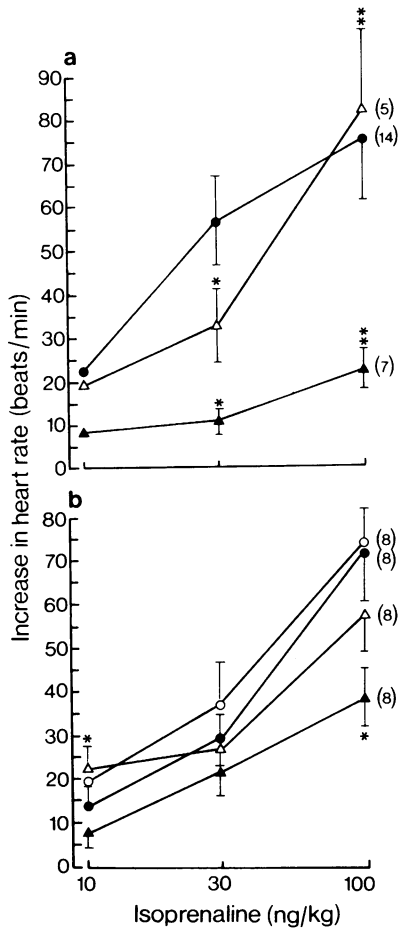

Figure 2 The effects of diabetes and starvation on the heart rate response to isoprenaline. Each point represents the mean of *n* observations indicated in parentheses. Vertical lines show s.e. mean. (●) Fed controls; (○) starved controls; (▲) fed diabetic; (△) starved diabetic rats. (a) Effect of streptozotocin diabetes and starvation; (b) effect of alloxan diabetes and starvation. Significant differences between fed diabetic and fed controls and between starved and fed diabetic are indicated by: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

colourimetric copper reduction method (Varley, 1963). Free fatty acids were determined by a modification of the method of Itaya & Ui (1965), in 0.2 ml plasma samples from rats which had received the same treatment as the test animals. Determination of plasma free fatty acid levels in the obese rats was not carried out since these and other metabolic abnormalities are already well documented (Bray & York, 1971).

Statistical significance was assessed by Student's *t* test, *P* values of 0.05 or less being taken to indicate significance.

Results

Table 1 shows blood glucose and free fatty acid levels in control, diabetic and obese rats.

Streptozotocin (60 mg/kg i.v.) and alloxan (50 mg/kg i.v.) both produced marked hyperglycaemia at the time of test but neither had a significant effect on blood free fatty acid levels. Starving for 20–28 h significantly reduced blood glucose in controls and

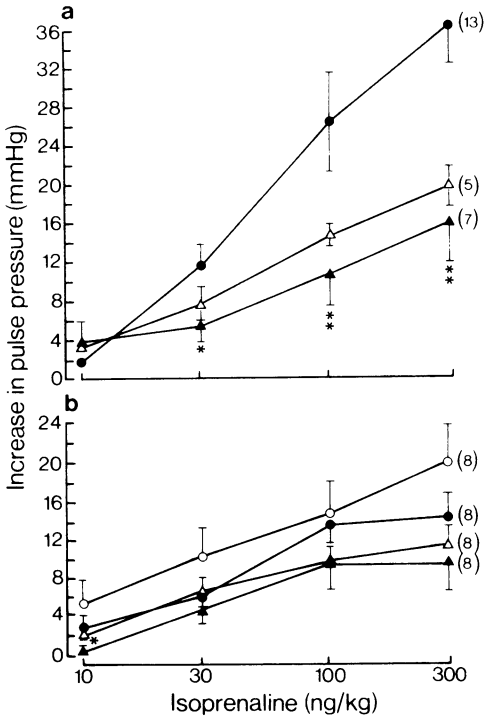

Figure 3 The effects of diabetes and starvation on the pulse pressure response to isoprenaline. Each point represents the mean of *n* observations indicated in parentheses. Vertical lines show s.e. mean. (●) Fed controls; (○) starved controls; (▲) fed diabetic; (△) starved diabetic. (a) Effect of streptozotocin diabetes and starvation; (b) effect of alloxan diabetes and starvation. Significant differences between fed diabetic and fed controls and between starved and fed diabetic are indicated by: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

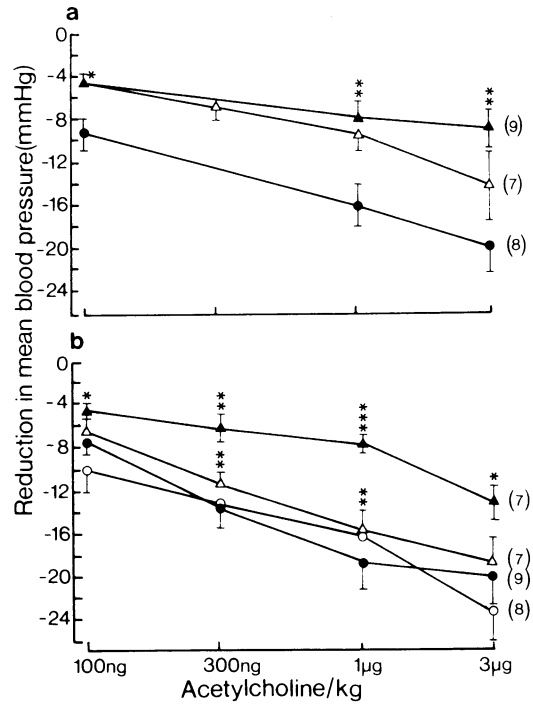

Figure 4 The effect of diabetes and starvation on the depressor response to acetylcholine. Each point represents the mean of *n* observations indicated in parentheses. Vertical lines show s.e. mean. (●) Fed controls; (○) starved controls; (▲) fed diabetic; (△) starved diabetic rats. (a) Effect of streptozotocin diabetes and starvation; (b) effect of alloxan diabetes and starvation. Significant differences between fed diabetic and fed controls and between starved and fed diabetic are indicated by: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

both diabetic groups but did not significantly change free fatty acid levels (Table 1).

There were no significant differences between any of the test groups in mean blood pressure. The fed streptozotocin diabetic group had a significantly reduced heart rate and increased pulse pressure compared to the fed controls. These values in the starved streptozotocin diabetic group were not significantly different from those of the fed controls (Table 2). Noradrenaline pressor responses (Figure 1), heart rate increases due to isoprenaline (Figure 2) and depressor responses to acetylcholine (Figure 4) were all significantly reduced at least at one dose level in both diabetic fed groups compared to their fed controls. Pulse pressure responses were also significantly reduced in the fed streptozotocin diabetic group (Figure 3a). Sensitivities to the pressor effect of noradrenaline (Figure 1) and the cardioaccelerator effect of isoprenaline (Figure 2) were raised significantly towards normal by starvation. Sensitivity to the depressor effect of acetylcholine was also significantly restored in the alloxan diabetic group (Figure 4b). The sensitivities of the obese rats to the agents used were not found to differ significantly from those of their controls. In the interest of simplicity therefore, results are not included.

Discussion

Sensitivity to all the three autonomic agents used was found to be reduced in both fed diabetic groups. The

reduction in noradrenaline sensitivity contrasts with some previous work showing increased sensitivity in rat hindquarter vasculature (Brody & Dixon, 1964) and isolated aortic strip of the rabbit (Cseuz *et al.*, 1973). The possibility that differences were due to different diabetic models being studied must be considered. The animals used in the present experiments may be regarded as relatively mildly diabetic since they did not have the raised free fatty acid levels which characterize the more severe diabetic state. Brody & Dixon used much higher doses of alloxan (150 mg/kg) but they used the subcutaneous route of administration which, due to the short half life of alloxan *in vivo* (Leech & Baily, 1945; Patterson, Lazarow & Levey, 1949) produces a very variable degree of diabetes as indicated by the proportions of their animals which either died or failed to develop the disease. Since the more severely affected were probably among those that died, and those relatively unaffected were rejected from their test group, it is reasonable to assume that metabolically, their animals were similar to those used in the present study. It is therefore postulated that parts of the cardiovascular system other than those of the hindquarters become less sensitive to noradrenaline resulting in an overall decrease in pressor response. The positive chronotropic responses to isoprenaline were reduced in both fed diabetic groups (Figure 2) and this probably contributed to the reduced noradrenaline pressor effect. Since the cardiac response contributes only a small part to the pressor effect of noradrenaline its reduction cannot entirely account for the large decrease in the latter seen particularly in the streptozotocin-treated group. It, therefore, appears that overall vasoconstriction was also reduced. Hyperglucagonaemia has been found to occur in all forms of endogenous hyperglycaemia where it has been measured and may even be necessary for its development (Unger & Ora, 1975). The effect of glucagon on various vascular beds has been investigated. It has been found to reduce resistance (Bashour, Geumei, Nagrawi & Downey, 1973) and reduce the constrictor effects of noradrenaline, angiotensin and vasopressin (Richardson & Withrington, 1975) in the dog hepatic vasculature possibly by increasing extracellular potassium concentration (Hulstaert, Beijer, Brouwer, Teunissen & Charbon, 1974). Recent findings suggest a similar action in the rat mesenteric arteries but no such effect even at high concentrations was seen in the rat hindlimb vessels (Mishra, Sharma & Kishore, 1975). Hyperglucagonaemia may, therefore, contribute to the decreased noradrenaline pressor responses seen in the fed diabetic groups and account for the contrast between these results and those of the perfused rat hindquarter preparation. However, unless glucagon acts via an intermediate which is reduced by starvation, the return of sensitivity towards normal after starvation casts doubt on this hypothesis since hyperglucagonaemia along with hypoinsulinaemia and elevated blood free fatty acid and ketone levels are abnormalities of diabetes which are, if anything, made worse by starvation.

The most obvious metabolic abnormality of diabetes which is returned towards normal by starvation, hyperglycaemia, must therefore be considered as a causative factor in the reduced noradrenaline sensitivity of the fed diabetic groups. A reduction in constrictor responses of perfused rat mesenteries to both sympathetic nerve stimulation and exogenous noradrenaline when high glucose perfusate was used has been reported (Malik & McGiff, 1974). The reduction in response to exogenous noradrenaline by glucose concentration increases similar to those occuring *in vivo* in the present experiments however was not great and could only partly account for the large decrease in sensitivity, unless other large vascular beds were much more severely affected.

The reduction in depressor response to acetylcholine in both fed diabetic groups (Figure 4) coupled with the finding that the acetylcholine content of vagal nerve endings is reduced in the diabetic rat (Kuntscherova & Vlk, 1970) indicate impaired vagal function. This may be analagous to the impaired vagal function reported in some diabetic patients (Wheeler & Watkins, 1973). The return of sensitivity towards normal after starvation suggests hyperglycaemia as a causative factor.

The lack of significant changes in the cardiovascular responses of obese rats to the agents used compared to their non-obese litter mates indicates that the metabolic derangements of the obese rats, e.g. hyperlipaemia, hypercholestrolaemia and hyperinsulinaemia (for review see Bray & York, 1971) have no effect on such responses. For several reasons, however, this conclusion must be treated with caution: firstly the fact that these animals consist of about 50% fat may have resulted in a proportionally smaller blood volume because of the usually poorer blood supply to fat compared to most other tissues. This would have caused equivalent doses/kg to result in higher blood levels so that the lack of apparent change in sensitivity may have indicated lower actual sensitivity. Secondly, changes in cardiovascular sensitivity in one direction due to alteration in blood composition may have been masked by opposing changes due to metabolic or other abnormalities in the other direction. The net result could therefore be no overall change.

In conclusion, sensitivities to the three agents used were reduced in the fed diabetic but not in the obese rats and sensitivity was raised towards normal in the former by starvation. One of the causes of the reduced noradrenaline sensitivity hypothesized, namely hyperglucagonaemia, suggests a redistribution of blood supply away from the limbs in favour of the viscera.

Further investigations are at present being carried out to determine if this is the case. If so it may have a bearing on the vascular complications of the human diabetic which typically involve reduced peripheral blood supply.

References

BASHOUR, F.A., GEUMEI, A., NAGRAWI, A.G. & DOWNEY, F. (1973). Glucagon: Its effects on the hepatic arterial and portal venous beds in dogs. *Pflugers Arch. Eur. J. Physiol.*, **344**, 83–92.

BAVLI, S. & GORDON, G. (1971). Experimental diabetic hyperosmolar syndrome in rats. *Diabetes*, **20**, 92–98.

BRAY, G.A. & YORK, D.A. (1971). Genetically transmitted obesity in rodents. *Physiol. Rev.*, **51**, 598–646.

BRODY, M.J. & DIXON, R.L. (1964). Vascular reactivity in experimental diabetes mellitus. *Circulation Res.*, **14**, 494–501.

CHRISTLIEB, A.R. (1974). Renin, angiotensin and norepinephrine in alloxan diabetes. *Diabetes*, **23**, 962–970.

CSEUZ, R., WENGER, T.L., KUNOS, G. & SZENTIVANY, M. (1973). Changes in adrenergic reaction pattern in experimental diabetes mellitus. *Endocrinology*, **93**, 752–755.

ELLENBERG, M. (1973). Current status of diabetic neuropathy. *Metab. Clin. Exp.*, **22**, 657–662.

HULSTAERT, P.F., BEIJER, J.H.M., BROUWER, F.A.S., TEUNISSEN, A.J. & CHARBON, G.A. (1974). Glucagon: Hemodynamic action related to the effect on sodium and potassium metabolism. *J. appl. Physiol.*, **37**, 556–561.

ITAYA, K. & UI, M. (1965). Colorimetric determination of free fatty acids in biological fluids. *J. Lipid. Res.*, **6**, 16–20.

KUNTSCHEROVA, J. & VLK, J. (1970). Influence of alloxan diabetes on acetylcholine synthesis in tissues of the albino rat. *Physiol. Bohemoslov*, **19**, 431–434.

LEECH, R.S. & BAILY, C.C. (1945). Blood alloxan and blood glutathione in rabbits injected with alloxan. *J. biol. Chem.*, **157**, 525–542.

MALIK, K.U. & McGIFF, J.C. (1974). Relationship of glucose metabolism to adrenergic transmission in rat mesenteric arteries. *Circulation Res.*, **35**, 553–574.

MANSFORD, K.R.L. & OPIE, L. (1968). Comparison of metabolic abnormalities in diabetes induced by streptozotocin or by alloxan. *Lancet*, **i**, 670–671.

MISHRA, S.S., SHARMA, A.L. & KISHORE, S. (1975). A study of glucagon on isolated vascular preparations. *6th Int. Cong. Pharmacol. Helsinki*, C 1067.

PATTERSON, J.W., LAZAROW, A. & LEVEY, S. (1949). Reactions of alloxan and dialuric acid with the sulfhydryl group. *J. biol. Chem.*, **177**, 197–204.

RICHARDSON, P.D.I. & WITHRINGTON, P.G. (1975). The effects of glucagon on the hepatic arterial vasculature of the dog: on inhibition of the effects of vasoconstrictor agents. *Br. J. Pharmac.*, **55**, 272–273P.

RIFF, E.R. & RIFF, K.M. (1974). Abnormalities of myocardial depolarization in overt, subclinical and prediabetes. *Diabetes*, **23**, 572–578.

SCHEIN, P.S., ALBERTI, K.G.M.M. & WILLIAMSON, D.H. (1971). Effects of streptozotocin on carbohydrate and lipid metabolism in the rat. *Endocrinology*, **89**, 827–834.

UNGER, R.H. & ORA, L. (1975). The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet*, **i**, 14–16.

VARLEY, H. (1963). Method of Asatoor and King. In *Practical Clinical Biochemistry*, 3rd edition, pp. 35–37. New York & London: William Heineman Medical Books Ltd.

WHEELER, I. & WATKINS, P.J. (1973). Cardiac denervation in diabetes. *Br. med. J.*, **4**, 584–586.

(Received October 10, 1975. Revised January 20, 1976)