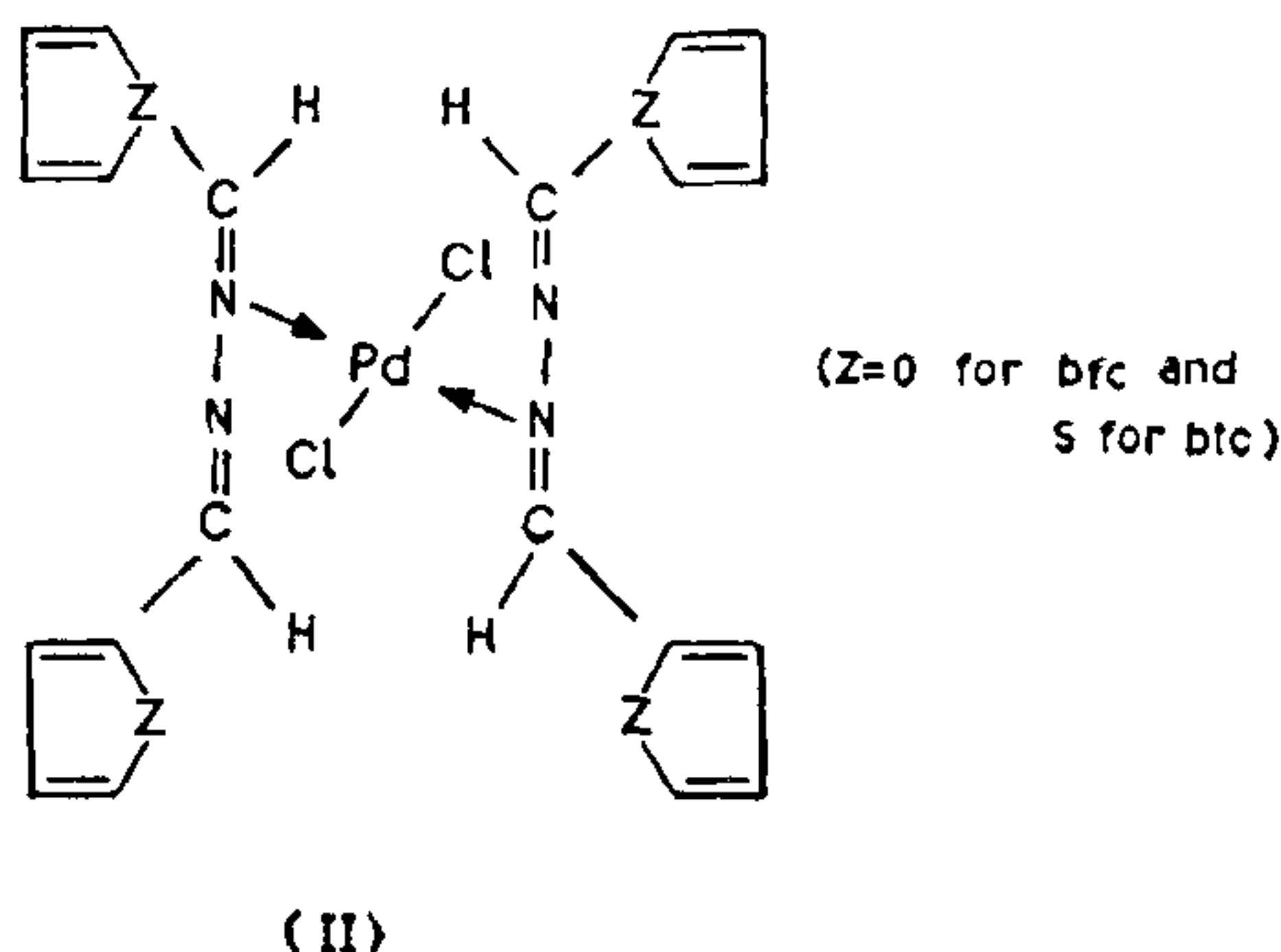


The infrared spectral studies thus indicate that bfc and btc behave as neutral ligands in their palladium(II) addition complexes, the bonding site being the azomethine nitrogen:



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1. Gupta, V. K. and Bhat, A. N., *J. Coord. Chem.*, 1979, 8, 183.
2. — and —, *Z. Naturforsch.*, 1977, 32, 225.
3. — and —, *Z. anorg. allg. Chem.*, 1979, 4.
4. Burger, K., *Coordination Chemistry: Experimental Methods*, Butterworths, London, 1973, p. 109.
5. Braibanti, A., Dallavalle, F., Pellinghelli, M. A. and Leoprati, E., *Inorg. Chem.*, 1968, 7, 1430.
6. Layton, R., Sink, D. W. and Durig, J. R., *J. Inorg. Nucl. Chem.*, 1966, 28, 1965.
7. Durig, J. R., Layton, R., Sink, D. W. and Mitchell, B. R., *Spectrochim. Acta*, 1965, 21, 1367.
8. Nakanishi, K., *Infrared Absorption Spectroscopy*, Holden Day, Inc., San Francisco, 1962, p. 50.
9. Nicholls, D. and Swindells, R., *J. Inorg. Nucl. Chem.*, 1968, 30, 2211.

EFFECT OF ALLOXAN DIABETES ON MYOCARDIAL LIPOLYSIS

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ABSTRACT

Lipolysis of heart triglyceride labelled with (14 -C) palmitate, which was increased in alloxan diabetes, was reduced by addition of free fatty acids in the incubating medium. Comparison of residual radioactivity after incubation of normal and diabetic hearts at 0.6×10^{-3} M (normal) and 1.2×10^{-3} M (diabetic) levels of palmitate respectively suggested decreased lipolysis *in vivo* as the contributory cause of enhanced accumulation of triglyceride in diabetic heart.

STATE of lipolysis of myocardial lipids in diabetes *in vivo* is not well defined. Although increased rate of lipolysis of heart triglyceride in diabetes during perfusion in the medium containing physiological level of free fatty acids (FFA) has been shown¹, the same may not be true in diabetes *in vivo* due to increased concentration of FFA in blood² which is known for inhibitory action on the myocardial lipolysis. We have therefore studied lipolysis of cardiac lipids prelabelled with (14 -C) palmitate in normal control, alloxan diabetic and insulin-treated diabetic rats in the absence as well as in the presence of physiological level of FFA and that level of FFA found in

blood of diabetic rats. In order to ascertain *in vivo* the state of lipolysis of heart lipids in diabetes, data of normal and diabetic rats were compared when heart slices were incubated in the medium consisting of FFA in the concentration which was observed in the blood of normal and diabetic rats respectively.

The rats were made diabetic by administering alloxan monohydrate (BDH Ltd., England) and were treated with lente insulin [Boots Co. (India) Ltd., India] as described recently⁴. All the animals, including controls which were treated only with physiological saline, were fasted for 6 hrs before they were killed. Myocardial lipids were labelled with (14 -C) palmitate of 30 mCi/m mole specific activity (Bhabha Atomic Research Centre, Bombay) by intravenously injecting with the tracer as palmitate-albumin complex at a dose level of 100 μ Ci per kg in the left ventricle of

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each rat under light anesthesia. After 5 min, the heart was removed, sliced and the slices (100 mg approximately) were incubated in air for 20 min at 37°C in 2.0 ml Krebs Ringer phosphate buffer (pH 7.4) containing 3% bovine serum albumin and various concentrations of palmitate. The reaction was stopped by injecting with 0.4 ml of 63% citric acid to each flask with air tight stopper. Lipid was extracted⁵ from slices which had been washed with cold physiological saline and stored at -20°C in chloroform containing butylated hydroxytoluene as an antioxidant. Phospholipids and triglyceride were separated by thin layer chromatography and radioactivity in each sample was measured by liquid scintillation counter.

TABLE I

Disappearance of myocardial triglyceride and phospholipids, prelabelled with (¹⁴C) palmitate, of normal, diabetic and insulin-treated rats in vivo. Data are expressed as decrease in percentage of initial radioactivity bound with triglyceride and phospholipids during 20 minutes incubation of heart slices. Each value is mean \pm SE of four animals. Difference between means of control and experimental groups is considered significant at $p < 0.05$

Addition of palmitate	Control	Diabetic	Insulin-treated
<i>Triglyceride</i>			
None	38.7 \pm 2.8	62.0 \pm 3.0 ^a	38.1 \pm 4.3
0.6 \times 10 ⁻³ M	20.9 \pm 2.0	28.4 \pm 1.9 ^b	20.7 \pm 3.1
1.2 \times 10 ⁻³ M	10.4 \pm 2.7	13.4 \pm 1.6	12.0 \pm 4.0
<i>Phospholipids</i>			
None	13.4 \pm 1.6	16.0 \pm 4.3	10.0 \pm 2.9
0.6 \times 10 ⁻³ M	7.4 \pm 1.1	13.0 \pm 1.7 ^b	9.3 \pm 2.5
1.2 \times 10 ⁻³ M	1.7 \pm 0.3	3.0 \pm 0.8	10.7 \pm 3.3

(a) $p < 0.01$; (b) $p < 0.05$.

Data on the decrease in percentage of initial radioactivity of heart triglyceride and phospholipid prelabelled with (¹⁴D) palmitate of normal control diabetic and insulin-treated diabetic rats are presented in the Table I. Although the heart triglyceride levels decreased in all the three groups of rats, with increased concentration of FFA in the incubation medium, they were always greater in diabetic heart and insulin treatment to diabetic rats restored the normal value. As compared to the lipolysis in heart triglyceride, heart phospholipids showed less lipolysis in all the three groups studied. Whereas hydrolysis

of myocardial phospholipids remained unaltered by increasing FFA concentration in the incubating medium, in the insulin treated diabetic rats, it was inhibited by increasing the fatty acid concentration in normal and diabetic rats. Reduced disappearance of radioactivity from heart triglyceride of diabetic rats at 1.2 \times 10⁻³ M palmitate (level of plasma FFA in diabetic rats as compared to that of normal rats) at 0.6 \times 10⁻³ M, the palmitate (physiological-level of plasma FFA in normal rats) points to a significant decrease in the rate of lipolysis. Demonstration of reduced uptake of FFA by diabetic heart⁷ and normal heart during perfusion in the medium containing ketone bodies⁸ ruled out the possibility of enhanced utilization of FFA for triglyceride accumulation in diabetic heart. Therefore, decreased breakdown of heart triglyceride in diabetes, as observed in this study may be a contributory factor of triglyceride accumulation in diabetic heart *in vivo*.

Turnover of heart phospholipid fatty acids is slower as compared to that of triglyceride and is further reduced by increasing FFA concentration in the medium. However, a significant decrease of radioactivity in heart phospholipids during incubation indicates that phospholipid fatty acids are not stagnant but in a dynamic state⁹. Comparison of the decrease of radioactivity in normal and diabetic heart at 0.6 \times 10⁻³ M and 1.2 \times 10⁻³ M palmitate respectively in the medium indicates a significant decrease in the hydrolysis of phospholipids in diabetic heart *in vivo* as observed in the case of heart triglyceride of diabetic rats.

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1. Shipp, J. C., Menahan, L. A., Crass, M. F. and Chaudhuri, S. N., *Rec. Advan. Stud. Cardiac Struct. Metab.*, 1973, 3, 179.
2. Rizza, R. A., Crass, M. F. and Shipp, J. C., *Metabolism*, 1971, 20, 539.
3. Crass, M. F., *Biochim. Biophys. Acta*, 1972, 180, 71.
4. Chauhan, U. P. S. and Singh, V. N., *Life Sci.*, 1978, 22, 1771.
5. Folch, J., Lees, M. and Sloane Stanley, G. H., *J. Biol. Chem.*, 1957, 226, 497.
6. Misra, U. K., *Biochem. Biol. Symp.*, 1968, 7, 57.
7. Kriesberg, R. A., *Am. J. Physiol.*, 1966, 210, 379.
8. Little, J. R., Gato, M. and Spitzer, J. J., *Am. J. Physiol.*, 1970, 219, 1458.
9. Shipp, J. C., Thomas, J. M. and Crevasse, L. E., *Science*, 1964, 143, 371.