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DIABETOGENIC EFFECTS OF CHRONIC ORAL CADMIUM ADMINISTRATION TO NEONATAL RATS

Z. MERALI & R.L. SINGHAL

Department of Pharmacology, Faculty of Health Sciences, School of Medicine, University of Ottawa, Ottawa, Ontario K1N 9A9, Canada

1 Chronic exposure of neonatal rats to oral cadmium (Cd) (0.1 and 1.0 μg/g daily for 45 days) disturbed glucose homeostasis, as reflected by hyperglycaemia, reduced liver glycogen and enhanced gluconeogenic potential of hepatic tissue.

2 This Cd-exposure regimen also increased hepatic cyclic adenosine 3',5'-monophosphate (cyclic AMP) which was accompanied by enhancement of basal, adrenaline and glucagon-stimulated form(s) of adenylate cyclase.

3 In order to assess the responsiveness of pancreatic beta cells to glucose, islets isolated from control as well as Cd-exposed animals were incubated *in vitro* and their rate of insulin secretion determined. In the presence of glucose 0.5 mg/ml, there was no significant difference in the rate of insulin release. However, at higher glucose concentrations (1.5 and 3.0 mg/ml), the islets from Cd-exposed rats released significantly less insulin than those of control animals.

4 The results are discussed in relation to the possible mechanism of the diabetogenic effect of Cd.

Introduction

Recent advancement in industrialization has resulted in man-made redistribution of trace elements in the environment; concentrated deposits which are generally protected and harmless to mankind are exploited and eventually dissipated in our biosphere (Luckey, 1975). Cadmium (Cd) is one such relatively rare metal with which man has been brought more and more into contact in recent years.

Evolution seems to have provided no effective homeostatic mechanism to deal with increasing intake of this heavy metal and so Cd tends to accumulate in the body with time. Thus, the toxicological potential of Cd has generated justified concern and emphasized the need for elucidation of the nature and mechanisms of physiological and toxicological reactions to this heavy metal.

Parental administration of Cd has been reported to elevate the concentration of blood glucose (hyperglycaemia) and/or to increase the urinary excretion of glucose (glucosuria) (Voinar, 1952; Havu, 1969; Sporn, Dinu & Stoonescu, 1970; Ghafghazi & Mennear, 1973; 1975; Ithakissios, Ghafghazi, Mennear & Kessler, 1975; Merali & Singhal, 1975; 1976). It is of interest that Ishizaki & Fukushima (1968) found glucosuria in well over 90% of Itai-Itai patients (population endemically exposed to Cd). Furthermore, glucosuria has also been documented in industrially exposed workers (Bonnell, Kazantzis & King, 1959; Kazantzis, Flynn, Spowage & Trott, 1963; Friberg, Piscator, Nordberg & Kjellstrom, 1974). Elevation of blood glucose or maintenance of normal blood glucose in presence of glucosuria could result not only from increased production and/or release of glucose into the circulation, but also from reduced tissue uptake and utilization of circulating glucose. Havu (1969) found that in fish (*Corpus scorpius*), intramuscular injection of Cd resulted in accumulation of this heavy metal in the islet tissue which was associated with necrotic lesions of beta cells, hyperglycaemia and glucosuria. Parenteral administration of Cd has also been found to impair glucose tolerance in the mouse and rat (Ghafghazi & Mennear, 1973; 1975; Ithakissios *et al.*, 1975; Merali & Singhal, 1975; 1976). The objectives of the present study were: (1) to assess the effects of chronic oral Cd administration on hepatic carbohydrate and cyclic adenosine 3',5'-monophosphate (cyclic AMP) metabolism in neonatal rats and (2) to elucidate if the pancreatic islets of Cd-exposed animals exhibit an altered responsiveness to glucose.

Our results demonstrate that chronic administration of oral Cd to developing rats results in significant alterations of hepatic carbohydrate metabolism which may be associated with enhanced cyclic AMP synthesis. Furthermore, since Cd treatment suppresses insulin secretory activity of the pancreatic beta cells, the observed diabetogenic effects may be related to a relative lack of insulin in Cd-exposed animals.

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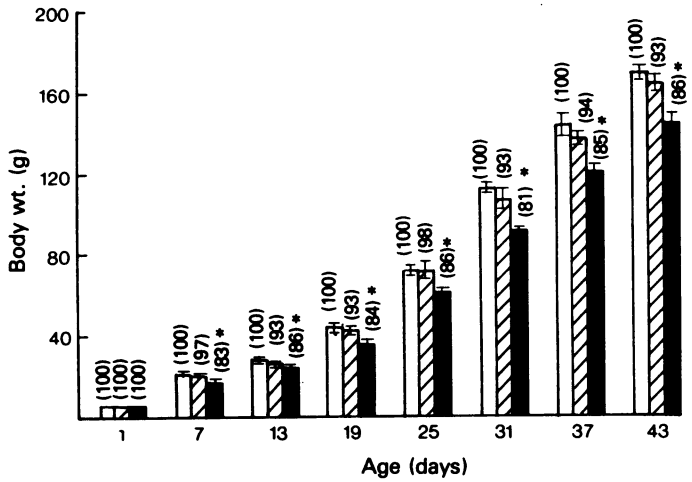

Figure 1 Influence of neonatal cadmium (Cd) exposure on body weight changes (growth). Control: open columns; hatched columns: Cd 0.1 μg/g; solid columns: Cd 1 μg/g. Each column represents the mean of 5–6 animals per group; vertical lines show s.e. mean. Data are also expressed in percentages (in parentheses) with values from the control group taken as 100%. *Significantly different from controls at $P < 0.05$.

Methods

Pregnant female Sprague–Dawley rats (Canadian Breeding Farm and Laboratories Limited, St. Constant, Quebec) were housed in individual cages, allowed free access to food (Master Laboratory Chow) and water and maintained under constant environmental conditions (24°C, 60% relative humidity and regular alternate cycles of 12 h light and darkness). Following parturition, each litter was reduced to a maximum of 8 males and the pups were weaned at 22 days of age. Newborn rats were administered Cd (0.1 or 1.0 μg/g daily) orally as Cd chloride solution by intubation, for 45 days. Control animals received an equal volume of 0.9% w/v NaCl solution (saline).

Before the rats were killed, unless indicated otherwise, all animals were food-deprived overnight. Islets of Langerhans were isolated from pancreas of non food-deprived rats by a modification of the collagenase digestion technique of Lacy & Kostianovsky (1967). Groups of 5 islets were preincubated for 30 min at 37°C in 1 ml of Krebs-Ringer bicarbonate (KRB) buffer containing bovine serum albumin (2 mg/ml) and glucose (0.5 mg/ml). The islets were then transferred into chambers containing 2 ml of incubation medium consisting of KRB buffer (pH 7.4), bovine serum albumin (2 mg/ml) and glucose (0.5, 1.5 or 3.0 mg/ml). The incubation was carried out for 90 min at 37° under constant gassing with 95% O_2 and 5% CO_2. At the conclusion of incubation, samples of the media were frozen for estimation of insulin content. Insulin was determined as immunoreactive insulin (IRI) by the method of Hales & Randle (1963) using the commercial kit available from Amersham/Searle Co. (Chicago). The amount of IRI in the sample was calculated by reference to a standard curve obtained with rat insulin (Novo Research Institute). The activities of gluconeogenic enzymes, levels of urea, protein, cyclic AMP and glucose were measured as described previously (Merali & Singhal, 1975). The activity of hepatic adenylate cyclase was assayed according to the procedure of Sutherland, Rall & Menon (1962). Adrenaline (50 μM), glucagon (10 μM) and Na fluoride (10 mM) were added to the incubation medium in order to measure the fluoride- and hormone-stimulated forms(s) of adenylate cyclase, the enzyme activity being expressed as pmol of cyclic AMP formed per mg tissue during a 10 min incubation period.

Statistics

The data were analyzed for significance of differences by Student's *t* test.

Results

Growth of young rats receiving daily oral doses of cadmium

Body weight changes (growth) of newborn rats exposed to two different doses of Cd are shown in Figure 1. There was no significant difference in body

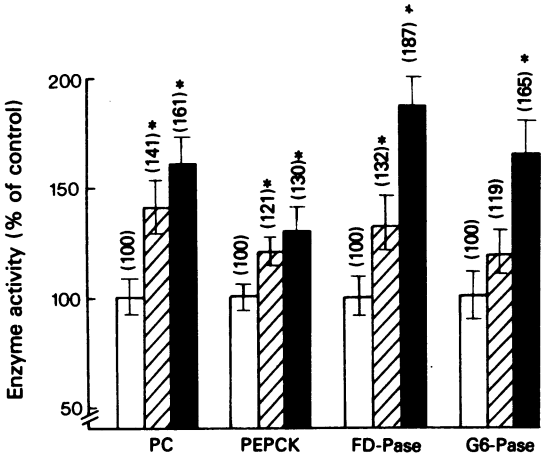

Figure 2 Stimulatory effect of orally administered cadmium (Cd) on hepatic gluconeogenic enzymes. Open columns: control; hatched columns: Cd 0.1 μg/g; solid columns: Cd 1 μg/g. Each column represents the mean of 5–6 values per group; vertical lines show s.e. mean. Data are given in percentages (in parentheses) with values from control rats taken as 100%. PC: pyruvate carboxylase; PEPCK: phosphoenolpyruvate carboxykinase; FD-Pase: fructose 1,6-diphosphatase; G6-Pase: glucose 6-phosphatase. *Significantly different from control values at $P < 0.05$.

weight gain of those rats receiving daily oral doses of 0.1 μg/g Cd over a period of 43 days. However, rats receiving the higher dose of Cd (1.0 μg/g daily) displayed a slight but significant depression in growth relative to the control group, from day 7 of treatment to the completion of Cd exposure regimen.

Effects of cadmium ingestion on blood glucose, serum urea and hepatic glycogen levels

Data presented in Table 1 demonstrate that exposure to both doses of Cd (0.1 and 1.0 μg/g daily) for 45 days caused significant elevation in blood glucose; however, serum insulin levels remained within the normal range. Both doses of Cd markedly reduced hepatic glycogen content and significantly elevated serum urea levels. The observed alterations in glucose, glycogen and urea were of a greater magnitude in animals exposed to the higher dose of the heavy metal.

Effects of cadmium ingestion on hepatic gluconeogenesis

Since Cd ingestion elevated blood glucose and urea and lowered hepatic glycogen content, we were prompted to examine whether orally administered Cd also enhanced the activities of various hepatic gluconeogenic enzymes. Data in Figure 2 demonstrate that long term oral administration of the low dose of Cd (0.1 μg/g daily) did indeed elevate the activities of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-diphosphatase (FD-Pase) and glucose 6-phosphatase (G6-Pase). Similarly, the 45 day high dose of Cd (1.0 μg/g daily) treatment resulted in significant enhancement of the activities of the four key gluconeogenic enzymes and the increment was of a greater magnitude than that seen in rats exposed to low doses of the heavy metal.

Effect of long term oral cadmium administration on hepatic adenylate cyclase activity

Daily administration of Cd at a dose of 0.1 or 1.0 μg/g for 45 days elevated hepatic cyclic AMP levels by 32%

Table 1 Effects of oral cadmium administration on blood glucose, serum insulin and urea and hepatic glycogen levels

Parameter	*Control*	*Cd (0.1 μg/g)*	*Cd (1.0 μg/g)*
Glucose (mg/100 ml)	79.8 ± 5.3 (100)	97.4 ± 5.8 (122)*	108.5 ± 9.2 (136)*
Glycogen (g/100 g)	1.47 ± 0.11 (100)	0.69 ± 0.08 (47)*	0.44 ± 0.06 (30)*
Urea (mg/100 ml)	20.0 ± 0.51 (100)	26.8 ± 0.74 (134)*	41.6 ± 3.14 (208)*
Insulin (IRI) (μu/ml)	16.35 ± 0.59 (100)	16.23 ± 0.78 (99)	15.04 ± 0.53 (92)

Each value represents the mean ± s.e. mean of 5–6 animals per group. Data are also given in percentages (in parentheses) with values from control animals taken as 100%.
* Significantly different from control values at $P < 0.05$.

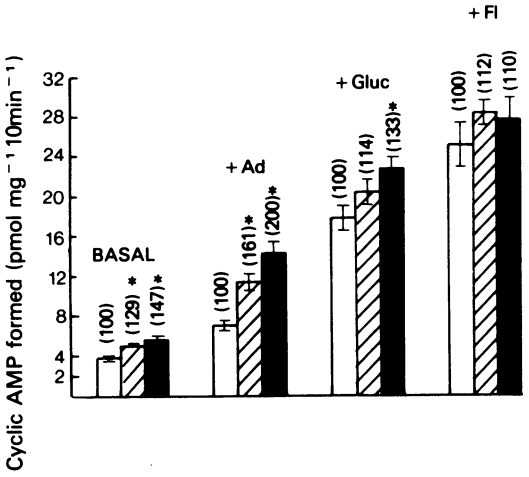

Figure 3 Influence of chronic oral cadmium treatment on the responsiveness of hepatic adenylate cyclase to adrenaline (Ad), glucagon (Gluc) or fluoride (Fl). Open columns: control; hatched columns: Cd 0.1 μg/g; solid columns: Cd 1.0 μg/g. Each column represents the mean of 5–6 animals per group; vertical lines show s.e. mean. Data are also given in percentages (in parentheses) with values from control rats taken as 100%. *Significantly different from control values at $P < 0.05$.

and 57%, respectively. This increase of hepatic cyclic AMP was accompanied by an enhancement in the activity of the cyclic AMP synthesizing enzyme, adenylate cyclase. Adrenaline, fluoride as well as glucagon were all able to stimulate adenylate cyclase activity in normal as well as Cd-exposed animals; however, in the latter group, both glucagon and adrenaline stimulated enzymatic activity to levels higher than those seen in corresponding controls (Figure 3). In contrast, the sensitivity of hepatic adenylate cyclase to fluoride stimulation remained similar in both control and Cd-exposed groups.

Influence of long term cadmium ingestion on insulin secretion from isolated rat islets

Since chronic exposure to oral Cd reduced the insulinogenic index, the question whether Cd-induced impairment of pancreatic function could be localized to the level of pancreatic islets was also investigated. Data in Table 2 illustrate the influence of Cd treatment on IRI release from isolated islets in response to varying glucose concentrations. In the presence of 0.5 mg/ml glucose, the rate of IRI release from islets of control animals was not significantly different from that noted in rats exposed to the low dose of Cd (0.1 μg/g daily) for 45 days. However, in the presence of 1.5 mg/ml glucose, although there was over 100% increase in the rate of IRI release from control islets, there was no appreciable change from the islets of low dose Cd-exposed rats. Similarly, in the presence of 3.0 mg/ml glucose, whereas the rate of IRI release from control islets increased by over 250%, this increment in IRI release from islets of low dose Cd exposed animals was only 113%. It is of interest that the islets obtained from rats exposed to the higher dose of Cd (1.0 μg/g daily) for 45 days also elicited a similar suppression of IRI release in response to different glucose concentrations.

Discussion

Previous studies have demonstrated that parenteral

Table 2 Effects of oral cadmium administration on insulin (IRI) release from isolated rat islets

	Rate of IRI release in presence of varying glucose concentrations (μu from 5 islets/90 min)		
	Glucose (mg/ml)		
Treatment	*0.5*	*1.5*	*3.0*
Control	128 ± 13 (n = 20) (100)	270 ± 14 (n = 20) (100)	450 ± 27 (n = 20) (100)
Cd (0.1 μg/g)	112 ± 15 (n = 20) (88)	131 ± 14 (n = 20) (49)*	239 ± 13 (n = 20) (53)*
Control	135 ± 14 (n = 20) (100)	287 ± 32 (n = 19) (100)	549 ± 53 (n = 20) (100)
Cd (1.0 μg/g)	132 ± 20 (n = 18) (98)	161 ± 20 (n = 18) (56)*	217 ± 32 (n = 18) (40)*

Each value represents the mean ± s.e. mean of n incubations from 4 rats per group and each n represents one batch of 5 islets. Data are also given in percentages (in parentheses) with control values taken as 100%.
* Significantly different from control values at $P < 0.05$.

administration of Cd can inflict biochemical or functional lesions in several organ systems (Singhal & Merali, 1979). However, chronic Cd poisoning in man is usually the result of inhalation or ingestion, presenting a rather slow but continuous exposure. Also it is of interest that the intestine of the newborn has the ability to absorb greater quantities of substances, including heavy metals, than that of the adult (Sasser & Jarboe, 1977). Indeed, Cd concentration in human body increases by more than 200 fold during the first three years of life (Kello & Kostial, 1977). It was thus of interest to investigate whether chronic exposure of newborn rats to oral Cd produces functional or biochemical lesions similar to those produced by the parental administration of relatively large doses of Cd. In order to simulate a more physiological mode of Cd exposure as well as to administer known amount of Cd right from birth, neonatal rats were given Cd (0.1 or 1.0 μg/g daily) by intubation as $CdCl_2$ solution. Like chronic injection of Cd (Merali, Kacew & Singhal, 1975), oral administration for 45 days was found to disturb glucose homeostasis in the rat, in that it reduced liver glycogen levels, increased the concentration of blood glucose and enhanced the gluconeogenic potential of hepatic tissue.

Cyclic AMP plays an important role in the control of carbohydrate metabolism as it elevates blood glucose and urea levels as well as enhances gluconeogenic and glycogenolytic potential of the liver (Menahan & Wieland, 1967; Wicks, 1969). In the present study, exposure to Cd resulted in elevation of hepatic cyclic AMP content which was accompanied by enhancement of basal, adrenaline as well as glucagon-stimulated form(s) of adenylate cyclase. As with chronic parenteral administration (Merali & Singhal, 1976), oral Cd treatment increased the responsiveness of hepatic adenylate cyclase to adrenaline by a degree greater than to glucagon. This is of some interest since the experimental diabetic state is also accompanied by enhanced responsiveness of adrenaline-sensitive adenylate cyclase of rat liver (Bitensky, Gorman & Neufeld, 1972).

Experiments were also designed to elucidate whether the effects of chronic Cd exposure could be localized at the level of pancreatic beta cells. In contrast to the parenteral mode of Cd exposure, oral administration of Cd (0.1 μg/g daily) for 45 days failed to alter significantly the basal IRI levels. However, this maintenance of IRI levels in presence of persistent hyperglycaemia could be indicative of suppressed pancreatic function. In order to assess the responsiveness of pancreatic beta cells to glucose, islets isolated from the pancreas of control as well as Cd-exposed animals were incubated *in vitro* and the rate of insulin release was determined in the presence of varying concentrations of glucose. In the presence of 0.5 mg/ml glucose, there was no significant difference in the rate of IRI release. However, in presence of higher glucose concentrations (1.5 and 3.0 mg/ml), the islets from Cd-exposed rats released significantly less IRI than those from control animals. At both doses of Cd, the rate of glucose-stimulated IRI release seemed to be impaired to a similar extent, indicating that a ceiling to pancreotoxic effects may have been reached already at the lower dose utilized.

These experiments indicate that long-term exposure to oral Cd inflicts islet lesions and more specifically, at the level of the pancreatic beta cells. Recently, Ithakissios *et al.* (1975) studied the influence of multiple injections of Cd on insulin secretion from perfused rat pancreas. It was found that whereas injection of 0.25 mg/kg dose of Cd every second day (70 doses) failed to alter significantly the amount of insulin secreted from perfused pancreas, treatment with 0.5 mg/kg doses caused a marked inhibition in the amount of insulin secreted in response to glucose stimulation. Earlier studies had also presented evidence indicating that Cd possesses a marked pancreotoxic potential. Barbieri, Colombi & Straneo (1961) reported that Cd administration resulted in accumulation of this metal in rabbit pancreas that was accompanied by a decrease in the ratio of beta to alpha cells. In addition, Havu (1969) observed necrotic lesions of pancreatic beta cells in the fish, *Cottus scorpius*, injected with Cd. Our present data provide evidence to suggest that exposure to this heavy metal produces functional impairment of pancreatic islets. It is possible that the diminished insulin secretory activity may be related to a net reduction in the number of pancreatic beta cells, as observed by Barbieri *et al.* (1961).

In order to confirm the structural viability of beta cells in the isolated islets, electron microscopic examination was carried out. The beta cells from Cd-exposed rats revealed some degenerative changes that included frequent occurrence of structures resembling degenerating mitochondria and of 'empty vacuoles' that appeared to be secretory saccules devoid of the dense granules containing insulin (unpublished data). These observations may provide additional evidence for the insular origin of the marked hyperglycaemic effect of Cd. However, further detailed examination using a larger number of animals has to be carried out for adequately documenting this observation.

Ghafghazi & Mennear (1975) demonstrated that perfusion of isolated rat pancreas with Cd inhibited secretory response to glucose, tolbutamide and potassium ions. Furthermore, perfusion of the inhibited organ with a combination of glucose and theophylline resulted in partial reversal of the observed inhibition. Since theophylline is believed to result in intracellular translocation of calcium, Ghafghazi & Mennear (1975) hypothesized that Cd-induced inhibition of glucose-stimulated insulin secretion may be mediated, at least in part, through an inhibition of calcium

uptake by beta cells. Indeed, supportive evidence for this hypothesis exists in the report of Esposito-Avella (1974) who demonstrated that incubation of isolated mouse islets with Cd (1×10^{-5} and 1×10^{-7} M) and glucose (3.0 mg/ml) reduced the uptake of ^{45}calcium. Mitochondria constitute the major source of intracellular calcium available for translocation into the cytosol. Thus, if following chronic Cd exposure, pancreatic damage extends to the level of the beta cell mitochondria, it remains possible that not only does Cd interfere with calcium uptake, but that it may also be interfering with the translocation of intracellular calcium.

In conclusion, the present study indicates that administration of relatively low doses of oral Cd to neonatal rats results in disturbances of glucose homeostasis as reflected by reduced liver glycogen, increased concentration of blood glucose and an enhanced gluconeogenic potential of hepatic tissue. These alterations in hepatic carbohydrate metabolism resemble those of exogenously administered cyclic AMP and may indeed be related to an enhanced formation of the hormone-stimulated cyclic nucleotide. It is also noteworthy that Cd-induced alterations of glucose homeostasis seem to resemble those caused either by insulin anti-serum or alloxan treatment. The presently observed diabetogenic effects of Cd may therefore be related to an abnormal insulin secretory response of the beta cells in Cd-exposed rats.

References

BARBIERI, L., COLOMBI, R. & STRANEO, G. (1961). Modificazioni istologiche delle isole pancreatiche del coniflio dopo intossicazione cronica de cadmio. *Folia Med.* (Naples), **44**, 1120–1133.

BITENSKY, M.W., GORMAN, R.E. & NEUFELD, A.H. (1972). Selective effects of insulin on hepatic epinephrine responsive adenyl cyclase activity. *Endocrinology*, **90**, 1331–1335.

BONNELL, J.A., KAZANTZIS, G. & KING, E. (1959). A follow up study of men exposed to cadmium oxide fume. *Br. J. Ind. Med.*, **16**, 135–147.

ESPOSITO-AVELLA, M. (1974). Studies on the pancreotoxic effects of cadmium: effects on insulin secretion and calcium uptake by isolated pancreatic islets. *Thesis, Purdue University*, pp. 1–65.

FRIBERG, L., PISCATOR, M., NORDBERG, G. F. & KJELLSTROM, T. (1974). *Cadmium in the Environment.* pp. 1–248. Cleveland: CRC Press.

GHAFGHAZI, T. & MENNEAR, J.H. (1973). Effects of acute and subacute cadmium administration on carbohydrate metabolism in mice. *Toxic. appl. Pharmac.* **26**, 231–240.

GHAFGHAZI, T. & MENNEAR, J.H. (1975). The inhibitory effect of cadmium on the secretory activity of the isolated perfused rat pancreas. *Toxic. appl. Pharmac.*, **31**, 134–142.

HALES, C.D. & RANDLE, P.J. (1963). Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.*, **88**, 137–146.

HAVU, N. (1969). Sulfhydryl inhibitors and pancreatic islet tissue. *Acta Endocrinologica Suppl.*, **139**, 1–231.

ISHIZAKI, A. & FUKUSHIMA, M. (1968). Studies on "Itai-Itai" disease. *Jap. J. Hyg.*, **23**, 271–278.

ITHAKISSIOS, D.S., GHAFGHAZI, T., MENNEAR, J.H. & KESSLER, W.V. (1975). Effect of multiple doses of cadmium on glucose metabolism and insulin secretion in the rat. *Toxic. appl. Pharmac.*, **31**, 143–149.

KAZANTZIS, G., FLYNN, F.V., SPOWAGE, J.S. & TROTT, D.G. (1963). Renal tubular malfunction and pulmonary emphysema in cadmium pigment workers. *Q. J. Med.*, **32**, 165–192.

KELLO, D. & KOSTIAL, K. (1977). Influence of age and milk diet on cadmium absorption from the gut. *Toxic. appl. Pharmac.*, **40**, 277–282.

LACY, P.E. & KOSTIANOVSKY, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes*, **16**, 35–39.

LUCKEY, T.D. (1975). Introduction to heavy metal toxicity, safety and hormology. In *Environmental Quality and Safety*, Suppl. Vol. 1. ed Coulston, F. & Korte, F. pp. 1–3. Stuttgart: George Theme Publishers.

MENAHAN, L.A. & WIELAND, O. (1967). Glucagon-like action of N^6,2'-O-dibutyryl cyclic AMP on perfused rat liver. *Biochem. biophys. Res. Commun.*, **29**, 880–885.

MERALI, Z. & SINGHAL, R.L. (1975). Protective effect of selenium on certain hepatotoxic and pancreotoxic manifestations of subacute-cadmium administration. *J. Pharmac. exp. Ther.*, **195**, 58–66.

MERALI, Z. & SINGHAL, R.L. (1976). Zinc protection against cadmium-induced biochemical alterations. *Br. J. Pharmac.*, **57**, 573–579.

MERALI, Z., KACEW, S. & SINGHAL, R.L. (1975). Response of hepatic carbohydrate and cyclic AMP metabolism to cadmium treatment in rats. *Can. J. Physiol. Pharmac.*, **53**, 174–184.

SASSER, L.B. & JARBOE, G.E. (1977). Intestinal absorption and retention of cadmium in neonatal rat. *Toxic. appl. Pharmac.*, **41**, 423–431.

SINGHAL, R.L. & MERALI, Z. (1979). Biochemical toxicity of cadmium. In *Cadmium Toxicity, Modern Pharmacology-Toxicology*, Vol. 15 ed. Mennear, J.G. pp. 61–112. New York: Marcel Dekker Inc.

SPORN, A., DINU, I. & STOONESCU, L. (1970). Influence of cadmium administration on carbohydrate and cellular energy metabolism in the rat liver. *Rev. Rouman. Biochem.*, **7**, 299–305.

SUTHERLAND, E.W., RALL, T.W. & MENON, R. (1962). Adenyl cyclase I. Distribution, preparation and properties. *J. biol. Chem.*, **237**, 1220–1227.

VOINAR, A.C. (1952). The existence and role of cadmium in the organism of animals and man. In *Vsesiouznaia Kon-*

ferentsiia Pomikroelementam 1st Moscow, 1950. Microelementy v. Zhizni Rastenii i Zhivotnykh. pp. 580–583. Moscow.

WICKS, W.D. (1969). Induction of hepatic enzymes by adenosine 3',5'-monophosphate in organ culture. *J. biol. Chem.*, **244**, 3941–3950.

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