STUDIES ON CERTAIN HEPATOTOXIC AND PANCREOTOXIC MANIFESTATIONS OF CADMIUM ADMINISTRATION IN RATS

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LIST OF ABBREVIATIONS

AA, amino acids

AC, adenylate cyclase

AcCoA, acetyl coenzyme A

ADP, adenosine 5'-diphosphate

ALA, alanine

ASP, aspartate

ATP, adenosine 5'-triphosphate

C, catalytic subunit of protein kinase

cAMP or cyclic AMP, adenosine 3',5'-monophosphate

CIT, citrate

EPI, epinephrine

F or FL, fluoride

FDP, fructose 1,6-diphosphate

FD-Pase, fructose 1,6-diphosphatase

F6P, fructose 6-phosphate

GIP, glucose 1-phosphate

GLL, glycerol

GLN or GLYCO, glycogen

GLU or GLUCO, glucose

GLUC, glucagon

GLY, glycine

G6P, glucose 6-phosphate

G6-Pase, glucose 6-phosphatase

IRI, immunoreactive insulin

LAC, lactate

MAL, malate

OAA, oxaloacetate

PC, pyruvate carboxylase

PDE, phosphodiesterase

PEP, phosphoenolpyruvate

PEPCK, phosphoenolpyruvate carboxykinase

PES, phosphorylated endogenous substrate

PGF, prostaglandin F

PYR, pyruvate

R, receptor subunit of protein kinase

SER, serine

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I. INTRODUCTION

I. 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 are eventually dissipated in our biosphere (1-5). Cadmium 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 thus, cadmium accumulates in the body with advancing age (6-9). The potential harm of cadmium to man has not only generated justified concern, but has emphasized the urgent need for elucidating the nature and mechanisms of physiological and toxicological reactions to cadmium (10-12). There is a worrisome void in our knowledge of the long-range consequences of cadmium exposure.

> "Cadmium is not simply of academic interest. While its role as an aetiological agent in disease is still speculative, there is no doubt that the ionised form of the element is an extremely powerful poison of many basic animal enzyme systems. In man there is a causal link with renal lesions, lung disease, bone lesions and hypertensive vascular disease. We simply do not know how dangerous it is - or how wide is the safety margin. It is alarming to learn that the cadmium content of the polluted Severn estuary amounts to 4-5 µg per litre. Clearly, urgent studies are needed lest we, like Cadmus, King of Thebes, are not in danger of sowing some very lethal dragon's teeth for our children to harvest. The Japanese pollution disaster should not pass unheeded in our cadmium-enriched society."
>
> Harvey (13).

In this sphere, pharmacology and biochemistry can play an instrumental role in exploration of the full toxicological potential of cadmium, in elucidation of mechanism(s) underlying the toxic effects of cadmium, and in assessing the effectiveness of various protective agents against cadmium toxicity.

The present dissertation is concerned primarily with elucidation of acute, subacute and chronic effects of cadmium on carbohydrate metabolism in rats. Since cadmium causes an elevation in blood glucose concentration as well as urinary glucose excretion (14,15), the influence of this heavy metal on glucose synthesizing capacity of both liver and kidney cortex was examined. In particular, the effect of cadmium on four key gluconeogenic enzymes, namely pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase was investigated. In addition, changes in the concentration of blood glucose and serum urea, a product of protein catabolism, were determined following cadmium treatment. Since blood glucose concentration can be elevated through hepatic glycogenolysis, the effect of cadmium treatment on hepatic glycogen levels also was monitored.

The exact mechanism(s) underlying hormonal modulation of hepatic and renal carbohydrate metabolism is not known. However, it has been proposed that hormonal stimulation of membranal adenylate cyclase and the consequent elevation of endogenous cyclic AMP levels may be involved in the control of glucose synthesis. Indeed, administration of exogenous cyclic AMP has been found to enhance the processes of gluconeogenesis and glycogenolysis, and result in elevation of blood glucose and urea levels (16). This apparent similarity in the effects of cyclic AMP and cadmium on carbohydrate metabolism prompted studies on the influence of cadmium treatment on hepatic and renal cyclic AMP system. Furthermore, effects of subacute cadmium treatment on the responsiveness of hepatic adenylate cyclase to hormonal stimulation was examined. Since testes are very sensitive to the toxic effects of cadmium and cyclic AMP has been implicated in several gonadal functions (17), the effects of chronic cadmium exposure on testicular and prostatic cyclic AMP-adenylate cyclase-protein kinase system also were studied. Cadmium has a long biological half life. It was thus of interest to examine if cadmium-induced alterations

persisted upon cessation of heavy metal administration. The influence of 2- and 4-week cessation of cadmium administration was examined on various metabolic alterations induced by 21- or 45-day pretreatment with cadmium.

It is of interest that cadmium-induced changes in carbohydrate metabolism resemble those of insulin deficiency caused by alloxan or insulin anti-serum (18). These observations suggested that metabolic alterations caused by cadmium exposure might be due, at least in part, to lack of insulin. To examine this possibility, rats pretreated with cadmium were challenged with a glucose load and the subsequent time-course of glucose clearance as well as alterations in serum insulin levels were monitored. Since cadmium treatment may suppress pancreatic function through adrenal catecholamine release, the influence of cadmium on phentolamine-stimulated insulin release also was assessed. Since cadmium-induced testicular degeneration can be prevented by zinc or selenium (19), experiments also were undertaken in an attempt to examine if these two metals could prevent the cadmium-induced alterations in hepatic carbohydrate and cyclic AMP metabolism as well as in pancreatic insulin release.

Chronic cadmium poisoning in man is usually the result of inhalation or ingestion, presenting a rather slow but continuous exposure. Furthermore, small amounts of cadmium are excreted through human milk. It was therefore of interest to examine if chronic exposure of neonatal rats to oral cadmium would produce functional or biochemical lesions similar to those induced by parenteral administration of this heavy metal. Thus, new-born rats were administered relative low amounts of cadmium (0.1 or 1.0 μg/g cadmium) by intubation and the changes in hepatic gluconeogenic enzymes, hormonal responsiveness of adenylate cyclase as well as endogenous cyclic AMP levels were determined. Furthermore, concentrations of insulin in pancreas and serum were measured as was the glucose-stimulated insulin release from islets of rats exposed to cadmium for 45 days.

Results of the present investigation demonstrate that cadmium possesses a wide toxicological potential as it can induce functional or biochemical lesions in hepatic, renal, pancreatic as well as gonadal tissues. The heavy metal-induced alterations in hepatic carbohydrate metabolism may be associated with enhanced cyclic AMP synthesis. Furthermore, since cadmium treatment suppressed insulin secretory activity, the cadmium-induced diabetogenic effects may be related to the relative lack of insulin observed in cadmium-exposed animals. The ability of zinc and selenium to prevent or modify the cadmium-induced hepatotoxic and pancreotoxic effects indicate that sulfhydryl groups may represent a possible site of cadmium action and that a clearer understanding of trace element interactions may help to further elucidate the etiology of cadmium toxicity.

II. LITERATURE REVIEW

1. METALS AS POLLUTANTS

About 10,000 years ago, following the Paleolithic (old stone) age, began the Neolithic (new stone) phase of human existence. The realization that a seed, when put into the ground, sprouted into a plant represented a great breakthrough which revolutionized human evolution and heralded the beginning of human civilization. People were freed from constantly moving and gathering food. Homo sapiens, after 2 million years of hunting and gathering, had become farmers with a settled mode of existence. With this development, human culture acquired a new significance in the biosphere, introducing new forces which were different from those that had controlled the ecology and evolution of species during the preceding 2,000 to 3,000 million years of life on earth (20). Following this phase, perhaps 6,000 or 7,000 years ago, another monumental discovery was made: neolithic man learned to use copper and gold (found in native form) to make ornamental and hunting implements. This era characterized the transition into the epoch of the Bronze where ancient smelters discovered a harder and more useful alloy of copper and tin called bronze (21). Thus, for almost 3,000 years, copper and bronze were the main metals used for making implements, weapons, vessels etc. As the availability of metals increased, the use of stone declined for these purposes. The use of metals was further accelerated in the Iron Age by the acquisition of technological know-how of iron smelting. Because of the abundance and cheapness of iron, it finally became possible for all social classes to have efficient metallic tools and utensils, instead of only the royalty and the affluent possessing them as in the times of bronze.

Iron is considered to be the most useful metal of material civilization, as it has made possible structural plants, tools, weapons, machinery, coined money as well as many of the products of the industrial revolution that form

the basis of our modern living. Thus, the use of metals has contributed immensely to our social and economic advancement over the centuries. However, since the neolithic revolution, man has modified his environment at an ever-increasing rate. Today, he lives in and must adapt to conditions markedly different from those in which he made the transition from man-ape to man, in which he lived for tens of thousands of generations (22). With the increasing rate of population growth and industrialization, the requirement for metals also has increased tremendously. As a result, metallic ores are brought from the depths of the earth to the surface to a much greater extent causing a disturbance in the natural balance of elements in the environment. Thus, man's increased effectiveness in industrialization has brought him into contact with rare minerals of the earth for which evolution has provided no effective homeostatic mechanisms. At the same time, man's contact with these agents has been accentuated by environmental pollution from industrial wastes discharged into water reservoirs, air and the food chain. The magnitude of disturbances in the ecosystem of the biosphere is rapidly approaching the level that ecologists believe threatens the life of our very planet. Heavy metals, including cadmium, lead and mercury are cumulative poisons of principal toxicological concern today. At one time, these were considered to pose major health problems only in industries which mined and processed them. Today however, the concern has shifted to their possible health effects in the general population of both man and animals.

2. ELEMENTS OF THE II-B SUBGROUP

Cadmium, mercury and zinc are grouped together in the periodic table. Their natural abundance in the lithosphere and hydrosphere is in decreasing order of atomic weights. Zinc predominates, while cadmium is present in relatively higher concentrations than mercury. Of these three, only zinc is

recognized as an essential element in plant and animal nutrition; the other two may act as its antimetabolites.

3. ZINC

A. Properties of Zinc

Zinc is a soft white metal with a bluish tinge. Its atomic weight is 65.38, specific gravity, 7.1, melting point, 419.5°C and the boiling point is 907°C. The main ores are saphalerite (ZnS) and calamine ($ZnCO_3$) (23).

B. Biological Essentiality of Zinc

Over 100 years ago, Raulin, a pupil of Pasteur, discovered that zinc was indispensable for the growth of a black bread mold, Aspergillus niger. Some 50 years later, the presence of zinc was demonstrated in egg yolk, human milk, and cows' milk, suggesting that zinc was of nutritive value (24). Although it was appreciated that zinc was essential for plant growth and that the ubiquitous occurrence of this element might denote important functions, distinct from any of its toxicological actions, zinc was studied intensively in the 1920's because its fumes were toxic to exposed workers (25). In 1934, Todd et al. (26) successfully accomplished a controlled zinc-deficient diet and demonstrated that zinc is essential for growth and development of rats. The disease of swine, porcine parakeratosis, which had been long recognized in animal husbandry was shown to be the result of zinc deficiency. These findings led to the general practice of supplementing animal feeds with zinc. At present, at least 15 animal species, man included, have been shown to require zinc (24).

In the early 1960's, reports appeared in the literature suggesting that endocrinopathies (growth and sexual retardation) observed in human dwarfism syndrome might indeed be caused by zinc deficiency. Daily oral supplementation with zinc sulfate in such individuals was reported to significantly improve their growth rate and sexual development. It thus appears that zinc, in

addition to other essential nutrients, may be a limiting factor in normal growth and well-being of certain populations in underdeveloped regions of the world (24).

C. Biological Functions of Zinc

The biochemical functions in which zinc has been implicated include: 1) enzymes and enzymatic function, 2) protein synthesis and 3) carbohydrate metabolism. Initially, zinc was demonstrated to be an integral and necessary component of carbonic anhydrase of red blood cells. Since then, at least 18 other metalloenzymes have been shown to contain zinc (27).

Zinc also has been shown to be related to protein synthesis in micro-organisms, animals and animal tissues. The synthesis of both DNA and RNA is inhibited in the absence of zinc (28). Protein synthesis appears to be reduced or altered in zinc-deficient rats. Furthermore, zinc-deficient lambs have been observed to excrete significantly more urinary nitrogen and sulfur, suggesting an impaired protein and amino acid utilization (29).

Zinc also seems to play an important role in carbohydrate metabolism. In 1937, Hove et al. (30) and more recently, Quarterman et al. (31) reported decreased glucose tolerance in rats that were zinc-deficient. However, Macapinlac et al. (32) could find no differences in glucose tolerance curves of zinc-deficient rats as compared to controls. It is also of interest that the insulin molecule contains two zinc atoms and although their necessity for biological activity of insulin has not yet been demonstrated, zinc-free insulin appears less stable than zinc insulin (33). It is evident that the exact role of zinc in carbohydrate metabolism is yet to be fully elucidated.

4. MERCURY

A. Properties of Mercury

Mercury is a silvery white metal and is liquid at room temperature. Its

atomic weight is 200.61, specific gravity, 13.55, melting point, -38.87°C and boiling point is 357.3°C. Cinnabar (HgS) is the chief natural ore (23).

B. Mercurial Toxicity

Mining and use of mercury have been going on for well over 2,000 years, however, in the last couple of decades, mercury has been outlawed from several industrial uses. Clinical aspects of mercury absorption can be traced back to times when the Romans shipped their slaves to mines in Almaden, Spain where these workers developed mercurialism. In Yugoslavia, a mine that was activated in 1500 A.D. at Idria is still in operation today. After a fire broke out in the mine in 1803 and vapor and clouds of smoke and dust scattered throughout the community, some 900 of the town's inhabitants and most of its animals developed the tremor so characteristic of mercury poisoning (34). Mercury became a notoriously controversial subject in the late 1930's when men's hats were being manufactured in great quantities. Many workers were involved in a process called carroting, in which pelts from rabbits were treated with mercury nitrate to form felt. The drying of freshly carroted hair caused considerable volatization of mercury, resulting in intoxication of hat workers involved in that step of the process. A pact outlawing the use of mercury nitrate was finally made in December, 1941 and a relatively nontoxic material, hydrogen peroxide, was substituted.

There was great demand for mercury for shielding when the atomic age emerged in 1942. Today, it is employed in dental laboratories in amalgam fillings, and there is considerable concern about the air content of mercury in such facilities as well as the body burden being carried by technicians. Concern of mercurial exposure also exists in industries manufacturing thermometers, barometers, batteries, fungicides, gold mining, mercury electrical switches, direct current meters and in vapor lamps that illuminate traffic arterials (34).

In the Japanese fishing village of Minamata, a mysterious illness was affecting 111 persons. Intensive clinical, pharmacological, as well as epidemiological research that was conducted in the period between 1953-1968, pin-pointed mercury salts as the cause of illness. Following absorption, certain organic mercury compounds, namely the methyl, ethyl and other alkyl derivatives, are retained in various organisms for longer periods than the inorganic derivatives (35). Furthermore, about 15% of the total ingested dose of organic mercury is accumulated in the brain. This is of interest because in addition to crossing the blood-brain barrier, methyl mercury also crosses the placental barrier. Thus, in the fetus, methyl mercury is found to damage the central nervous system and cause teratogenic effects during early stages of development. This disease was first reported from Minamata, and thus the name "Minamata disease" (36).

In adults, methyl mercury also causes irreversible damage to the central nervous system. In order of increasing severity, the following manifestations are observed: paresthesia of mouth, lips, tongue, hands and feet; fatigue; difficulty in speech; concentric constriction of visual fields with abnormal blind spots; hearing difficulty; emotional instability; difficulty in writing; lack of memory; uncoordinated movements. Paralysis, coma and death may result in extreme cases (37). The inorganic form of mercury is universally distributed in tissues and a high accumulation is found in the kidneys. In contrast to the organic forms, the uptake of inorganic mercury by the brain is a slow process and may lead to tremor and erethism in affected individuals. In addition, gingivitis and proteinuria are frequently observed.

In fish, mercury is found mostly in the methyl form. The half-life of methyl mercury in fish is about 200 days, which is three times higher than in man (38). It appears that industrial waste is a direct source of mercury in fish. Industrial discharge and fossil fuel smoke are largely inorganic

mercurials. However, this is converted to methyl mercury compounds under anaerobic conditions by a bacterium, Methanobacterium omelianskii, which lives in mud at the bottom of lakes and rivers. Methyl mercury from the bacterium is taken up by plankton and thus enters the food chain of fish and subsequently that of man (39). This biological transformation from the relatively 'safe' form to the highly toxic form of mercury is a grave problem facing industries, society and pollution control authorities today.

5. CADMIUM

A. Properties of Cadmium

Cadmium is a blueish white metal that was first isolated by Gottingen in 1817. Its atomic weight is 112.41, specific gravity 8.65, melting point 321°C and boiling point 770°C. In nature, cadmium exists as greenockite (CdS), but the major source is zinc and lead ores which contain cadmium as an impurity. Cadmium is thus obtained as a by-product during processing of these ores (23).

B. Uses of Cadmium

The recognition of metallurgical and other properties of cadmium has increased the demand for this metal. The production of cadmium has thus increased from about 100,000 lb per year in 1910 (40) to about 40,000,000 lb in 1974. With time, there has also been a significant rise in the incidence of both acute and chronic cases of clinically identifiable cadmosis (41).

Cadmium is used for electroplating iron, steel and copper as protection from corrosion, in making alloys with other metals such as copper, lead, bismuth, nickel, silver and aluminum for use as electrical conductors, solders, antifriction and bearing metals, alkaline storage batteries, and jewelry, in glass, paint and plastic industry as a pigment (CdS), and as an insecticide in the form of the oxide and hydroxide (23,42). Furthermore, cadmium stearate

is used as a stabilizer to increase the longevity of plastic products (43).

C. Sources and Routes of Exposure to Cadmium

Cadmium is emitted into air and water by mines, metal smelters and by industries using cadmium in alkaline accumulators, alloys, paints and plastics. It is estimated that in 1968 alone, a total of 4.6 million pounds of cadmium escaped into our biosphere. Cadmium from air or dust gains access to the body through the respiratory system (Figure 1). Estimates indicate that a person inhales anywhere from 0.02 µg to 10.0 µg of cadmium per day, depending upon the distance from the cadmium source (42). The highest concentration of cadmium in air is found near mines and smelters. Soot and smoke from incinerators and from industrial plants using fossil fuel also increases the level of cadmium in the atmosphere. Coal, for example, contains 250 ng Cd/g (44). The maximum allowable limit of cadmium in the air of industrial workshops is internationally accepted as 0.1 mg/m^3 (42). Air samples collected from various cities in the United States contained 0.002 - 0.37 µg Cd/m^3 (45). In addition to industry, the general population contributes to atmospheric pollution by burning petroleum products in automobiles and heating furnaces. The extent of pollution from these sources can be visualized by comparison of cadmium analyses in samples of melted snow picked from two different locations in New Hampshire, U.S.A. The first sample taken 20 meters from a street in Hanover had 1.5 µg Cd/liter, and the second from a hill top 100 meters from the road, which was comparatively less exposed to motor vehicle exhaust, contained only 0.35 µg/liter (45).

Cigarette smoking can also contribute appreciable amounts of cadmium via the respiratory tract. A pack of 20 cigarettes, according to Schroeder and Balassa (46) contains 30 µg cadmium. Lower values, with an average of 22.7 µg, were reported by Nandi et al. (47). These investigators calculated that after passing through the filter, the smoke still retained 69% of the

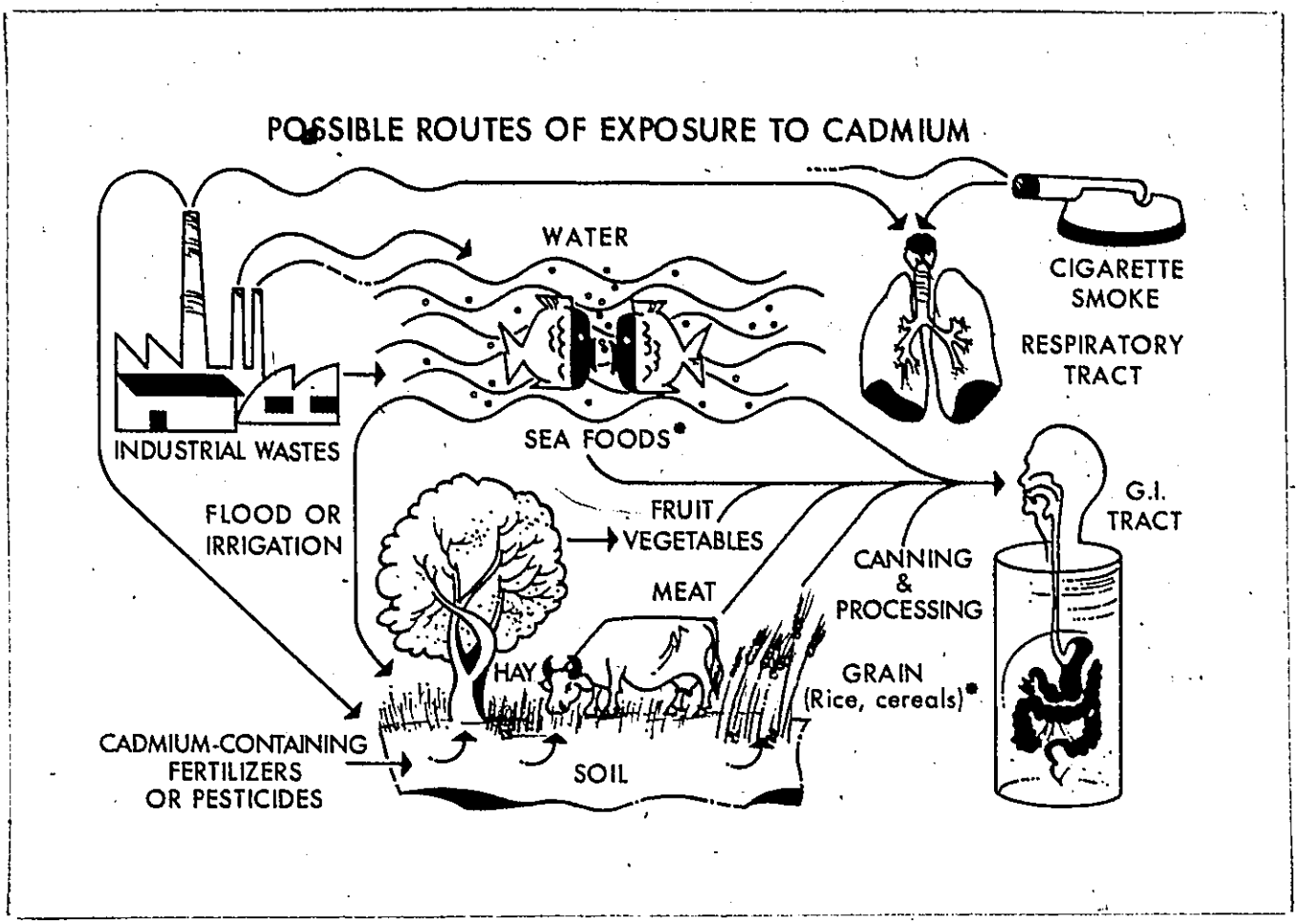

FIG. 1

Routes and sources of exposure to cadmium

total cadmium. Although quantitative data on human beings are lacking, animal experiments indicate retention of 10 to 40% of the inhaled cadmium (42). This would mean that a person smoking a pack of 20 cigarettes per day may absorb about 1-8 µg cadmium, in addition to that from other sources. Indeed, Lewis et al. (48) have reported that smokers accumulate larger amounts of cadmium in their organs than non-smokers and that accumulation in smokers is related to the number of pack-years smoked. Most of the cadmium emitted into the air however, is deposited in soil or water. Increased amounts of cadmium in water can also be due to dumping of contaminated industrial discharges into rivers, or due to passage of water through cadmium-containing pipes. Examination of fresh water reservoirs by Durum et al. (49) indicated high concentrations of cadmium in water near larger cities. Out of 720 samples of water obtained from lakes and rivers in the United States, 42% of the samples had cadmium concentrations ranging from 1-10 µg/liter. About 4% of the samples had more than 10 µg/liter cadmium which surpasses the United States Public Health upper limit. However, concentrations as high as 130 µg/liter also have been detected. Transport of water through galvanized iron, copper or plastic pipes increases water cadmium content (45). Municipal water supplies utilize soft water which tends to corrode metal pipes and makes soluble salts with metals. Stagnant water accumulates considerably more cadmium; concentrations as high as 77 µg/liter have been observed (45). Hard water, on the other hand, tends to form insoluble residues that coat metallic pipes and thus prevent corrosion.

Thus, consumption of cadmium-containing water contributes to the body burden of cadmium. But of more importance is the fact that crustaceans and fish can concentrate cadmium from water. Analysis of various sea foods by Schroeder et al. (45) indicated that oysters contained 3.14-3.66 µg Cd/g. Canned anchovies had 5.39 µg/g. The average of other sea foods excluding oysters

was 0.79 µg Cd/g. Consumption of these cadmium-laden sea foods is one of the most important contributors of cadmium. Cadmium from water can, in the case of irrigation or floods, increase the soil cadmium content. Furthermore, deposition of airborne cadmium as well as the use of various cadmium-containing pesticides and fertilizers can also elevate the concentration of cadmium in the soil. Cadmium from the soil can ultimately end up in various food products by mechanisms not yet entirely clear. Meat, especially liver, from cattle consuming cadmium-enriched hay is an important source of this metal; the data of Kropf and Mallinckrodt (50) showed that the highest concentration of cadmium was found in beef kidneys (ranging from 1-40 µg Cd/g). Cereals, grains and dairy products averaged 0.16 µg Cd/g. Vegetables had lower values ranging from 0.01-0.45 µg Cd/g. Japanese rice analyzed by Schroeder et al. (45) had 0.077 µg Cd/g and among Unites States varieties, the rice from Mississippi contained as high as 0.137 µg Cd/g. The upper limit set by the Japanese Ministry of Health and Welfare is 1.0 µg Cd/g for unpolished and 0.9 µg Cd/g for polished rice. This is supposed to keep daily intake of cadmium below the maximum safe limit of 300 µg. Data on gastrointestinal absorption of cadmium in humans are still scarce and estimates have therefore been based upon animal experiments, which indicate that anywhere from 1 to 10% of an oral dose of cadmium is actually absorbed (42,45,51).

D. Metabolism of Cadmium

Cadmium within an organism is neither degraded nor biosynthesized. Since the term metabolism is applied to biosynthetic as well as degradative processes within living cells (52), in the strict sense, the usage of this term with regard to elements would be incorrect. However, for convenience, the scientific literature accepts the term "trace element metabolism," in biological systems to replace the terms absorption, transport, cellular deposition, molecular binding, turnover and excretion. In the present study, therefore,

the term metabolism has been used to represent all processes involved in the biological cycle of an element.

(i) Placental transfer

In principle, transplacental passage of cadmium must be distinguished from placental binding as the latter may involve both maternal and fetal tissues. Transplacental transfer gives an initial body burden which will be augmented by later environmental exposure. Several human studies have shown that cadmium is bound to and crosses the placenta (42,51,53). Schroeder et al. (45) could not detect cadmium in the livers and kidneys of stillborns, newborns or infants upto the age of 5 months. However, in children over the age of one year, cadmium was always present in these organs. Henke et al. (54) analyzed liver and kidney samples from newborns in West Germany and found between 4 and 20 ng Cd/g wet weight in kidneys and less than 2 ng Cd/g in the liver. This would indicate that the total cadmium content in the newborn will be less than 1 µg. It would also mean that the fetal liver and kidney concentrations are about 1,000 times lower than what could be expected in the mother. Concentrations in the placentas of 44 Swedish women were found to be less than 0.01 µg Cd/g wet weight (42). This indicates that not more than about 5 µg will accumulate in a placenta weighing about 500 g. Thus, in humans, it appears that the passage of cadmium over the placental membrane takes place only to a limited degree.

Similarly, laboratory studies involving experimental animals have shown a limited passage of cadmium across the placental barrier. Berlin and Ullberg (77) gave pregnant mice a single tracer dose of ^{109}Cd on the 18th day after conception. They could not detect any cadmium in the fetus, but noted an accumulation of cadmium in the placenta. Tanaka et. al. (55) gave pregnant mice single intravenous injections of ^{115}Cd 24 to 36 hr before delivery. The mean uptake in newborns was 0.09% of the dose. Parizek (56)

showed that large doses of cadmium (4.5 mg Cd/mg) when given on the 17th and 21st days of gestation, destroyed the placenta. In pregnant hamsters, a single intravenous dose of 0.5 to 0.85 mg Cd/kg on the 8th day of gestation resulted in relatively high concentrations of cadmium both in the placenta and in the fetus. The variable penetration observed may be explained by differences in experimental species, challenge compound and dose used, the route of administration and the gestational age.

(ii) Excretion through mammary gland

Exposure of the newborn through milk can constitute a hazard, especially in cases where the initial body burden is already raised due to transplacental transfer (57). Murthy and Rhea (58) found that human milk in the United States contained, on the average, 0.019 ppm of cadmium, which is higher than the range of 0.001-0.010 ppm found in cows' milk in different countries (42).

Tanaka et al. (55) cross fostered sucklings born to female mice not exposed to radioactive cadmium with those given radioactive cadmium before delivery. Whole body measurements of the sucklings showed that after 14 days, 0.3% of the dose given to sucklers could be found in the sucklings. As some cadmium must have been excreted by the sucklings, the total amount excreted via the sucklers' milk can be thought to exceed 0.3%. Lucis et al. (59) administered a single subcutaneous postpartum injection of cadmium to female rats. It can be calculated from their results that less than 0.05% of the administered dose is excreted daily per g of milk. It should be pointed out that relatively large amounts of cadmium were retained in the mammary tissue. However, there is again a lack of similar human data.

(iii) Gastrointestinal absorption

In man, data on gastrointestinal absorption are very limited, even though the major portal of entry of cadmium into man is through the diet. Rahola et al. (60) studied the fate of ^{115m}Cd given orally to 5 human male volunteers

(ages 19-50 years). They received single doses of 4.8 to 6.1 μCi of ^{115m}Cd mixed with a calf kidney suspension. The total ingestion of cadmium was about 100 μg. During the first 3-5 days after administration, about 70% of the activity was eliminated, primarily in the faeces. A rapid elimination continued until about 6% of the dose was retained in the body. This indicates an average absorption of at least 6%. Balance studies on "normal" intake of inorganic cadmium compounds via food in human beings can theoretically be expected to provide figures on whole-body retention of the metal. However, it is difficult to derive a precise figure for cadmium because of the low and variable intake of this element and the low gastrointestinal absorption. However, with this approach, gastrointestinal absorption of cadmium in humans has been estimated to range from 2-10% (42).

Several studies on the uptake of cadmium in animals have been conducted to determine the retention of cadmium after its ingestion. Moore et al. (61) administered a single dose of 50 μCi of ^{115m}Cd by stomach tube. The whole body retention curve declined rapidly during the first 4 days to about 2.3% of the initial dose and then approximated a first order exponential curve. Most of the ^{115m}Cd was eliminated in faeces and only an extremely small amount was eliminated in the urine. Richmond et al. (62) gave ^{109}Cd to mice by intubation. They found a mean retention of about 1% (range 0.5 to 3%) after a few days. Nordberg (63) gave ^{109}Cd to mice by the oral route and found about 1.6% retention 14 to 16 days after dosing. Similarly, Ogawa et al. (64) gave mice a single oral dose of 1 μCi of ^{115m}Cd. The whole body retention after 24 and 48 hr was 7.3 and 2.7%, respectively. Retention studies conducted on monkeys (Saimiri Sciureus) after ingestion of ^{115}Cd through a stomach tube also indicated absorption of 2.5 to 3.2% (average: 2.9%) of the ingested dose, 10 days after dosing (42).

It has been shown that dietary factors may play an important role in influencing the absorption of cadmium from the intestine. Larsson and Piscator (65) kept female rats on low and high calcium diets for 1 to 2 months. They found that rats on low calcium diet had accumulated about 50% more cadmium in liver and kidney than those on high calcium diet. Furthermore, the uptake of an oral dose of ^{115}Cd was greater in rachitic chickens under treatment with vitamin D than in untreated chickens (66). It also is of interest that low protein diet gave considerably higher levels of cadmium in kidney, liver and whole body, irrespective of the calcium content in the diet. The whole body retention of the oral dose of ^{115}Cd was 9% and 4.5% in mice fed on low and high protein diets, respectively (67).

Thus, most animal data indicate an absorption of about 2% of ingested cadmium. However, the absorption is increased considerably by a low protein or low calcium intake. As indicated earlier, average absorption of about 6% has been reported in five humans (60). However, a retention rate of 10% or higher remains possible where there is a dietary deficiency of calcium or protein.

(iv) Absorption by inhalation

Pulmonary absorption of cadmium is another major route for the entry of this heavy metal into the human body. Metals may be taken into the respiratory system in the form of particles, gases or vapours. Unfortunately, data on transpulmonary absorption of cadmium in man are scarce. Friberg et al. (42) reviewed observations on cadmium uptake in humans exposed to this metal and concluded that quantitative data were not obtainable. However, significantly more cadmium is found in the organs of industrially exposed workers as compared to those of "unexposed" subjects. Thus, absorption via inhalation does take place (42).

In animal experiments, absorption and retention appears to range between

10-40% of the inhaled cadmium (42). However, the number of animal inhalation studies on cadmium are very limited, and some of the data do not lend themselves to detailed calculations for the percentage absorption. The contribution from direct absorption by the lungs and secondary absorption via the gastrointestinal tract following mucociliary clearance has not been determined. Moore et al. (61) exposed rats to an atmosphere containing 1800 µg Cd/m^3 for 4 hr. The cadmium aerosol was a mixture of ^{115m}Cd chloride and stable cadmium chloride (final specific activity 104.2 µCi/mg). The whole body retention curve showed an initial rapid decline during the first 4 days. This initial loss was attributed primarily to mucociliary clearance of cadmium from the lungs and passage of the non-absorbed cadmium through the digestive tract. Thus, approximately 59% of ^{115m}Cd was lost through the gastrointestinal tract by the 4th day of post-exposure. The second or slower component of the retention curve represented the summation of primary absorption (or deposition) by the lungs and secondary absorption by the gastrointestinal tract. Thirty days post-exposure, rats retained approximately 41.1% of the total inhaled ^{115m}Cd. Friberg (68) exposed 25 rabbits for 3 hr a day (for 20 days per month) for 8 months to about 8 mg cadmium iron oxide dust/m^3 (approximately 5 mg cadmium/m^3). It can be calculated that the rabbits had inhaled, on the average, a total of 190 mg of cadmium iron oxide dust (120 mg of cadmium). It seems that about 30% of the inhaled cadmium was retained (42,68). Thus, cadmium is absorbed and retained to a considerable degree in the body after inhalation. Data on human beings are again scarce, but the information available on smokers indicates that 25 to 50% of the cadmium in the fumes may be absorbed. Animal experiments would seem to speak in favour of between 10 to 40% retention of inhaled cadmium.

(v) Transport, distribution and excretion of cadmium

The total body burden of cadmium in a "standard man" in the United States has been calculated to be about 30 mg (46). Since the newborn contains

less than 1 µg of cadmium, it has been estimated that the human organism retains about 3 µg of cadmium per day. Cadmium absorbed via ingestion or inhalation is subsequently transported by the blood to various organs. Since it is difficult to determine the low concentrations of cadmium accurately in biological materials, large discrepancies exist among blood cadmium values reported by different authors. On the average, the normal level in whole blood and in serum seems to be well below 10 ng/g (42). Although both plasma and blood cells can take part in the transport of metal compounds, cell-bound metals do not exchange with other tissues as easily as do the metals bound to plasma constituents. Plasma-to-blood cell ratio of cadmium in man is believed to be 1.0 (i.e. equal concentration of cadmium in erythrocytes as in plasma) (51).

Different organs manifest striking variations in the fraction of the total body burden of cadmium that they contain. From blood, most of the cadmium is taken up by the kidneys and liver. It has been estimated that about 33% of the total cadmium in the body is in kidneys, 13.8% in liver, 2.3% in lungs, 0.3% in pancreas and the remainder in other tissues at low concentrations (45). It was shown that accumulation of cadmium in the kidneys occurred until the fourth decade of life. Older individuals showed a lower cadmium content in various organs (46). In these subjects, a decrease in zinc concentration and cadmium to zinc ratio also was observed (45). Curry and Knott (69) attempted to establish an age-linked trend for cadmium accumulation; however, their results failed to reveal any definite pattern.

Tissue concentrations and distribution patterns of cadmium vary with the exposure level. Variations in cadmium intake are related to several factors including environmental levels, dietary habits, geographic location as well as occupational environment. Data from various investigations of cadmium in the kidney and liver in relation to age have been assembled for "normals" in different countries, "occupationally-exposed" workers as well as for Itai-Itai patients (42). It is apparent that the hepatic values for "normals" in

the United States, the United Kingdom and Sweden are comparatively low, and the average values do not exceed 2 µg Cd/g wet weight. The normal Japanese values were 5-10 times those for the Unites States, United Kingdom and Sweden. However, in the "occupationally-exposed" workers and in Itai-Itai patients, hepatic cadmium concentrations were noted that were as high as 300 µg/g.

Similarly, it seems apparent that there is an almost linear increase in renal accumulation of cadmium in the age interval of 5 to 50 years. The peak renal cortical values in the "normal" American of about 50 µg/g wet weight were attained at the age of 40-50 years. Those "occupationally-exposed" had values scattered over a wide range, with about 67% of the values between 100 and 500 µg/g and the rest between 20 and 100 µg/g. It has been hypothesized that concentrations in some "occupationally-exposed" workers and in Itai Itai patients that are even lower than "normals" may be due to cadmium-induced renal damage and a consequent increase in renal excretion of the heavy metal (42).

Gastrointestinal excretion represents the major route of cadmium elimination from the body. There is a lack of adequate human data on total gastrointestinal excretion of cadmium. Rahola et al. (60) studied 5 male volunteer subjects (ages 19-50) who had ingested ^{115m}Cd mixed with calf kidney proteins. They estimated that the net gastrointestinal excretion of the amount retained in the body was less than 0.1% per day. Gastrointestinal excretion can take place by a number of routes: (i) active secretion or simple passive loss of metal with secretion, from various glands into the alimentary tract i.e. salivary glands, pancreas and glands located in the intestinal epithelium, (ii) loss by the shedding of epithelial cells, and (iii) biliary excretion (51). Further research is therefore needed for gaining adequate knowledge of source(s) of cadmium eliminated via the gastrointestinal tract.

Under normal circumstances, only a small quantity of cadmium is excreted through the urine. As in the case of blood, large variations exist in the reported values for normal urinary concentrations of cadmium. However, most of the recent work indicates an average normal excretion of 1 to 2 μg/day (42). As expected, the urinary excretion of cadmium in "exposed workers" can increase substantially. Smith and Kench (70) found urinary cadmium levels of 14-428 μg/l in workers industrially exposed to cadmium. Similarly, increased urinary excretion was found in a population endemically exposed to cadmium (71). Although the precise mechanism(s) of cadmium excretion is not known, Nordberg and Piscator (72) have proposed a model for renal accumulation and excretion of this metal which implies a role for metallothionein.

The earliest work on the metabolism of cadmium in animals was published in 1867 by Marme (73) where cadmium was detected in blood, liver, kidneys, heart and brain. In most of the early work, it was measured chemically in animal tissues after oral (74) or parenteral (75) administration of cadmium salts, or after the inhalation of cadmium dust and fumes (68,76). The distribution of cadmium has been found to be dependent, to some extent, on the mode of administration. Intravenous injection usually gives relatively higher liver concentrations than oral ingestion, with subcutaneous and intraperitoneal administration as well as aerosol inhalation probably inbetween (42).

Moore et al. (61) conducted whole body retention studies of ^{115m}Cd in rats following four different routes of administration: oral, inhalation, intraperitoneal and intravenous. The retention curve for each route of administration was divided into two components. The first component reflected the initial rapid clearance of ^{115m}Cd primarily by the gastrointestinal tract and the second component indicated the absorption and turnover of ^{115m}Cd. Extrapolation of the second component to the intercept gave initial absorption

values of 93%, 91%, 41% and 2.3% for intraperitoneal, intravenous, inhalation and oral routes, respectively. The route of administration did not significantly affect the rate of elimination as well as the biological half-life of the second component of the whole body retention curve which varied from 173 to 252 days.

Berlin and Ullberg (77) using the technique of whole body sagittal section autoradiography, studied the distribution of intravenously injected ^{109}Cd. In mice, 1 hr after intravenous injection of ^{109}Cd, the highest radioactivity appeared in the liver, followed by the kidneys and pancreas. The distribution pattern of radioactivity remained unchanged for 16 days. In kidneys, the cortex contained more ^{109}Cd than the medulla. Radioactivity was also detected in hair follicles and in interstitial tissue of the testes. More recently, Horner and Smith (78) examined the distribution of tracer doses of cadmium, both on short-term and long-term basis. In this experiment, cadmium distribution was studied for a 60-day time-course at 17 different points ranging from 5 min to 60 days post-injection. Twenty-two different organs and tissues were examined for radioactive cadmium content at each of the 17 time points. Faecal and urinary cadmium excretion was carefully monitored in all animals for the 60 day period. It was demonstrated that initial high plasma levels of intravenously administered ^{109}Cd decreased rapidly and that most of the cadmium which remained in the blood after 24 hr was associated with red cells.

The tracer dose of ^{109}Cd was shown to be distributed throughout the body in a characteristic pattern. The organs that accumulated the largest amounts of cadmium at all times were the liver, kidneys and pancreas. In most organs and tissues, a level was reached (usually within 24 hr) at which the percent of the body burden recovered remained relatively stable for the 60-day time-course. The amount of cadmium in every organ, except kidney, decreased slowly as time progressed, but the percent of total body burden of cadmium

in those organs remained approximately the same. In kidney, the percent of the body burden of cadmium increased progressively over the entire time-course. By 60 days, liver, kidneys and pancreas contained 71.75, 6.83 and 1.64% respectively of the total body burden. Excretion of cadmium was found to take place predominantly *via* the faeces. Twenty-five to 28% of the dose was excreted by that route in 60 days, the most rapid phase occurring during the first 3 days. Only less than 0.15% of the dose was excreted *via* the urine. Furthermore, it was observed that excretion of cadmium proceeded according to a triphasic pattern, and that the rate declined with time. Cadmium half-life values for the 3 phases were found to be 32, 103 and 231 days, respectively. It may be of interest that the cadmium half-life of 231 days is in agreement with that reported by Moore *et al.* (61).

Lucis *et al.* (79) investigated cadmium concentrations for 14 days following a tracer dose of ^{109}Cd to rats by the subcutaneous route. They found that already 2 hr after the injection, kidney values were slightly higher than liver levels and by 10 hr, the concentration in the kidneys was about 50% higher than that in the liver. Matsubara-Khan and Machida (80) studied the accumulation in mouse organs during sequential injections of ^{109}Cd. On 130th day, the end of the experimental period, the relative concentration of ^{109}Cd in kidneys was about 3 times that of liver. Similarly, Nordberg studied the distribution of ^{109}Cd after repeated exposure to the metal (42). In their experiment, mice were exposed to 0.25 mg or 0.5 mg Cd/kg subcutaneously for 6 months. In animals administered the lower dose of cadmium, the concentrations in the liver, kidney, pancreas and spleen were 281, 162, 71 and 10.1 µg/g wet weight, respectively. In the group with the highest exposure level, cadmium concentration in the spleen was twice as high as in the group with lower exposure. Pancreatic concentration was about 50% higher in the high exposure group. However, liver and renal levels of cadmium were about the same in both groups. The lack of any further increase in hepatic and renal concentrations

was attributed to renal tubular dysfunction accompanied by a sharp increase in the excretion of cadmium.

Miller et al. (81) compared the distribution of cadmium administered orally with that injected intravenously in goats. Fourteen days after intravenous dosing, highest concentrations were observed in the liver, kidney and spleen, in descending order. In contrast, following oral dosing, highest concentrations of the metal were found in the small intestine wall, kidney and liver. Furthermore, much higher levels of ^{109}Cd were seen in liver, heart and spleen of intravenously dosed animals relative to those noted in orally dosed ones. The distribution among various tissues after long-term oral exposure also has been studied by Stowe et al. (82) in rabbits given 160 µg/g of cadmium chloride in drinking water for 6 months. The mean exposure was 15.5 mg Cd/kg body weight per day. Mean cadmium concentrations in liver, kidney, pancreas and spleen were 188, 170, 29 and 10 µg/g wet weight, respectively.

Although the distribution pattern of cadmium is quite well characterized, the mechanisms of transport, distribution and excretion still remain obscure. It is not known in what form is cadmium transported from the lungs, intestines or injection sites to various organs. Horner and Smith (78) reported that initially, following its intravenous administration, most of the cadmium in blood was mainly in plasma. However, the cadmium that did remain after 24 hr was found in the red cells. This may mean that the initial uptake by kidneys is the result of glomerular filtration and reabsorption in the tubules of low molecular weight compounds (42). Metallothionein is probably one of the proteins responsible for cadmium transport in the blood. The selective accumulation in kidneys has been thought to be due to reabsorption of metallothionein in renal tubules (42,83). As proteins in normal kidneys are almost completely reabsorbed, only trace amounts of protein can be expected to appear in the urine. When the kidney has become saturated with cadmium, the

reabsorption decreases and tubular proteinuria appears. If metallothionein is filtered, less will be reabsorbed and it could be expected to appear in the urine (42). Nordberg and Piscator (72) have indeed shown the existence of a low molecular weight protein fraction containing cadmium in urine from chronically exposed mice.

More recently, Tanaka et al. (84) studied the fate of ^{109}Cd-labelled metallothionein in rats. The distribution of intravenously injected ^{109}Cd-labelled metallothionein in rats revealed an overwhelming concentration in the kidney at 1 hr. Liver and kidney accumulated 66.51 and 1.85% of the administered ^{109}Cd, respectively, 1 hr after intravenous injection. However, 1 hr following the administration of ^{109}Cd-metallothionein, liver and kidney accumulated 0.94 and 26.75% of the administered dose. It has been proposed that when cadmium is absorbed in the body, it is mainly taken up by the liver, and biosynthesis of thionein occurs within several hours. In the kidney, on the other hand, scarcely any induction of the thionein takes place probably because of the low cadmium uptake. Metallothionein produced in the liver is then gradually transferred to the kidney where it is retained (84).

E. Cadmium Toxicity

The isolation of cadmium in 1817 was soon followed by the discovery of its acute toxic effects on the gastrointestinal tract (after ingestion) and on the lung (after inhalation) (85,86). Despite the early recognition of these local effects of cadmium, the specific systemic effects of this heavy metal remained virtually unknown until recent years. With increasing production and utilization of cadmium, certain industrial workers became acutely exposed to high concentration of cadmium oxide fumes and developed pulmonary edema. However, the possible long-term effects of this heavy metal on the industrial workers received no attention. Stephens (87) and later Hardy and Skinner (88) were among the first to suggest that serious disease(s) might result in

industrial workers undergoing chronic exposure to cadmium, but the hypothesis at that time lacked empirical proof. It was not until 1948 when Friberg (68, 89, 90) conducted several biochemical and toxicological studies that the specific features of chronic cadmium poisoning in the industrial workers gradually became recognized. He noted emphysema and renal damage characterized by excretion of low molecular weight proteins in workers exposed to cadmium oxide dust over long periods in a battery plant. Prolonged industrial exposure to cadmium is now known to cause damage to the respiratory system, as characterized by emphysema, to the renal tissue, as indicated by increased excretion of glucose, proteins, amino acids, calcium, phosphorus and uric acid, and to the olfactory nerve, often causing total anosmia (91).

Probably the greatest concern was triggered by recent evidence indicating that chronic cadmium poisoning may not be restricted to industrial workers only, but may also constitute a serious health hazard to the general population. Reports of extensive non-industrial exposure to cadmium emerged only in the last decade such as the one from the Toyama area of Japan. The residents were exposed to relatively high levels of cadmium for several years as a result of contamination of river water by a mine discharging cadmium-laden waste-water, and increased cadmium concentrations in crops of that area. The unique disease was first described in English by Tsuchiya (92) and is called "Itai-Itai Byo" (which can be translated as the "ouch ouch disease.") The main features of the fully developed syndrome included severe pain in the bones and pathological fractures (hence the name of the disease), waddling gait, aminoaciduria, glycosuria, altered pancreatic function and severe osteomalacia. Following a carefully executed study, the Japanese Ministry of Health and Welfare concluded that based on available data, the cause of the disease was related to exposure to cadmium from a nearby mine factory (92,93). Around the same time when these studies on Itai-Itai disease were being carried out

in Japan, cadmium was incriminated as a possible factor in the etiology of hypertension in human beings (94). Later, Carroll (95) reported that there was a positive correlation between cardiovascular disease and cadmium concentrations in the air of 28 American cities studied.

(i) Renal lesions in cadmium poisoning

Marme, in a report published over a century ago, was one of the first to comment on the nephrotoxic effects of cadmium in animals (73). He reported that small doses of cadmium (actual dosage not mentioned) produced diffuse inflammation of the kidneys in addition to infarction of the lungs and fatty degeneration of liver and heart. Several subsequent reports confirmed and elaborated on the ability of cadmium to inflict morphological damage to the renal tissue. However, it was not until the mid nineteen hundreds that the effects of cadmium on human renal function began to be recognized. In 1950, Friberg investigated a group of industrial workers chronically exposed to cadmium oxide dust and found that there was a high incidence of kidney damage in these men. Prolonged exposure to cadmium produced renal damage reflected in proteinuria and decreased ability to concentrate the urine (68). Studies on the nature of proteinuria revealed that as in Fanconi syndrome, cadmium-induced renal damage was tubular in origin. The proteinuria was characterized by the presence of relatively small albumin fraction and dominance of proteins with mobility of α, β and γ-globulins in paper electrophoresis (96-98).

Further evidence of renal dysfunction was offered by the presence of glycosuria (98,99), aminoaciduria (98,100), impaired ability to excrete an acid load and probably as a result of this, hypercalciuria and renal stone formation, all of which have been observed in cadmium workers (98). A cadmium-induced syndrome similar to that described above has also been found to occur in non-industrially exposed population. Almost 100% of the Itai-Itai patients characteristically displayed proteinuria and glycosuria. Other common findings

included increased urinary excretion of amino acids such as alanine, glycine and lysine, as well as an increase in urinary calcium. Phosphorus excretion was somewhat reduced (92).

In rabbits subacutely exposed to cadmium, increases in the excretion of protein, alkaline phosphatase and acid phosphatase were found to be early warning signs, suggestive of proximal tubular injuries (101). Temporal dysfunction of distal tubules, accompanied by warning signs and renal dysfunction appeared later. It has been claimed that enzymuria is the most sensitive and earliest warning sign of renal tubular injury seen in cadmium nephropathy. To test out this hypothesis in a more physiological model, signs of cadmium intoxication were studied in rabbits fed on a diet containing 300 ppm cadmium for up to 54 weeks. The earliest signs of cadmium intoxication were aminoaciduria and enzymuria, both of which were detected after 14-16 weeks. Anemia was observed after 27 weeks, and later, proteinuria and glycosuria appeared. These data suggest that early cadmium intoxication can be detected by determining urinary excretion of amino acids and enzymes. Proteinuria and glycosuria indicate a later stage of cadmium intoxication (102).

Although chronic cadmium poisoning has been characterized by renal injury and proteinuria, the mechanism of the nephrotoxic effect of cadmium still remains obscure. It is evident that cadmium is predominantly localized in the kidney cortex, more specifically in the proximal tubules (63,103). This selective accumulation in kidneys has been thought to be due to reabsorption of metallothionein in renal tubules (83,104,105). Metallothionein is a metal-binding protein, the synthesis of which is induced by the presence of cadmium (106,107). It has been suggested by Piscator (83,104,105) that cadmium is transported to the kidneys by means of glomerular filtration of metallothionein-bound cadmium and thereafter accumulated in renal tubules by reabsorption from the filtrate.

From the Karolinska Institute, a hypothesis has been advanced suggesting that when more cadmium accumulates than can be bound by metallothionein, cadmium will exchange with zinc in enzymes necessary for reabsorption and catabolism of proteins (42). Leucine-aminopeptidase is one such zinc-dependent enzyme thought to play a role in the renal handling of proteins. Interestingly, Chiappino et al. (108) found inhibition of leucine-aminopeptidase activity in renal cortex of cadmium treated rats and rabbits. It is conceivable that because of the interference with the enzymatic actions, less protein is catabolized or reabsorbed causing tubular proteinuria. Furthermore, cadmium excretion would be enhanced as less metallothionein would be absorbed (42). Although metallothionein has been shown to exert protective effect against cadmium toxicity, efforts to identify the function of this metal-binding protein have thus far been largely unsuccessful (25). Recently, Nordberg et al. (109) suggested that metallothionein may have a paradoxical role in the pathogenesis of cadmium toxicity. Animals injected with metallothionein-bound cadmium did not exhibit testicular necrosis, a condition invariably encountered in animals injected with corresponding doses of non-metallothionein bound cadmium. It was also demonstrated that cadmium-metallothionein complex injected into mice resulted in higher cadmium levels in renal cortex and more severe renal tubular cell injury than when a comparable dose of the metal was administered by the same route. It is of interest that the type of renal damage observed in this study was similar to that observed in animals given repeated injections of cadmium for several months. These observations are concordant with the hypothesis that metallothionein is transported to the kidney tubules by means of glomerular filtration and subsequent tubular reabsorption (109). Another possible site of action of cadmium could be at the level of phospholipids. Cadmium has been shown to interact with phosphatidylethanolamine and phosphatidylserine monolayers and is effective in expanding them (110). Such an

interaction at the membranal level could provide a potential biochemical basis for the toxic effects of cadmium in mitochondria and kidney tubules (25). Since cadmium is also known to inhibit sulfhydryl enzymes in vitro (111,112), such an interaction could result in modification or blockade of the cellular metabolic activity. Finally, cadmium has been noted to markedly affect the conformation of polyriboadenylic acid as well as the physical properties of DNA (113,114). Since small amounts of cadmium are bound to RNA from equine kidney (114), the possibility remains that cadmium-induced renal damage might be a consequence of interference with the processes of transcription and translation. Although as discussed above, several potential models for elucidation of the mechanism responsible for the nephrotoxic effect of cadmium exist, the precise mechanism(s) for the development of renal injury still remains obscure.

(ii) Effects of cadmium on liver

As liver is known to accumulate large amounts of cadmium during exposure, functional disturbances could be expected in hepatic function. However, liver dysfunction has not been a common finding in exposed workers. Friberg (42) found that Takata reaction was positive in 2 and the thymol test in 3 of 19 workers exposed to cadmium oxide dust with a mean exposure time of 20 years. Increases in serum gamma globulin levels were found in several workers. In most other investigations, liver function has been little studied, but it seems apparent from the reports of Bonnell (115), Kazantzis et al. (98) and Adams et al. (116) that compared to the pronounced alterations in renal function, gross changes in liver function are rather unusual in cadmium-exposed workers.

Prodan in 1932 (117) reported changes in the livers of cats exposed to cadmium via the respiratory or the oral route. Similar observations were also made in rats given cadmium orally, 250 to 500 µg/g in the diet for several months (118). Friberg (68) injected cadmium sulfate (0.65 mg Cd/kg) into

rabbits 6 days a week for 2 to 4 months and found cirrhotic changes. Axelsson and Piscator (119) determined the serum glutamate oxaloacetate transaminase (GOT) activity in serum from rabbits given injections of cadmium chloride (0.25 mg Cd/kg) 5 days a week for 11 to 29 weeks. They noted that compared with controls, there was no difference after 11 weeks of exposure, whereas there were significant increases in activity after 17 weeks. At that time, the concentration of cadmium was around 450 µg/g wet weight. Piscator and Axelsson (103) made similar determinations in a group of rabbits exposed in the same way for 24 weeks and thereafter followed for another 30 weeks. At that time, the GOT activity was the same in serum from the exposed rabbits and controls. The concentration of cadmium in the liver was about 180 µg/g. There was no difference in alkaline phosphatase activity of serum. These findings suggest that the liver damage was reversible. Kimura (120) has reported increased levels of serum GOT and glutamate pyruvate transaminase (GPT) in rabbits exposed to 2 mg Cd/kg body weight subcutaneously 6 days a week for 2 weeks. He also reported a slower clearance of galactose from blood in these rabbits compared with controls. All of these changes are suggestive of hepatic involvement.

Long-term studies on effects of cadmium on activities of certain liver enzymes have been performed by Sporn et al. (121). In one experiment, rats were given cadmium chloride (1 µg/g cadmium) in drinking water for 335 days. An increase in the activity of phosphorylase a and a decrease in the aldolase activity were found, indicating that cadmium may interfere with carbohydrate metabolism in the liver. When rats were given larger amounts of cadmium (10 µg/g Cd in food) for shorter periods, an influence of cadmium on the oxidative phosphorylation in the liver mitochindria not seen in the above mentioned long-term experiment was noted.

When zinc was administered simultaneously via the oral route to rats given 10 µg/g cadmium in food for 60 days, it was found that whereas zinc

prevented the action of cadmium on the oxidative phosphorylation in the mitochondria, it did not prevent changes in the activity of phosphorylase a or aldolase (122). In an experiment by Decker et al. (123), it was found that animals receiving 0.5 µg/g cadmium in drinking water for 1 year had ingested a total amount of 5.8 mg. In these animals the mean cadmium concentration in the liver was 1.1 µg/g. It is conceivable that in the above mentioned experiment by Sporn et al. (121), cadmium levels in livers were not much different from the ones reported by Decker et al. (123). This indicates that cadmium might act upon certain enzyme activities at a liver concentration of the same magnitude as can be found in normal human adults (42). More recently, Stowe et al. (82) demonstrated that after 6 months of oral exposure to cadmium, mean liver concentrations were 188 µg/g wet weight. Light microscopy revealed that, in contrast to controls, the cadmium-exposed rabbits showed depletion of glycogen and deposits of collagen. Inflammatory cell infiltrates were frequent in the portal regions and biliary hyperplasia was often present. Electron microscopy revealed that the most striking changes took place in the endoplasmic reticulum, which was increased in exposed animals. Liver function tests such as determination of activity of GOT, GPT, alkaline phosphatase and lactic dehydrogenase isoenzymes in serum, bromosulphalein retention (BSP) test, and blood coagulation tests did not show any difference between controls and exposed. These results indicate that negative liver function tests do not exclude morphological liver changes and that some earlier conclusions regarding liver damage might not be valid. It would therefore appear that the commonly used clinical tests for hepatic function may not be suitable for evaluation of cadmium effects on the liver (42).

(iii) Pancreotoxic effects of cadmium

The pancreas contains a fairly high concentration of cadmium, surpassed only by that of kidneys and liver. Furthermore, during prolonged exposure, accumulation of cadmium in the pancreas continues even after it has ceased in

liver and kidneys (42). These findings are of particular interest in light of the reports indicating that cadmium has injurious effects on the cellular architecture as well as on the functional capacity of the pancreatic tissue. Voinar (124) was the first to demonstrate that administration of cadmium (i.v.) into rabbits produced hyperglycemia. Furthermore, he observed that administration of cadmium also decreased the hypoglycemic and convulsant effects of insulin. Barbieri et al. (125) presented further evidence to support the observation that cadmium induces alterations of pancreatic function. Upon histological examination, the authors found evidence of increased ratio of alpha to beta cells. This shift in the cell type ratio was considered to be indicative of diminished insulin-secretive activity. In concordance with this report, Havu (126) found that in fish (Corpus Scorpius), intramuscular injection of cadmium resulted in accumulation of this metal in the islet tissue. Furthermore, he found that like alloxan, cadmium accumulation was associated with necrotic lesions of beta cells, hyperglycemia and glycosuria. Although all these reports implicated altered insulin secretory activity, the effects of cadmium on the pancreatic function remained unassessed at that time.

Ghafghazi and Mennear (127) initially demonstrated that in addition to causing acute hyperglycemia, administration of a single dose of cadmium (6 mg/kg, i.p.) reduced glucose tolerance in intact mice. Furthermore, these authors found reduced serum immunoreactive insulin (IRI) concentrations in mice treated with cadmium prior to the glucose tolerance test. In contrast to acute effects, repeated injections of cadmium (4.0 mg/kg/day; 15 days) did not produce glucose intolerance; however, the subacute treatment with cadmium did decrease the resting IRI concentration. It was pointed out that the pancreatic damage was not reflected in the change in glucose tolerance because cadmium also lowered renal threshold for excretion of glucose by damaging proximal renal tubules. Furthermore, although chronic administration of cadmium failed

to produce persistant hyperglycemia during glucose tolerance test, the animals excreted significantly more glucose in their urine than did the control animals (128). These data provide support to the work of Ghafghazi and Mennear (127) who suggested that renal abnormalities may be associated with a 'masking' of cadmium-induced hyperglycemia.

More recently, Mennear's laboratory offered evidence utilizing isolated perfused rat pancreas to demonstrate that cadmium indeed inhibits pancreatic insulin secretory activity. In their initial experiments, pancreata were perfused with either high concentration of glucose (300 mg/100 ml) alone, or glucose combined with cadmium (1×10^{-4} - 1×10^{-3} M) (129). The perfusion of pancreata with glucose resulted in characteristically biphasic release of insulin. However, concentrations of either 1×10^{-3} or 5×10^{-5} M cadmium in perfusion medium resulted in a complete inhibition of glucose-induced insulin secretion; 1×10^{-4} M cadmium significantly attenuated this effect, resulting only in a partial inhibition of insulin secretion. Their experiments also demonstrated that presence of cadmium interfered with the tolbutamide- or potassium-stimulated secretion of insulin, indicating that the inhibitory action of cadmium may not be specific for glucose-induced hormone release. Although the inhibitory effects of cadmium on pancreatic insulin secretory activity have been demonstrated, the concentrations of cadmium used in these experiments were far in excess of those achieved in chronic in vivo situations. Lower concentrations of cadmium probably could not inhibit pancreatic secretory activity because of the fact that beta cells were exposed to the metal for short periods only (up to 60 min); longer perfusion periods could not be used because of development of pancreatic edema (129). However, it is of interest that long-term exposure of rats to cadmium has indeed been found to inhibit the amount of insulin secreted from the perfused pancreas of these animals (130). In their experiments, rats were treated with cadmium (0.25 or 0.5 mg/kg;

i.p.) every second day for 70 doses. They noted that based on total insulin released, 70 doses of 0.5 mg/kg markedly reduced insulin secretion during perfusion of the pancreas, whereas the 0.25 mg/kg doses did not. The above experiments demonstrated that exposure of intact animals or perfused pancreas to cadmium results in impairment of pancreatic insulin secretory activity. However, it seems that fairly large doses are required to induce pancreatic dysfunction. Furthermore, under certain circumstances, even when rats are exposed to large doses of cadmium, the pancreas can secrete basal levels of insulin; however, upon challenge with a glucose load, the impaired pancreatic function becomes apparent.

These reports could be of significance because as compared to normal pancreatic levels of cadmium which are usually below 2 μg/g wet weight (42), those in exposed workers and Itai-Itai patients range from 30 to 80 μg/g wet weight (i.e. 20 to 40-fold increase)(41,131,132). Interestingly, these pancreatic cadmium levels are within the range of cadmium levels reported by Ithakissios et al. (130) in the pancreatic tissues of chronically exposed rats displaying impaired pancreatic function. These findings may be concordant with the observations on humans suffering from cadmium intoxication (Itai-Itai disease) in whom decreased pancreatic function has been noted (133).

(iv) Cadmium-induced lesions of reproductive system

The prominent effects of cadmium on testicular tissue are well recognized. As early as 1919, Alsberg and Schwartze (134) had noted that administration of cadmium salts in animals caused a "bluish discoloration of the testicles." However, this observation escaped further scrutiny for almost 4 decades. It was not until the mid 1950's that Parizek (135) rediscovered and thoroughly studied the destructive effects of cadmium on testicular tissue. He observed that following parenteral administration of cadmium (2.2 mg/kg), the testes became swollen, dark red or purple. The weight then rapidly decreased and the

testicles turned small, hard and yellowish. Thus, the testes became hemorrhagic, edematous and in time, necrotic. The sterilizing effect of cadmium is very rapid and animals can become permanently sterile as early as 24 hr after its injection (135,136). Although the selective destruction of rat testis and its associated vasculature by cadmium is now well documented, the precise mode of action of cadmium in the gonad still remains unresolved.

Studies on the early sequential changes after cadmium administration revealed that circulatory disturbances precede any morphologically detectable damage to the seminiferous tubules (135,136,137). The entry of cadmium into the capillary endothelium of the testis and its ability to damage endothelial cells has been demonstrated (138,139). The injury of endothelium is subsequently followed by an increase in the vascular permeability resulting in a decrease in blood flow and the failure of blood vessels to fill adequately (140). It would appear that cadmium directly insults the testicular vasculature, rather than by acting indirectly through the release of chemical mediators, because cadmium-induced testicular injury is not prevented by various permeability inhibitors such as antihistaminics and antihyaluronidases (141,142). In addition, administration of vasodilators or anticoagulants failed to block the cadmium effect (143).

Parizek, on the other hand, demonstrated that large doses of zinc salts could prevent the action of cadmium on the testes (135). Similar findings have since been reported by several other authors (136,144-147). Zinc deficiency in animals affects the gonads and it has been found that cadmium administration can displace significant amounts of testicular zinc. Since zinc is essential for the maintenance of germinal epithelium, it has been suspected that cadmium might exert its initial injurious effects on zinc-dependent spermatogenic elements. However, the fact that cadmium evoked typical hemorrhagic necrosis even in testes devoid of any germinal epithelium

(as in the cryptorchid) would seem to oppose this hypothesis (136). On the other hand, zinc might be essential for normal metabolic activity and/or tissue integrity at some other testicular site(s), where cadmium could be displacing the zinc. Unfortunately, however, very few studies on cadmium-induced vascular injury have examined the biochemical alterations prior to the onset of generalized necrosis. In one study, succinic dehydrogenase activity of the internal spermatic artery-pampiniform plexus complex decreased 4 hr after the administration of cadmium (148). In contrast, alkaline phosphatase in testicular capillaries increased substantially (143). Although the function of these enzymes in vasculature is still obscure, alkaline phosphatase is suspected to be involved with active transport across the capillary wall (149). Dimow and Knorre (150) reported that 6 hr after cadmium administration, there was a decrease in the activities of three enzymes that are primarily associated with seminiferous tubules. Activities of succinic dehydrogenase, lactic dehydrogenase and DPN-diaphorase were depressed. This may not necessarily suggest a direct effect of cadmium on the germinal epithelium, since it could be a secondary reaction due to loss of nutritives following interference with testicular blood supply. It is of interest that the enzymes (alkaline phosphatase and dehydrogenases) which are altered by cadmium, are zinc metalloenzymes (i.e. zinc requiring enzymes). Hodgen et al. (151) studied the effects of cadmium on another zinc-containing enzyme, carbonic anhydrase in the testes. They described an isoenzyme of carbonic anhydrase which was found to be specific to the testis (isoenzyme T); it was absent in both erythrocytes and kidneys. Activity of this isoenzyme was decreased as early as 30 min after cadmium injection. Conversely, testes taken from animals exposed to cadmium for 4 hr contained no isoenzyme T, but exhibited demonstrable amounts of carbonic anhydrases I, II and IV, apparently due to haemorrhage of the testes and consequent invasion of carbonic anhydrase-rich erythrocyte into

interstitial spaces (151,152). Since the testicular isoenzyme was found to be present in both the testicular artery and the testicular parenchyma (152), these studies failed to reveal whether the action of cadmium took place in the interstitium or in the tubules of the rat testicle (42).

It may be pointed out that zinc is not the only element implicated in the etiology of cadmium-induced testicular damage. Selenium has been found to be even more efficient than zinc in preventing cadmium-induced gonadal damage (137,144,153). Initially, it was suspected that selenium might be diverting cadmium away from the testis, but it was later found that selenium actually increased the testicular uptake of cadmium. The mechanism by which selenium affords protection against the necrotizing action of cadmium is, of course, still obscure. Other agents which have also been reported to protect against cadmium-induced testicular injury include manganese, cobalt, copper, calcium, iron, lithium, nickel, arsenic and uranium (140). Metallothionein has also been implicated to play a protective role against cadmium-induced testicular necrosis. Nordberg et al. (109) were able to demonstrate that whereas cadmium-metallothionein complex was without effect, a comparable dose of cadmium chloride administered by the same route resulted in testicular necrosis. It therefore seems possible that some elements might afford protection against cadmium-toxicity by inducing metallothionein formation.

Recently, Lee and Dixon (154) reported that a single injection of cadmium at a dose that did not cause any morphological change in testicular vasculature, produced a significant decrease in fertility for a period of 55 days. They were also able to demonstrate that the late elongated spermatids had an affinity for cadmium that was 2.5 to 2.9 times that of zinc at equimolar concentrations; the incorporation of these two metals into the late elongated spermatids appeared to be competitive. Furthermore, cadmium administration (1.0 mg/kg, i.p.) inhibited thymidine uptake into spermatogonial cells by

42% and 52% of the control values on days 1 and 7. Pretreatment of mice with zinc chloride (1 mg/kg) prior to cadmium treatment completely blocked the biochemical and functional effects of cadmium on spermiogenic cells but not on spermatogonial cells. Since the effect of cadmium on spermatogonia, where DNA synthesis occurs, was not reversed by zinc, they postulated that this persistent cadmium-induced biochemical lesion might be due to the inhibitory effect of cadmium on DNA synthesis (154). However, this is only a postulation and more work is required in this area. Nevertheless, their study has shown that (a) biochemical lesions precede histological abnormalities of seminiferous tubules or vasculature and (b) cadmium has at least two actions on spermatogenic cells. The most likely are a direct interaction of cadmium with DNA and competitive inhibition of essential enzymes. The first action is not reversed by zinc while the second is.

Thus far, only the acute effects of cadmium have been discussed. However, from the point of view of environmental exposure, acute effects of large doses are of limited interest. Histological changes in the testicles of rats as a result of long-term administration of cadmium in food were reported by Pindborg (155) and Ribelin (156). Pindborg gave 150 to 610 µg/g cadmium and Ribelin, 50 to 1,270 µg/g cadmium in the diet. Richardson et al. (157) studied the histological sequelae of feeding 75 mg Cd/kg of diet from hatching to 4 or 6 weeks in Japanese quail. Amongst other effects, testicular hypoplasia was one of the most obvious lesions produced by dietary cadmium. It is of interest that similar failure of normal spermatogenic development also was observed in birds weaned on zinc-deficient diet, suggesting that cadmium may interfere with zinc metabolism (157). On the other hand, Piscator and Axelsson (103) could not observe any histological abnormalities in testicles of rabbits injected with cadmium for 24 weeks and maintained for another 30 weeks without any cadmium administration. They suggested that absence of testicular changes could be due to the relative insensitivity of rabbits to

the necrotizing action of cadmium, or by formation of metallothionein in the liver. It is of interest that repetitive injection of small quantities of cadmium into mice increased the amount of cadmium in the testes without inducing degenerative changes, and furthermore, subsequent exposure of these animals to normally toxic doses of cadmium produced no testicular damage (157). Cadmium, perhaps as a result of "priming" of metallothionein production renders the otherwise toxic dose of cadmium innocuous by causing a rapid incorporation of the metal into the protein (159).

The available reports indicate that acute or chronic exposure of experimental animals to cadmium can induce biochemical and/or functional alterations in the male reproductive organs. Furthermore, the observations in a great number of animal species implicate that similar effects are likely to occur in human beings (42). So far however, testicular necrosis as a result of cadmium exposure has not been reported for man. Industrially exposed workers do seem to accumulate considerable amounts of cadmium in the testis and some histological changes of rather unspecific nature have been noted upon post mortem examination (131). In addition, Favino et al. (160) investigated fertility of 10 cadmium workers and found only one case of impotency with abnormally low urinary testosterone levels (160). It is therefore obvious that further studies are needed on the chronic effects of cadmium on male reproductive function in both the experimental animals and the industrially exposed humans.

(v) Cardiovascular effects of cadmium

The ability of cadmium to produce hypertension has received considerable attention during recent years. Chronic arterial hypertension has been induced in rats by feeding or injecting small amounts of cadmium over a period of a few months (161). Furthermore, Carroll (95) observed a positive correlation between the cadmium content of air and the incidence of hypertension and

arteriosclerosis in 28 North American cities. However, there is still no firm evidence to show that there is an increased prevalence of cardiac disease or hypertension in workers industrially exposed to cadmium (68,98,99,115,162). Similarly, although Schroeder (94) reported that kidneys of hypertensive patients had higher cadmium levels (or cadmium to zinc ratio) than those of normotensive ones, Morgan (163) found no evidence of excessive kidney cadmium loads in hypertensive patients. It may also be pointed out that in certain areas of Japan where there has been a considerable exposure to cadmium for decades, hypertension has not been associated with this heavy metal (42).

Results arising from animal experiments are equally ambiguous. Hypertension has been produced in rats of Long Evans strain by giving cadmium in drinking water for long periods of time (164). Hypertension also has been induced by parentral injection of cadmium. Thind et al. (165) induced hypertension in rabbits by giving weekly intraperitoneal injections of cadmium acetate (2 mg/kg) for 7 weeks. However, negative findings also exist in regard to the relationship between cadmium exposure and hypertension. Lener and Bibir(166) failed to produce hypertension in Wistar rats by giving cadmium in drinking water (5 µg/g) for 16 months. Similarly, injections of cadmium chloride (0.5 mg Cd/kg) 3 days a week for 6 months could not induce hypertension in Sprague-Dawley rats (42). Due to controversial reports on this subject, it is not possible to reach any definitive conclusion and further work is obviously needed to prove or disprove the role of cadmium in the pathogenesis of cardiovascular diseases. As far as the biochemical effects of cadmium in the myocardium are concerned, very few studies seem to have been published. Flavoproteins and dehydrogenases of the respiratory chain are targets for cadmium inhibition because it binds specifically with the functional dithiol groups (167). Cadmium has been shown to depress oxidative phosphorylation in heart mitochondria (168) and in pulmonary alveolar

macrophages (169). It was also reported that cadmium produced a 50% inhibition of ATPase activity in macrophage mitochondria and plasma membranes. Furthermore, cadmium was found to induce cardiac atrophy which was accompanied by decreases in ATP levels, AMP formation and ATPase activity. In contrast, the inorganic phosphate levels were increased by cadmium (170). These studies indicate that cadmium may interfere with the availability of high energy phosphates essential for normal cardiac function.

(vi) Toxic effects of cadmium to the central nervous system

Histological evidence indicates that administration of cadmium (1 mg $CdCl_2$/ 100 g, s.c.) in newborn rats causes hemorrhagic lesions in the brain (171). In older animals, and during adult life, the lesions were produced in spinal sensory ganglia of rats, rabbits, guinea pigs and hamsters, indicating the importance of age with respect to the central nervous system site of cadmium toxicity (172,173). This is of interest since data by Lucis et al. (59) indicate that cadmium penetrates the blood-brain barrier with more ease in fetal rats. However, cadmium has also been demonstrated to accumulate in adult brain (77,82). Recently, Kasuya et al. (174) demonstrated that cadmium stearate inhibited the outgrowth of cerebellar cells from newborn rats in tissue culture and produced degenerative changes at a concentration of 0.58×10^{-6} M. Cadmium stearate at a concentration of 2.3×10^{-6}M completely inhibited the outgrowth of nerve fibers, glial cells and fibroblasts (174). These in vivo and in vitro studies using relatively high concentrations of the heavy metal indicate that cadmium can be potentially toxic to the central nervous system. Indeed, iontophoretic administration of cadmium into cerebral cortex or brain stem of the cat has been found to markedly depress spontaneous neural firing (175). Cearley and Coleman (176) observed "abnormal behavioural patterns" in fish intoxicated with cadmium. The "abnormal behavioural patterns" were first observed during the third week of exposure in bass exposed to 0.85 mg Cd/liter. The same symptoms could be noted at 7 weeks when exposed to 0.080 mg

Cd/liter and also at 12 weeks if exposed to 0.008 mg/liter level of cadmium. Identical behavioural patterns were observed in the bluegill at the 0.85 mg Cd/liter exposure level after 13 weeks of exposure. The bass and bluegill exhibited erratic, uncoordinated swimming movements, muscle spasms and convulsions, followed by loss of equilibrium with periods of quiescence and paralysis. The "abnormal behaviour" exhibited by bass and bluegill may suggest that the nervous system was the site of damage. Furthermore, the toxic manifestations would be consistent with the inhibition of acetylcholinesterase (AChE) causing death by paralysis of the muscles of respiration and/or depression of the respiratory centre (176). Although cadmium has been reported to alter neuronal function (175) and the behavioural pattern (176), neurochemical alterations associated with the observed pathological changes are lacking. Preliminary experiments indicate that neonatal exposure of rats to cadmium increased the spontaneous motor activity and altered the central metabolism of norepinephrine and 5-hydroxytryptamine. In contrast, the changes in the endogenous levels of dopamine were not quite as marked (170). To what extent these changes reflect the pathological damage to the central nervous system as well as the cadmium-induced behavioural aberations, remains to be elucidated. Furthermore, it would be important to investigate if, like lead, cadmium also induces minimal brain dysfunction and also whether the damage to the central nervous system is reversible.

6. CARBOHYDRATE METABOLISM IN MAMMALIAN TISSUES

The primary function of carbohydrates in the body is to provide a source of chemical energy as many of the reactions coupled to ATP generation are found in the pathways involved in the metabolism of carbohydrates. The major carbohydrate in the body is glucose, which is metabolized in the presence of oxygen to carbon dioxide and water with the release of 686 kcal/mole of energy.

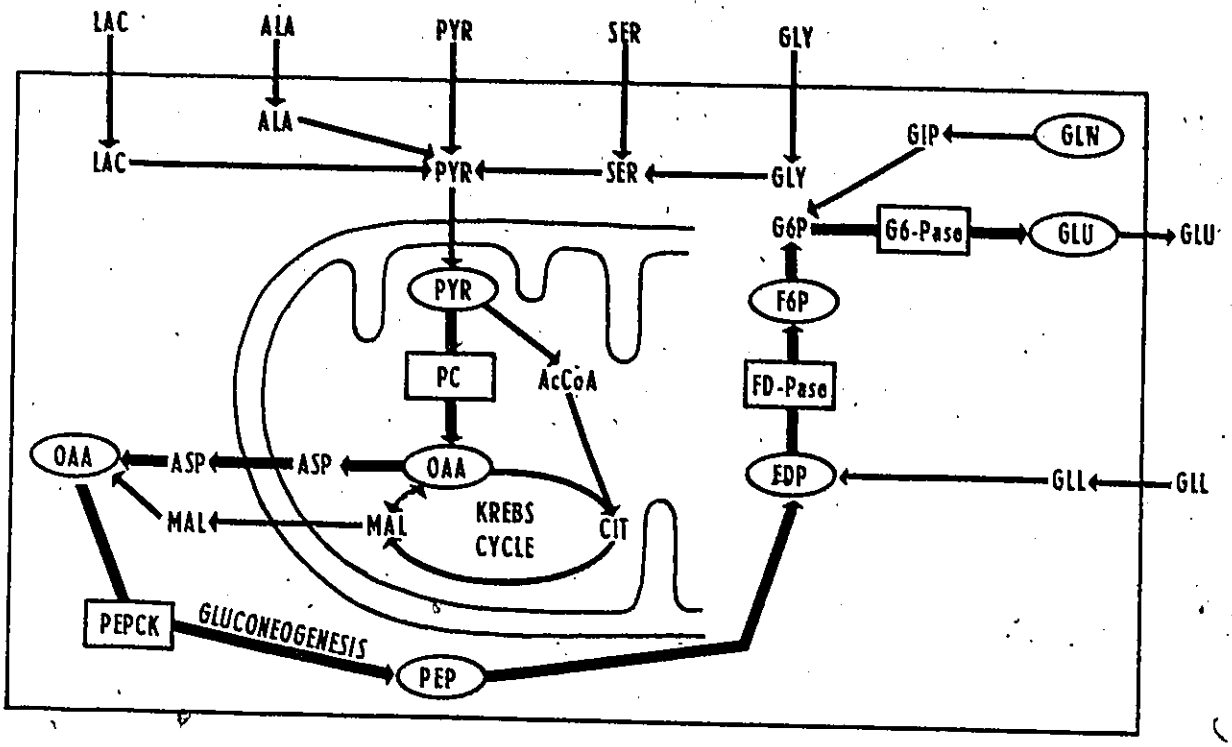

FIG. 2

Gluconeogenesis in the rat liver cell; the location of key rate-limiting enzymes. Abbreviations are: LAC, lactate; PYR, pyruvate; PC, pyruvate carboxylase; ALA, alanine; SER, serine; GLY, glycine; AcCoA, acetyl CoA; CIT, citrate; MAL, malate; OAA, oxaloacetate; ASP, aspartate; PEPCK, phosphoenolpyruvate carboxykinase; PEP, phosphoenolpyruvate; FDP, fructose 1,6-diphosphate; FD-Pase, fructose 1,6-diphosphatase; G6P, glucose 6-phosphate; G6-Pase, glucose 6-phosphatase; GLU, glucose; GLN, glycogen; GIP, glucose 1-phosphate; GLL, glycerol.

The maintenance of glucose homeostasis is of fundamental importance to the existence of mammalian organisms, since energy provided by carbohydrate metabolism is essential for the cellular activities in many tissues.

A. Role of Liver and Kidney in Glucose Metabolism

In higher organisms, the kidney cortex and liver appear to be unique in that they possess the full enzymatic potential for both glucose synthesis from noncarbohydrate precursors, and glucose degradation via the glycolytic pathway of Embden, Meyerhof and Parnas. In the process of glycolysis, glucose is not only used to generate biological energy in the form of adenosine triphosphate, but also supplies precursors for fatty acid, protein and nucleic acid synthesis. Glucokinase, phosphofructokinase and pyruvate kinase are believed to be the three key enzymes in the glycolytic sequence since they play a rate-limiting role in determining the overall activity of this metabolic pathway (177,178).

Gluconeogenesis in man and other nonruminants comprises the synthesis of glucose and glycogen from lactate, pyruvate, glycerol and certain amino acids. The liver is the major site of gluconeogenesis with the kidney becoming an important site during starvation and acidosis. Gluconeogenesis performs several functions. First is the provision of glucose to the body during starvation and other situations when carbohydrate intake from the alimentary tract is limited and the body glycogen stores are depleted. Second is the reutilization of lactate and glycerol produced in small amounts under basal conditions and in increased amounts during exercise and heightened sympathetic activity. Third is the provision of NH_3 in the kidney to counteract acidosis, e.g. in prolonged starvation. Fourth is the metabolism of amino acids absorbed from the alimentary tract or released during protein breakdown in muscle and other tissues (179).

Figure 2 shows the sequence of key reactions involved in the conversion of lactate, pyruvate, glycerol and other major amino acids into glucose and glycogen. Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase are the quartet of enzymes which play a rate-limiting role in glucose formation from non-carbohydrate sources, since they catalyze irreversible reactions, are involved in circumventing thermodynamic barriers and are located almost exclusively in organs (liver and kidney cortex) capable of gluconeogenesis (180). The amino acids and possibly other substrates enter the cell by membrane transport systems. Lactate, alanine, serine and glycine are converted to pyruvate in the cytosol. This substrate enters the mitochondrion where it is converted to oxaloacetate by pyruvate carboxylase (PC). However, small portion of pyruvate may also be converted to acetyl-CoA by pyruvate dehydrogenase. Oxaloacetate is then converted to malate, aspartate and citrate which is mainly metabolized in Krebs cycle. In the cytosol, malate and aspartate are reconverted to oxaloacetate which is then converted to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK). Phosphoenolpyruvate is converted to fructose 1,6-diphosphate by the same cytosolic enzymes that are involved in the Embden-Meyerhof glycolytic pathway. Fructose 1,6-diphosphate is hydrolyzed to fructose 6-phosphate by fructose 1,6-diphosphatase (FD-Pase), an enzyme present in large amounts exclusively in tissues carrying out gluconeogenesis. Finally, glucose 6-phosphate is converted to glucose by glucose 6-phosphatase (G6-Pase), another enzyme specific for gluconeogenesis (179).

(i) Factors regulating gluconeogenesis

There are several factors that are known to be involved in controlling gluconeogenesis under physiologic conditions. Gluconeogenesis may be modulated by the availability of substrates essential for this process. The blood levels of gluconeogenic substrates are subject to elaborate control and vary

greatly in different metabolic states (181). Levels of several amino acids can be altered by diet, fasting, obesity, pregnancy, exercise, renal failure, as well as by administration of insulin, corticosteroids, glucagon, catecholamines and growth hormone. The lactate, pyruvate and glycerol supplies are also under hormonal control involving chiefly catecholamines and insulin (179).

From the circulation, substrates must enter specific cells for transformation into glucose. This process of uptake of substrates by hepatic cells can also be influenced by several hormones such as glucagon, insulin, cortisol and growth hormone. Glucagon stimulates net uptake of alanine, glycine, arginine, lysine and phenylalanine by the liver (182). Similarly, growth hormone, cortisol and epinephrine have been found to increase hepatic accumulation of various amino acids (183). Insulin, on the other hand, seems to depress the hepatic uptake of amino acids. The transport of metabolites across the inner mitochondrial membrane which appears to be carrier-mediated, may also be subjected to modulation. It has been suggested that pyruvate entry into liver mitochondria is a control point for gluconeogenesis which is influenced by epinephrine, glucagon and cortisol (184).

Gluconeogenic activity may also be controlled via modulation of enzymic activities of various pathways. Alterations in the activity of the enzymes involved directly in synthesis of glucose or those competing for gluconeogenic substrates may influence the rate of gluconeogenesis. Pyruvate carboxylase is a key enzyme in gluconeogenesis in that it is instrumental in determining whether pyruvate is converted to oxaloacetate or is oxidized to acetyl-CoA. The mitochondrial level of acetyl-CoA may be a major factor influencing the flow of pyruvate to glucose since this intermediate is an allosteric activator of pyruvate carboxylase (185). Furthermore, several additional effectors have been reported to influence pyruvate carboxylase activity including Ca^{++}, Mg^{++}, pyruvate, acetoacetyl-CoA, β-hydroxybutyryl-CoA, malonyl-CoA, CoA and

aspartate (179). Regulation of pyruvate dehydrogenase is also important in the control of gluconeogenesis because this enzyme competes with pyruvate carboxylase for pyruvate. This enzyme has recently been found to be subject to regulation by a phosphorylation dephosphorylation reaction sequence (186). Phosphorylation occurs on the pyruvate dehydrogenase component of the enzyme complex and causes a proportional decrease in overall enzyme activity. Phosphorylation and inactivation are catalyzed by an ATP-specific kinase, and dephosphorylation and activation are catalyzed by a phosphatase. Both the phosphatase and the kinase enzymes are Mg^{++} dependent. The phosphatase requires about a 10-fold higher Mg^{++} concentration for optimal activity than does the kinase. It is thus possible that the enzyme phosphatase is regulated by the intramitochondrial level of free Mg^{++} which in turn, may depend on the ATP/ADP ratio (186).

The enzyme, phosphoenolpyruvate carboxykinase, has been suggested to be a major site at which cyclic AMP acts indirectly or directly to activate gluconeogenesis (187). Control of phosphoenolpyruvate carboxykinase synthesis is probably an important factor in long-term adaptations of gluconeogenesis such as during diabetes, starvation, birth and glucocorticoid administration. Shrago et al. (188) were the first to show that phosphoenolpyruvate carboxykinase activity is increased in the liver by glucocorticoids, fasting, glucagon, lactate or during diabetes induced by alloxan, mannoheptulose or pancreatectomy. Their results have been confirmed many times and are apparently due to increased enzyme synthesis (188). Insulin is effective in suppressing phosphoenolpyruvate carboxykinase, perhaps through decreased cyclic AMP levels (189), altered hepatic amino acid supply or by primary action at the gene level (178). Stimulation of phosphoenolpyruvate carboxykinase by glucocorticoid (190), glucagon (191) and isoproterenol has also been reported. Furthermore, glucagon and isoproterenol stimulation of phosphoenolpyruvate

carboxykinase has been shown to be mimicked by cyclic AMP (191).

The reaction sequence between pyruvate and phosphoenolpyruvate represents another major site of control for the process of gluconeogenesis. It is possible that control may partly involve alterations in pyruvate kinase. This enzyme exists in multiple forms in liver, some of which are allosterically activated by fructose 1,6-diphosphate (192). Krebs and Eggilston (193) have shown that starvation, diabetes and birth decrease the activity of pyruvate kinase in liver tissue.

Fructose 1,6-diphosphatase is another potential site of control of gluconeogenesis and is subject to allosteric control by AMP and fructose 1,6-diphosphate. The inhibition of fructose 1,6-diphosphatase by these compounds would complement their stimulatory action on phosphofructokinase and could play a role in the inhibition of gluconeogenesis by anoxia (179). It is of interest that administration of corticosteroids to rats has been reported to increase the activity of fructose 1,6-diphosphatase in rat liver (194).

Glucose 6-phosphatase is known to be almost exclusively confined to liver and kidney. It catalyzes the conversion of glucose 6-phosphate into glucose, liberating a phosphate moiety. This reaction is inhibited by citrate, palmityl-CoA, phosphate, bicarbonate, ATP and ADP. Nordlie et al. (195) have suggested that some of these agents may play a role in the physiological regulation of glucose 6-phosphate hydrolysis.

Any detailed presentation of the control of glycogen metabolism is beyond the scope of this dissertation. However, it is of interest to consider the factors determining whether a gluconeogenic substrate is converted to glucose or glycogen. A prime factor appears to be blood glucose level since glucose can activate glycogen synthetase and inhibit phosphorylase in isolated liver (197). Another factor influencing the flow of substrate to glucose or glycogen is the level of glycogen itself in the liver. A high glycogen level limits glycogen synthesis by inactivating and thus favours glycogenolysis

(198). Many hormones influence the disposition of gluconeogenic substrates between glucose and glycogen in the hepatic tissue. Glucagon and catecholamines increase glycogenolysis and decrease glycogen synthesis. These effects seem to be related to increase in cyclic AMP causing activation of a protein kinase which phosphorylates and activates phosphorylase kinase which in turn, inactivates glycogen synthetase (199). It is now well established that insulin promotes net liver glycogen deposition. The action of insulin to activate glycogen synthetase and inactivate phosphorylase in the liver under in vivo conditions is explicable in terms of a lowering of cyclic AMP (189). However, Shen et al. (200) have proposed that insulin may also act by inhibiting the action of cyclic AMP on protein kinase, while Bishop (201) obtained evidence that insulin activates glycogen synthetase. Similarly, glucocorticoids have also been known to cause glycogen deposition in the liver. Starvation and diabetes exert profound effects on hepatic glycogen metabolism which are probably on account of increased cyclic AMP levels due to decreased insulin and enhanced secretion of glucagon (202).

Thus, gluconeogenesis is subject to several control processes which include supply of substrate from peripheral tissues, substrate uptake by the liver, transport of metabolites across the mitochondrial membranes and altered activity of enzymes involved in glucose metabolism. Furthermore, changes in ATP, ADP, AMP, cyclic AMP, Mg^{++} and Ca^{++} also seem to play an important role in the control of gluconeogenesis.

Schimmel and Knobil (203) found that starvation enhanced glucose-C^{14} production from labeled alanine, lactate, pyruvate and glycerol in hepatic tissue slices as well as serum glucose levels. Young and Scrimshaw (204) showed that starvation also stimulated the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-

phosphatase. The rise in various gluconeogenic enzyme activities was accompanied by a fall in the concentration of valine and alanine (204). During starvation, there is a striking change in the role of the kidney cortex since it contributes almost as much as the liver to overall glucose production (205). This shift is believed to be attributed, at least in part, to changes in the acid-base balance in the blood caused by an increase in the body's production of ketone bodies.

Hypoxia and the consequent loss of oxygen supply to the liver also exerts profound effects on carbohydrate metabolism. Ballard (206) found that C^{14}-lactate, C^{14}-serine and C^{14}-aspartate was not incorporated into labeled glucose in 2-day old rats exposed to a 100% nitrogen atmosphere. Similarly, Philippidis and Ballard (207,208) found that gluconeogenesis was suppressed in fetuses subjected to hypoxia. Inhibition of gluconeogenesis and activation of glycolysis has also been demonstrated following hypoxia in perfused rat livers (179). Although the mechanism by which hypoxia suppressed gluconeogenesis is unknown, it is believed to be due to a fall in the concentrations of hepatic pyruvate and oxaloacetate (209,210).

Pregnancy is associated with altered metabolic state since the fetus is dependent on maternal substrates (211,212). Herrera et al. (213) found an enhanced gluconeogenesis in livers of pregnant rats administered C^{14}-pyruvate and suggested that the observed increase in gluconeogenesis was probably related to the large demand made by the fetus on the maternal glucose supply. Lactation makes extra demands on glucose supply in mammals and the gluconeogenic pathway is the major route by which the organism is supplied with glucose in this condition (179). Alterations in carbohydrate metabolism have also been observed during exercise. Krebs et al. (214) demonstrated an enhanced rate of gluconeogenesis from lactate, pyruvate and fumarate following muscular

exercise. Sanchez-Medina et al. (215,216) not only found an increased production of glucose from lactate and pyruvate in exercised rats, but also stimulation of hepatic and renal phosphoenolpyruvate carboxykinase activity.

Disturbances in acid-base balance also exert profound effects on carbohydrate metabolism in the kidney. Goodman et al. (217) demonstrated that in ammonium chloride-induced metabolic acidosis, there was an enhanced renal gluconeogenesis. They observed an increased incorporation of several non-carbohydrate precursors into glucose in rat renal cortical slices. In addition, Alleyne and Scullard (218) noted a marked stimulation in the activity of renal phosphoenolpyruvate carboxykinase during metabolic acidosis induced by ammonium chloride. In contrast, metabolic alkalosis induced by sodium bicarbonate loading was found to significantly depress the synthesis of glucose by renal cortical slices (217). As expected, Pilkington and O'Donovan (219) found that net glucose production in dog kidneys was decreased in metabolic alkalosis.

Human as well as experimental diabetes is characterized by a disturbance in carbohydrate metabolism and is associated with secondary alterations in protein and fat metabolism (220). Weber et al. (177) demonstrated a marked decrease in the activities of various glycolytic enzymes in alloxan diabetic rats. Recently, Singhal et al. (221) found that administration of streptozotocin induced diabetes in rats and significantly lowered the activities of key glycolytic enzymes in liver tissue. In contrast, the activities of various gluconeogenic enzymes were markedly elevated in livers of alloxan- and streptozotocin-induced diabetic rats (178,188,221-223). It is interesting that insulin treatment prevented the diabetes induced rise in various gluconeogenic enzymes (178). Thus, changes in gluconeogenesis are important in the metabolic adaptations of man and other mammals to many physiologic and pathologic conditions. The process of gluconeogenesis is altered under

situations including exercise, fasting, diabetes, pregnancy, lactation as well as altered state of acid-base balance. In most physiologic situations, the hormones and other factors responsible for the changes are still not clearly defined.

B. Pancreas and Glucose Homeostasis

The pancreas is a large gland composed of two main elements: exocrine tissue and endocrine tissue. Exocrine tissue, which makes up the bulk of pancreas, consists of acinar cells that secrete various enzymes important to the digestive process. Scattered throughout the exocrine tissue are small, isolated pockets of endocrine tissue known as the islets of Langerhans. These islets may be composed of several different cells, but the predominant constituents are the alpha and beta cells. Both types of cells contain small hormone producing organelles, known as granules. Granules in the beta cells produce insulin, while those in the alpha cells produce glucagon. Both hormones are excreted directly into the bloodstream. Surgical removal of the pancreas leads, within 24 hr, to high concentrations of glucose in the blood. This hyperglycemia is followed in a short time by all other symptoms of diabetes (224).

(i) Diabetes - Metabolic consequences

Diabetes mellitus, generally called simply diabetes, is a disease in which metabolism of carbohydrates is impaired and that of proteins and fats is enhanced. Ingested glucose or glucose metabolized from body's reserves cannot be assimilated into fat and muscle cells for use, and thus builds up to high levels in the blood (hyperglycemia) and is excreted in the urine (glycosuria). Both hyperglycemia and glycosuria persist even when the diabetic individual is fasting. Breakdown of tissue proteins is sharply accelerated in diabetics with a concomitant increase in the excretion of nitrogen in the

urine. Breakdown of lipids and fatty acids is also accelerated, leading to production of large quantities of the so-called ketone bodies. Excretion of glucose and ketone bodies causes loss of water and salt from the body, producing dehydration and severe thirst. In severe cases, build-up of ketone bodies can produce a condition known as ketoacidosis; the accumulation of acids in the blood pushes its pH below the critical level of 7.4, thereby producing coma and if the condition is not corrected by the administration of insulin, eventually to death (225).

It has been recognized for many years that one of the major metabolic abnormalities of diabetes is the accelerated breakdown of body protein to yield glucose. Enhanced gluconeogenesis is probably as important as impaired peripheral glucose utilization in the genesis of hyperglycemia characteristic of diabetes (179). Perfused livers from diabetic rats synthesize glucose from lactate at almost three times the normal rate (226). Human diabetics and alloxan diabetic rats also show enhancement of gluconeogenesis from lactate, pyruvate, alanine or glutamate in vivo (227,228). The various mechanisms involved in the enhanced rate of gluconeogenesis in diabetic liver are not well defined.

(ii) Role of insulin in control of carbohydrate metabolism

Banting and Best (229) discovered insulin and demonstrated that administration of insulin could correct diabetic abnormalities. It is of interest that insulin treatment of diabetic rats restored changes in blood glucose and hepatic glycogen as well as in various enzyme activities (177,221). Prior administration of either actinomycin, ethionine, or cycloheximide effectively blocked the insulin-induced rise in the activities of hepatic glucokinase, phosphofructokinase and pyruvate kinase suggesting that the action of insulin on diabetic livers involved de novo enzyme biosynthesis (177,221). In contrast to hepatic glycolytic enzymes, the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-

diphosphatase and glucose 6-phosphatase were markedly elevated in livers of alloxan and streptozotocin-induced diabetic rats (178,188,221-223). It is interesting that insulin treatment prevented the diabetic-induced rise in various gluconeogenic enzymes (178). Furthermore, insulin produces striking changes in plasma levels of most amino acids and alters the uptake of several amino acids by the tissues of the forearm or the splanchnic bed (230). The hormone produces a decrease in total plasma amino acids with declines in threonine, isoleucine, valine, leucine, tyrosine and phenylalanine levels being the most consistent. This decrease is probably due to increased amino acid uptake by muscle because of facilitated protein synthesis. Intraarterial injection of physiologic doses of insulin into the forearm in man produces significant inhibition of the release of threonine, glycine, α-aminobutyrate, isoleucine, leucine, tyrosine and phenylalanine, but no significant change in alanine, the amino acid released in greatest amount (230). The overall decline in amino acid output was 74%. The failure of insulin to inhibit alanine output from the muscle is probably due to the concurrent enhancement of pyruvate formation through glycolysis.

Infusion of glucose to elicit endogenous insulin secretion in post-absorptive man produces a marked decrease in the splanchnic uptake of alanine, glycine, phenylalanine, lactate and pyruvate and a reversal of splanchnic glucose balance from output to uptake (182). The changes occur in the absence of alterations in the peripheral levels of gluconeogenic substrates indicating a primary action of insulin on the liver. The changes in splanchnic metabolism produced in vivo are similar to those induced by insulin in isolated rat liver perfused with a physiologic mixture of substrates (182). They are, of course, consistent with the inhibition of gluconeogenesis and glycogenolysis consequent to a fall in cyclic AMP. Tissue metabolite analysis points to the reaction sequence between pyruvate and phosphoenolpyruvate as a major site

of insulin control of gluconeogenesis. Insulin has been found to inhibit protein breakdown in liver, but it is not known whether this is related to the decrease in cyclic AMP (179).

Thus, a major part of the hypoglycemic action of insulin is due to inhibition of amino acid gluconeogenesis. The action is exerted both on the liver to slow flux through the gluconeogenic pathway and at the periphery to inhibit amino acid release. The hepatic component is probably more important since insulin does not decrease the peripheral release of alanine, which is the major gluconeogenic amino acid.

(iii) Role of glucagon in control of carbohydrate metabolism

Although metabolic abnormalities in diabetes have largely been attributed to the lack of insulin, glucagon has also been recognized as an important factor in the metabolic consequences of diabetes (224). Recent evidence indicates that plasma glucagon levels are normal or high in subjects with diabetes and in animals made insulin deficient with alloxan or antiinsulin serum, despite the existence of marked hyperglycemia (231). The failure to suppress glucagon secretion in the diabetic state no doubt contributes to the gross imbalance between plasma glucagon and insulin and hence to the increased level of cyclic AMP in the liver (179).

The action of glucagon on hepatic carbohydrate metabolism is believed to be antagonistic to that of insulin (232). Administration of glucagon was shown to augment hepatic gluconeogenesis from noncarbohydrate precursors in man (233) and in perfused livers (234). Glucagon treatment produced a rise in phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase whereas the activities of hepatic phosphofructokinase and pyruvate kinase were decreased in the rat (188,232). A similar effect on these liver enzymes was found when glucagon was administered to humans (232). It is of interest that the concentration of glucose rises during glucagon perfusion and drops when the hormone is removed (234).

From physiologic point of view, the balance between the effects of glucagon and insulin may be the major governing influence on glucose output by the liver. As regards to the effect of insulin on gluconeogenesis per se, Menahan and Wieland (235) have shown in perfused rat liver preparation that the activation of endogenous gluconeogenesis by glucagon could be suppressed by insulin. Lewis et al. (236) have shown that the activation of gluconeogenesis from lactate by glucagon can be suppressed by insulin under essentially the same conditions in which insulin suppressed glycogenolysis. It has been proposed that glucagon-insulin balance may regulate hepatic glucose output and that the effects may be associated with the modulation of hepatic cyclic AMP content (236,237). This concept of glucose output control by the liver influenced by the balance of glucagon-insulin interactions is supported by studies on acute diabetes induced by injecting insulin antibodies (189). Injection of insulin antiserum was associated with stimulation of glycogenolysis and gluconeogenesis. Presumably, the activation of glucose output was due to neutralization of insulin thereby leaving unopposed the influences of glucagon and/or the basal discharge of the hepatic sympathetic system (237). Thus, interaction of glucagon and insulin may govern the hepatic glucose output and these effects may be associated with changes in hepatic cyclic AMP.

7. CYCLIC AMP

In 1957, while studying the hyperglycemic effects of epinephrine and glucagon, Rall et al. (238) isolated a small, heat-stable compound capable of stimulating glycogenolysis. This compound was identified as cyclic adenosine 3',5'-monophosphate (cyclic AMP) and was proposed to serve as intracellular mediator in the glycogenolytic effect of epinephrine and glucagon. Since then, it has become clear that this nucleotide functions as an intracellular second

messenger mediating many actions of a number of hormones in mammalian tissues (239).

A. Metabolism of Cyclic AMP

The cellular level of cyclic AMP is dependent upon the activities of the enzymes regulating its synthesis and degradation, as well as those factors which are responsible for its release into the extracellular fluid. Adenylate cyclase, the enzyme catalyzing the formation of cyclic AMP from ATP has been found in most animal cells to be localized in the plasma membrane or certain membranous fractions within cells such as the sarcoplasmic reticulum (240). Thus, adenylate cyclase generates cyclic AMP on the inner surface of cell membrane and subsequently brings about the appropriate physiological responses. One of the most striking features of adenylate cyclase in mammalian cells is its ability to respond to hormones. Hepatic adenylate cyclase can be stimulated by adrenal catecholamines (241,242) as well as glucagon (243,244). Glucagon is much more potent than any of the catecholamines, not only in the sense that smaller concentrations of glucagon are required to stimulate the enzyme, but also in that the maximal response in this case is much greater than the maximal response to catecholamines. It is also of interest that only certain effectors can modulate adenylate cyclase activity in a given tissue. Thus, Rodbell and co-workers (245) have proposed that the hormone-sensitive, membrane-localized adenylate cyclase has multiple components; there is a site on the external surface of the cell membrane which serves as a receptor or binding site for the hormone. Specificity in these binding sites appears to provide the means by which tissues discriminate among the various hormones. There is a catalytic site on the interior surface of the cell membrane which has access to ATP and can generate cyclic AMP. Furthermore, there appears to exist an intermediate coupling or transducer mechanism that translates hormone binding to cyclase activation. Finally, there are probably regulatory sites which may

modify the binding of hormone to receptor (239,245).

Other enzymes known to influence the level of cyclic AMP are the ones responsible for the metabolic breakdown of the cyclic nucleotide, phosphodiesterase (PDE). Cyclic nucleotide phosphodiesterases are the only enzymes known to directly terminate the activity of cyclic nucleotides. Despite the fact that they represent another potential site for hormonal or metabolic control of cell function, much less is known about these enzymes than about the cyclases which form the cyclic nucleotide. This is in part due to the fact that there are multiple cyclic nucleotide phosphodiesterases in each cell type, and they have been rather difficult to separate and purify (246). Several metabolites including ATP, pyrophosphate, other nucleoside triphosphates and citrate have been shown in vitro to act as inhibitors of cyclic AMP phosphodiesterase activity and it seems likely that these agents act by chelating Mg^{++} (239). Similarly, enzymic activity can be inhibited by methyl xanthines and other compounds such as adenosine, puromycin, diazoxide and papaverine (239). Furthermore, it is generally true that the effects of hormones which act by stimulating adenylate cyclase are potentiated in the presence of one of these compounds.

One of the central issues in the field of cyclic nucleotide research has been the elucidation of the mechanism(s) whereby these low molecular weight nucleotides achieve such a diversity of effects, ranging from the mediation of hormone action in vertebrates to the stimulation of enzyme synthesis in bacteria. In recent years, it has been postulated that cyclic AMP exerts its influence on many cell functions by activating a class of enzymes called cyclic AMP-dependent protein kinases. Indeed, Kuo and Greengard (247,248) postulated that all of the wide variety of effects elicited by cyclic nucleotides are mediated by stimulation of protein kinase, which in turn, using ATP as a substrate, phosphorylate endogenous substrates within target cells.

In the past few years, there has been increasing evidence in support of this hypothesis (249). The molecular events involved in the activation of protein kinase by cyclic AMP has been elucidated in several tissues. The holoprotein kinase consists of two subunits: a cyclic AMP-binding protein or regulatory subunit, and a catalytic subunit. In the absence of cyclic AMP, the enzyme exists as the holoenzyme and it is essentially devoid of any activity. When present, cyclic AMP binds to the regulatory subunit, and in so doing, causes the dissociation of catalytic component. The free catalytic subunit is considered to be the active species of the enzyme. It is well appreciated that the protein kinase system may be very complex; there appears to be multiple forms of cyclic AMP-dependent protein kinases where the catalytic subunits are similar in molecular weight, but the regulatory subunits seem to differ among various kinases (239). Furthermore, it has been shown that in addition to cyclic AMP, certain substrates of protein kinases such as histones or protamine may dissociate and activate the enzyme. However, the significance of this activation by substrates is unclear (239).

B. Cyclic AMP and Carbohydrate Metabolism

Considerable data have accumulated implicating a direct relationship between cyclic AMP and alterations in hepatic and renal carbohydrate metabolism. Some of the more prominent changes produced by cyclic AMP in liver include stimulation of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase (250-253), and an enhanced incorporation of noncarbohydrate precursors into glucose (254-257). An increase in the incorporation of gluconeogenic precursors into glucose also was found in kidneys of rats treated with cyclic AMP (258-262). Recently, Weiss et al. (263) demonstrated that cyclic AMP augmented the entry and cumulative uptake of several amino acids into rat kidney cortex slices. From studies with gluconeogenic precursors, Guder et al. (264) suggested that the rate-limiting step in cyclic AMP-stimulated renal gluco-

neogenesis involved an enhanced formation of phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. Since pretreatment with actinomycin D and cycloheximide prevented the cyclic AMP-induced rise in renal and hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, evidence suggested that de novo enzyme biosynthesis was involved in the observed action of this cyclic nucleotide (250-253,265). It is of interest that cyclic AMP also produced a fall in hepatic glycogen content (255,266,267) and an elevation in the concentration of blood glucose (268-270).

Since the classic reports of Sutherland and co-workers provide evidence for a role of cyclic AMP in the action of epinephrine on hepatic glycogenolysis, the role of cyclic AMP in the metabolic actions of several other hormones has been demonstrated in perfused liver preparations (179). In these experiments, catecholamine-stimulated hepatic gluconeogenesis and glycogenolysis was accompanied by elevation of intracellular levels of cyclic AMP. However, more recently, Sherline et al. (271) and Tolbert et al. (272) have pointed to the existence of an α-receptor mechanism for catecholamine activation of glycogenolysis and gluconeogenesis in the liver parenchymal cell that apparently does not involve the adenylate cyclase-cyclic AMP system. However, much controversy still exists regarding the nature of the hepatic adrenergic receptor. Thus, the role of cyclic AMP in the hormonal modulation of overall hepatic carbohydrate metabolism is still not fully understood (273).

In liver therefore, there seems to exist a dual system for hormonal and neural control of the hepatic release of glucose by catecholamines which probably has a survival value. The liver plays a vital role in glucose homeostasis and in the rapid release of a fuel that can be readily used by the muscle in a "fight or flight" situation. The presence of the two types of adrenergic receptors producing the same end result could be thought of as an example of "failsafe" principle commonly seen in the control systems of higher organisms (273).

III. MATERIALS AND METHODS

1. ANIMALS

Male rats of the Sprague-Dawley strain, used throughout the course of this investigation, were obtained from Canadian Breeding Farm and Laboratories Limited, St. Constant, Quebec. Animals were maintained on Master Laboratory Chow and water ad libitum. For experiments on the effects of neonatal exposure, pregnant females of the same strain were obtained and after parturition, each litter was reduced to a maximum of 6-8 pups. The litters were weaned at 22 days of age. All animals were maintained under a constant environment of 24°, 60% humidity and alternate cycles of 12 hr of light and darkness. Prior to sacrifice, unless mentioned otherwise, all animals were starved overnight (16 hr).

2. PREPARATION OF PANCREATIC TISSUE FOR DETERMINATION OF INSULIN RELEASE IN VITRO

A. Isolation of Rat Islets of Langerhans

Islets of Langerhans were isolated from pancreas by a modification of the collagenase digestion technique of Lacy and Kostianovsky (274). Non-starved rats were anesthetized with ether and the pancreas distended with 10 ml of Krebs-Ringer bicarbonate (KRB) buffer introduced through a polyethylene cannula inserted into the common bile duct. The pancreas was then removed, fat trimmed off and pieces of the tissue were chopped into 0.5 mm squares using a McIlwain Tissue Chopper (Brinkmann Instruments, Rexdale, Ontario). The chopped pancreatic tissue was then washed twice in chilled KRB buffer and fat tissue aspirated off. The volume of pancreatic tissue was then adjusted to 7 ml with KRB buffer and transferred to a siliconized glass vial containing 40 mg of collagenase. Digestion of acinar tissue was affected by immediate submersion of the vial under water at 37° with agitation provided by vigorous shaking for 7-8 min. The partially digested pancreatic tissue was then washed 4 times with 10 ml

volumes of KRB buffer, the islets being allowed to sediment on each occasion before aspiration of the supernatant. The remaining collagenase-free digest was suspended in 14 ml of KRB buffer. One ml aliquots were transferred to a chilled Petri dish and examined under a dissecting microscope. Individual islets were picked from the digestion mixture with an Eppendorf pipette and transferred into fresh, chilled KRB buffer. The pancreatic tissue from a single animal yielded about 150-200 islets which were used immediately.

B. Tissue Preparation for Electron Microscopy

The freshly isolated islets were subjected to double fixation: first the islets were incubated for 1 hr in 0.18 M sodium cocodylate buffer (pH 7.4), containing 1% glutraldehyde and 1% paraformaldehyde. The islets were then submerged in 0.18 M sodium cocodylate buffer (pH 7.4) containing 1% osmium for 1 hr. After fixation, the specimens were dehydrated in a series of graded ethanol concentrations. The islets were then carried through styrene and suspended in vestopal. The capsules containing islet suspension in vestopal were heated in an oven at 60° for 72 hr. Sections of about 400Å thickness were obtained using a OmU2-Reichter ultramicrotome and mounted on supported copper grids. Electron micrographs were then taken using a Siemens Elmiskop 101 electron microscope.

C. Determination of Insulin Release From Rat Islets In Vitro

Groups of 5 islets were preincubated for 30 min at 37° in 1 ml of 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 O_2 + CO_2 (95:5). At conclusion of the incubation, samples of the media were frozen for estimation of insulin content by radioimmunoassay.

D. Insulin Determination

Insulin was determined as immunoreactive insulin (IRI) by the method of Hales and Randle (275) using the commercial kit available from Amersham/ Searle Co. (Chicago). This assay was essentially an isotope dilution technique in which serum IRI and a known amount of ^{125}I-labeled insulin was allowed to react with anti-insulin serum to form an insulin-antibody complex. The complex was recovered by filtration and the amount of ^{125}I-labeled insulin in the complex was inversely proportional to the concentration of IRI in the serum sample. The amount of IRI in the sample was calculated by reference to a standard curve obtained with rat insulin (Novo Research Institute).

3. TISSUE PREPARATION FOR ASSAYING INSULIN, GLUCOSE, GLYCOGEN AND UREA

Animals were killed by decapitation and freely bled. Blood was allowed to stand at room temperature for 45 min. Following coagulation, the blood was spun at 1000 x g for 5 min and the serum aspirated out. Glucose and urea assays were carried out on freshly prepared sera whereas aliquots of serum samples were stored frozen for determination of insulin at a later date. A small portion of liver was rapidly removed, weighed and immersed in 1.0 ml of 30% boiling potassium hydroxide for the assay of glycogen.

A. Determination of Blood Glucose, Serum Insulin and Urea and Hepatic Glycogen

Blood glucose was determined by the glucose oxidase method using a Beckman Glucose Analyzer and expressed in terms of mg glucose per 100 ml of blood. The concentration of serum urea was measured according to the urease method of Bernt and Bergmeyer (276), and expressed as mg per 100 ml serum. For glycogen estimation, the anthrone method of Seifter et al. (277) was used and the concentration expressed as g per 100 g of liver.

4. PREPARATION OF SAMPLES FOR ASSAYING THE ACTIVITIES OF VARIOUS GLUCONEOGENIC ENZYMES

After decapitation, liver and kidney cortices were rapidly dissected out,

weighed and homogenized. The 5% homogenates were prepared in 0.15 M potassium chloride (pH 7.4), using a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a Teflon plastic pestle spinning at about 700 r.p.m. for exactly 90 sec. Aliquots of the homogenate were frozen for estimation of glucose 6-phosphatase and protein. The remainder of the homogenate was spun for 30 min at 100,000 x g at 0° in a refrigerated ultracentrifuge (International Preparative Model B-60). The supernatant fluids were then decanted and stored in chilled glass vials immersed in ice until the time of the assay.

A. Estimation of Various Gluconeogenic Enzymes

The activities of 3 gluconeogenic enzymes, namely pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase, were estimated in the supernatant, whereas glucose 6-phosphatase activity was determined in the whole homogenate. Preliminary experiments were conducted to establish linearity with regard to time and amount of the tissue in each case. Enzyme activities were assayed under strictly linear kinetic conditions at 37° and calculated as micromoles of substrate metabolized per mg protein per hr. Protein was determined according to the phenol (Folin) method of Lowry et al. (278).

(i) Pyruvate carboxylase

The activity of pyruvate carboxylase was determined by the method of Scrutton et al. (279). The assay was based upon oxidation of NADH in an assay system coupled with malic dehydrogenase. The reaction mixture (final volume, 1.0 ml) consisted of Tris buffer (pH 7.8), 100 mM; $MgCl_2$, 5 mM; ATP, 1 mM; Na pyruvate, 20 mM; $KHCO_3$, 15 mM; cysteine, 20 mM; acetyl coenzyme A, 0.1 mM; NADH, 112.5 mM; malic dehydrogenase, 50 µg of protein. The reaction was initiated by addition of an appropriate dilution of the supernatant fluid and changes recorded at 340 mµ in a Unicam spectrophotometer (Model SP 800)

with the cuvette chamber held at 37°. The changes in optical density were recorded for at least 5 min against a blank devoid of pyruvate.

(ii) Phosphoenolpyruvate carboxykinase

The activity of this enzyme was estimated by the method described by Phillips and Berry (190). The assay was based on the conversion of oxaloacetate to phosphoenolpyruvate which was subsequently allowed to react with hypoiodide to produce inorganic phosphate. The reaction mixture (final volume, 2.0 ml) contained Tris buffer (pH 7.5), 88 mM; $MgCl_2$, 40 mM; KF, 200 mM; inosine triphosphate, 45 mM. The blanks were devoid of the substrate, oxaloacetate. The reaction was initiated by adding supernatant fluid (equivalent to 5 mg wet weight tissue) to the assay medium at 37° and arrested with trichloroacetic acid (10%). The supernatant of the reaction medium was then reacted with hypoiodide and centrifuged. In the resulting supernatant, inorganic phosphate level was estimated by the colorimetric method of Fiske and Subbarow (280), using a Klett Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.)

(iii) Fructose 1,6-diphosphatase

The activity of fructose 1,6-diphosphatase was determined by estimating the release of inorganic phosphate from fructose 1,6-diphosphatase, as described by Weber and Cantero (281). The reaction mixture (final volume, 1.0 ml) contained the following: glycyl glycine buffer (pH 7.4), 10 mM; $MgSO_4$, 10 mM; sodium borate (pH 7.4), 30 mM; fructose 1,6-diphosphate, 50 mM. Blanks were devoid of fructose 1,6-diphosphate. The reaction was initiated by the addition of supernatant fluid (equivalent to 5 mg tissue wet weight) and terminated by trichloroacetic acid (10%). Inorganic phosphate was measured in the supernatant by the method of Fiske and Subbarow (280).

(iv) Glucose 6-phosphatase

The activity of glucose 6-phosphatase was assayed in the whole homogenate by the method of Cori and Cori (282). The assay was based on measuring the rate of glucose and inorganic phosphate formation from glucose 6-phosphate.

The reaction mixture (final volume 1.0 ml) contained potassium citrate buffer (pH 6.7), 33 mM and glucose 6-phosphate, 10 mM. Blanks contained no substrate, glucose 6-phosphate. The reaction was initiated by adding the homogenate (equivalent to 5 mg wet weight of tissue) and terminated by trichloroacetic acid (10%). The inorganic phosphate formed was determined by the method of Fiske and Subbarow (280).

5. DETERMINATION OF CYCLIC AMP LEVELS AND THE ACTIVITIES OF ADENYLATE CYCLASE, PHOSPHODIESTERASE AND PROTEIN KINASE

A. Preparation of Cyclic AMP-Dependent Protein Kinase

The procedure described by Kuo and Greengard (283) was used for the preparation of cyclic AMP-dependent protein kinase from bovine heart. All steps used in the preparation of the enzyme were carried out at 4°. A fresh bovine heart (about 1 kg) was obtained from a local slaughterhouse and cut into small pieces. The tissue was then homogenized with three volumes of neutral 4 mM ethylenediaminetetraacetic acid (EDTA) solution for 2 min using a Waring Blendor. The homogenate was centrifuged at 27,000 x g for 20 min and the pH of the supernatant was adjusted to 5.0 by acetic acid (1 M). The precipitate was then removed by centrifugation at 27,000 x g for 30 min and the pH of the clear supernatant readjusted to 6.5 with 1 M potassium phosphate buffer (pH 7.2). All buffers used in the succeeding steps of purification contained 2 mM EDTA.

Protein kinase activity was precipitated from the neutralized supernatant solution by the gradual addition of solid ammonium sulfate (32.5 g/100 ml). After stirring for 30 min, the precipitate was collected by centrifugation at 27,000 x g for 20 min and dissolved in 120 ml of 5 mM potassium phosphate buffer (pH 7.0). The resulting solution was dialyzed overnight against 20 volumes of the same buffer with two changes of buffer. After dialysis, the

solution was centrifuged at 27,000 x g for 30 min and the precipitate discarded.

The enzyme solution was applied to a column (10 x 40 cm) on DEAE-cellulose equilibrated with 5 mM potassium phosphate buffer (pH 7.0). After the enzyme application, the column was washed with two bed volumes of 0.05 M potassium phosphate buffer (pH 7.0); 0.3 M phosphate buffer (pH 7.0) was then applied to elute the enzyme. The active fractions were pooled and dialyzed overnight against 20 volumes of 5 mM potassium phosphate buffer (pH 7.0) with two changes of buffer. The dialyzed enzyme solution contained the required binding protein. After dialysis, Tris HCl buffer, pH 7.5, EDTA and bovine serum albumin (BSA) were added to give the following concentrations: Tris-HCl buffer pH 7.5, 50 mM; EDTA, 4 mM and bovine serum albumin, 0.1%. The concentration of binding protein was adjusted so that 100 µl of the solution would bind 55% of the activity (zero dose binding) when incubated with 0.9 pmoles of tritiated cyclic AMP in a final volume of 200 µl.

B. Determination of Endogenous Cyclic AMP Levels

(i) Tissue preparation

Approximately 100 mg of the tissue was quickly frozen in liquid nitrogen and homogenized in 3 ml of 5% trichloroacetic acid. Homogenization was effected with a chilled Potter-Elvehjem homogenizer, fitted with a plastic Teflon pestle, spinning at 700 r.p.m. for exactly 90 sec. The homogenate was then spun for 15 min at 1,000 x g and 0.2 ml of 1 N HCl added to the supernatant. Trichloroacetic acid was removed by extraction with anhydrous ether and the remaining supernatant was freeze-dried using a Virtis Lyophilizer (Gardiner, N.Y.). The freeze-dried residue was dissolved in 0.5 ml of distilled water and the concentration of cyclic AMP determined as follows:

(ii) Cyclic AMP determination

For the assay of cyclic AMP, the modified protein binding method of Gilman-

(284), as described by Tovey et al. (285), was used. Cyclic AMP from the sample was allowed to compete with a known amount of tritiated cyclic AMP for binding sites on fully activated cyclic AMP-dependent protein kinase. The amount of radioactivity bound in the labeled complex decreased as the amount of unlabeled compound was increased. The separation of protein bound cyclic AMP from unbound nucleotides was achieved by absorption of the free nucleotide on charcoal (Norit GS Extra, J.T. Baker Chemical Co., N.J.) followed by centrifugation at 10,000 x g for 1 min, as described by Brown et al. (286). Samples of supernatants were removed and radioactivity determined by liquid scintillation counting.

To each tube, components of the reaction mixture were added in the following sequence: 50 µl of tritiated cyclic AMP reagent (0.9 pmoles), 50 µl of unlabeled cyclic AMP (Calibration standard or unknown) and 100 µl of binding protein solution. After mixing, the tubes were incubated for 2 hr (±30 min) in an ice bath. 100 µl of charcoal suspension was added to each tube which was then vortex-mixed for 10 sec. After centrifugation (10,000 x g for 1 min at room temperature), 200 µl samples of supernatants (bound fraction) was removed and radioactivity determined by scintillation counting in PCS (Amersham/Searle), a liquid scintillation cocktail for aqueous samples. The ratio of counts per min (cpm), bound in the absence of unlabeled cyclic AMP to those bound in the presence of unlabeled cyclic AMP, yielded a straight line calibration curve when plotted against pmoles of standard cyclic AMP (0.5 to 16 pmoles cyclic AMP/incubation tube). The amount of cyclic AMP in the unknown sample was then read off this line using the appropriate ratio. The concentration of cyclic AMP was expressed as pmole cyclic AMP per mg tissue.

C. Measurement of Adenylate Cyclase Activity

(i) Tissue preparation

The tissue samples were homogenized in 50 volumes (weight to volume) of

buffer consisting of 2 mM Tris (hydroxymethyl) aminomethane-maleate buffer (pH 7.4) and 2 mM EGTA. Homogenization was effected on ice in a Potter-Elvehjem homogenizer fitted with a plastic Teflon pestle spinning at about 700 r.p.m. for exactly 90 sec. An appropriate aliquot of the homogenate was then used for the assay of adenylate cyclase.

(ii) Assay for adenylate cyclase activity

Adenylate cyclase activity was determined by the method of Kebabian et al. (287). The assay system (final volume 0.5 ml) contained Tris (hydroxymethyl) aminomethane-maleate, 80.2 mM; ATP, 1.5 mM; $MgSO_4$, 6.0 mM; theophylline, 10 mM; EGTA, 0.6 mM; plus homogenate (0.05 ml). In some cases, epinephrine (5×10^{-5} M), glucagon (1×10^{-5} M) or sodium fluoride (10 mM) was added to the incubation medium to measure the fluoride- and hormone-stimulated form(s) of adenylate cyclase. The reaction was initiated by addition of ATP. Incubation was for 2.5 or 10 min in a shaking water bath at 30°. The reaction was terminated by placing the tubes in a boiling water bath for 2 min, followed by centrifugation at 1000 x g for 5 min to remove insoluble material. The amount of cyclic AMP formed was measured in duplicate 50 µl aliquots by the protein binding method described above. Adenylate cyclase activity was calculated as pmoles of cyclic AMP formed per mg tissue or per protein, over the incubation period.

D. Determination of Phosphodiesterase Activity

(i) Tissue preparation

5% homogenates of kidney cortex and liver were prepared in a homogenizing buffer containing NaCl, 20 mM; $MgSO_4$, 1 mM; and glycyl-glycine buffer (pH 7.6), 2 mM. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a plastic Teflon pestle spinning at about 700 r.p.m. for exactly 90 sec. An appropriate aliquot of the homogenate was then used for the assay of phosphodiesterase activity.

(ii) Assay of phosphodiesterase activity

This enzyme was assayed according to the procedure of Weiss (288), based on the rate of conversion of cyclic 3',5'-AMP to 5'-AMP, which was reacted with alkaline phosphatase to yield inorganic phosphate. The assay medium contained the following in a total volume of 1.0 ml at the designated final concentration: Tris buffer (pH 8.0), 50 mM; cyclic AMP, 1 mM; $MgSO_4$, 3 mM; alkaline phosphatase, 0.1 mg of protein. The reaction was started by the addition of homogenate fluid corresponding to 5 mg wet weight of the tissue and stopped by the addition of 55% trichloroacetic acid. Inorganic phosphate was measured in the supernatant according to the method of Fiske and Subbarow (280) using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.). Phosphodiesterase activity was calculated as nmoles of cyclic AMP hydrolyzed per mg protein per min.

E. Measurement of Protein Kinase and Cyclic AMP Binding Activity

(i) Tissue preparation

Tissues were weighed and homogenized immediately in 2 vol of buffer containing 0.05 M Tris-HCl, pH 7.4, 0.25 M sucrose, 0.05 M KCl, 5 mM $MgCl_2$. The homogenate was centrifuged at 100,000 x g for 1 hr. The cytosol fraction was used for assaying protein kinase and cyclic AMP binding activity.

(ii) Protein kinase assay

Protein kinase was assayed by measuring the incorporation of ^{32}P into histone mixture (type IIA) by modification of the method of Sanborn et al. (290). The assay medium (final volume of 0.13 ml, pH 6.5) contained 46 mM sodium acetate, 1.5 mM sodium fluoride, 3.1 mM theophylline, 0.24 mg histone, 17 mM magnesium chloride, 85 µM ATP and 5 µM cyclic AMP (when measuring the activity of cyclic AMP-dependent enzyme). The reaction was initiated by adding (γ-^{32}P) ATP-$MgCl_2$ (2×10^{-5} cpm) followed by incubation at 30° for 7 min and terminated by the addition of 5 ml of 20% trichloroacetic acid. The

protein precipitate was separated by filtering on Millipore Filters (HAWP, 0.45 µm) and washed 3 times with the same volume of 5% trichloroacetic acid. The filter was then counted using a scintillation cocktail containing 7 parts Omnifluor solution (4 g Omnifluor per 1 scintanalyzed toluene) to 3 parts ethylene glycol monoethyl ether. Appropriate blanks without protein kinase (enzyme preparation) were run simultaneously along with the experimental samples. The protein kinase activity was expressed as pmoles ^{32}P incorporated per mg protein.

(iii) Cyclic AMP binding assay

The cyclic AMP binding studies were performed as described by Sanborn et al. (290). The assay mixture, consisting of 0.05 M sodium acetate and 1 mM EDTA (pH 5.0), 5 mM theophylline, 2.4 nM cyclic (^{3}H) AMP and 100 µg of sample, was incubated at 0° for 3 hr, passed through a Millipore filter (HAWP, 0.45 µM) which was then counted for radioactivity in scintillation "cocktail" described for protein kinase assay. The cyclic AMP binding activity was expressed as pmole bound/mg protein.

6. MEASUREMENT OF PROSTAGLANDIN F (PGF) LEVELS IN RAT TESTES

A. Tissue Preparation

Rats were stunned, decapitated and the testes rapidly excised and freed of all extraneous tissue. The tissues were then cooled to 0° and minced with scissors. A 10% (w/v) homogenate was prepared in 1 N formic acid (pH 3.0) using a chilled Kontes Duall glass-glass homogenizer with the pestle spinning at 700 r.p.m. for exactly 90 sec. A 0.5 ml aliquot of the homogenate was extracted twice with 5 volumes of ethyl acetate. The organic phase was evaporated to dryness under nitrogen before being dissolved in 0.5 ml of De Jalon's solution (296). Under these conditions, the recovery of added labelled prostaglandin (PG) $F_{2\alpha}$ was better than 95%. The identification of PGF in the

extracts (0.1 ml) was then carried out as follows.

B. Prostaglandin F Assay

For the assay of PGF, the radioimmunoassay technique described by Salmon and Amy (297) was used. The following components were added to each tube: 0.5 ml of 1:5000 dilution of rabbit $PGF_2\alpha$ antiserum (R#6, courtesy of Dr. Karim and Dr. Salmon), relative cross reactivity for $PGF_1\alpha$ = 12.2% and PGE_1 = 0.4%), 0.1 ml of ^3H-$PGF_2\alpha$ (approximately 2500 d.p.m., specific activity 7.5 Ci/mmol; New England Nuclear) and 0.2 ml of De Jalon's solution (pH 7.5). The reaction mixture was allowed to equilibrate for 1 hr at room temperature. The protein bound PGF was recovered by filtering on Millipore filter (HAWP, 0.45 μm) and washed three times with 10 ml of De Jalon's solution. The filter containing ^3H-labeled prostaglandin antibody complex was then submerged in a cocktail containing 7 parts Omnifluor solution (4 g Omnifluor/l scintanalyzed toluene) to 3 parts ethylene glycol monoethyl ether and subjected to liquid scintillation counting. A calibration graph was constructed using standard solutions containing 20 pg to 5.2 ng of $PGF_2\alpha$/ml. Since the anti-$PGF_2\alpha$ serum cross-reacted with $PGF_1\alpha$ (12%) and 15-keto $PGF_2\alpha$ (10%), values were expressed as PGF (rather than $PGF_2\alpha$) per mg tissue.

7. STATISTICAL ANALYSIS

All data were analyzed statistically using Student's t-test (2-tailed) and statistically significant differences between mean values are indicated where $p \leq 0.05$. The data from the individual *in vitro* experiments with isolated islets were first subjected to a 3-way analysis of variance: as inter-day variability was low, values for the different days were subsequently pooled and analyzed by Student's t-test.

IV. RESULTS

1. EFFECT OF CHRONIC CADMIUM ADMINISTRATION ON HEPATIC CARBOHYDRATE AND CYCLIC AMP METABOLISM

A. Changes in Tissue and Body Weights After Chronic Cadmium Treatment

Results presented in Table 1 demonstrate that daily administration of cadmium chloride (0.25 or 1.0 mg/kg) for 21 days, failed to significantly alter the body, liver, kidney, thymus, testes, ventral prostate and heart weights however, there was an increase in adrenal weight. A more prolonged treatment with cadmium chloride (1 mg/kg) for 45 days increased adrenal as well as thymus weights, but failed to affect total body, kidney or heart weight. In contrast, however, a significant decrease in hepatic, testicular and ventral prostatic weights was observed. It is of interest that a 45-day exposure to a dose of 0.25 mg/kg of cadmium chloride failed to produce any significant change in body and organ weights. Although during the first week of cadmium treatment the rats appeared to be lethargic, they were indistinguishable from untreated controls in the later part of the exposure period.

B. Effects on Liver Glycogen, Blood Glucose and Serum Urea

Data in Figure 3 show that daily injection of cadmium chloride (0.25 and 1 mg/kg) for 21 days significantly elevated the concentration of blood glucose and serum urea. In contrast, hepatic glycogen levels were reduced in rats given either amount of the heavy metal for 21 days. When the period of exposure to cadmium was extended to 45 days, both doses produced similar increases in blood glucose and urea and a decrease in liver glycogen content. In the present study, chronic heavy metal treatment also was found to increase the urinary excretion of glucose and protein above control values, as measured by the Dextrostix and Combistix tapes, respectively (Ames Co., Canada Ltd., Toronto, Ontario).

C. Influence of Chronic Cadmium on Liver Gluconeogenic Enzymes

Since cadmium administration caused hyperglycemia and elevated serum urea

TABLE 1

CHANGES IN TISSUE AND BODY WEIGHTS AFTER CHRONIC TREATMENT WITH CADMIUM

Each value represents the mean ± s.e.m. of 5-6 animals in the group. Animals were injected with cadmium chloride (0.25 or 1.0 mg/kg/day, i.p.) for 21 or 45 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Treatment	Dose (mg/kg per day)	Body Wt. (g)	Liver (g)	Kidney (g)	Adrenal (mg)	Thymus (mg)	Testes (g)	Ventral Prostate (mg)	Heart (g)
	0.0 (Control)	204±10.4 (100)	7.0±0.5 (100)	1.90±0.1 (100)	25±3.0 (100)	693±43 (100)	2.38±0.07 (100)	157±29.1 (100)	1.06±0.08 (100)
Cadmium (21 days)	0.25	192±9.6 (94)	7.4±0.3 (106)	1.92±0.1 (101)	32±2.5 (128)*	778±63 (112)	2.37±0.07 (100)	185.5±17.7 (118)	1.06±0.07 (100)
	1.0	211±3.6 (103)	7.9±0.3 (113)	1.97±0.12 (104)	40±3.0 (160)*	770±54 (111)	2.16±0.07 (91)	147.5±5.9 (94)	1.08±0.08 (102)
	0.0 (Control)	317±10.1 (100)	11.8±0.7 (100)	2.92±0.15 (100)	37±3.0 (100)	455±59 (100)	3.2±0.1 (100)	465±24 (100)	1.17±0.10 (100)
Cadmium (45 days)	0.25	305±6.3 (96)	10.4±0.6 (88)	2.60±0.03 (89)	37±2.7 (100)	535±17 (118)	2.8±0.2 (88)	382±23 (82)	1.13±0.10 (97)
	1.0	318±12 (100)	9.4±0.9 (80)*	3.12±0.03 (107)	53±4.0 (143)*	680±32 (149)*	2.1±0.2 (66)*	279±27 (60)*	1.18±0.09 (101)

*Significantly different from control values at $p < 0.05$

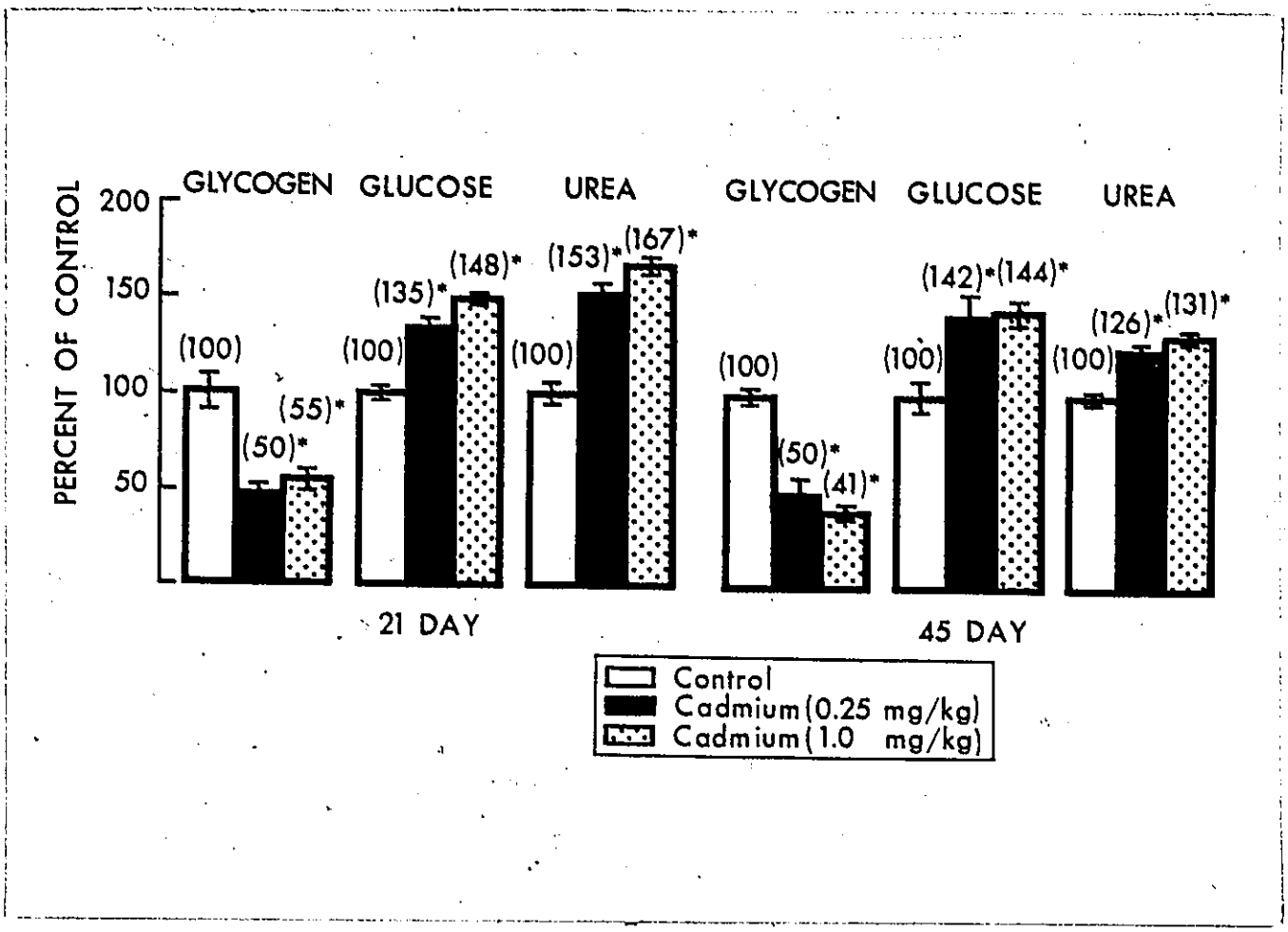

FIG. 3

Influence of chronically administered cadmium on liver glycogen, blood glucose, and serum urea. Each bar represents the mean ± s.e.m. of 5 animals in the group. Cadmium chloride (0.25 or 1.0 mg/kg) was administered i.p. to animals daily for 21 or 45 days. The control values for the 21-day experiment were 94±3 mg glucose per 100 ml blood, 15.0±0.6 mg urea per 100 ml serum, and 2.04±0.15 g glycogen per 100 g liver; and for the 45 day experiment, the control values were 81±5 mg glucose per 100 ml blood, 26±0.4 mg urea per 100 ml serum, and 2.20±0.06 g glycogen per 100 g liver. Data are expressed in percentages (in parentheses) with values from control rats as 100%.

*Significantly different from control values at $p < 0.05$.

levels, it was of interest to examine if cadmium also produced stimulation of the four key gluconeogenic enzymes. Results in Table 2 show that cadmium chloride given for 21 days at a dose of 0.25 mg/kg failed to alter markedly any of the four hepatic gluconeogenic enzymes studied. However, when treatment was extended to 45 days, a significant rise in PC to 125%, PEPCK to 153% and G6-Pase to 139% of the control values was noted. It may be noteworthy that the higher dose (1 mg/kg) of cadmium chloride enhanced the activities of all four gluconeogenic enzymes after 21 days, although the degree of stimulation was generally greater in rats exposed to this dose of the metal for 45 days.

D. Effects on Hepatic Cyclic AMP-Adenylate Cyclase System

To examine whether the cadmium-induced changes in hepatic carbohydrate metabolism are related to stimulation of the cyclic AMP-adenylate cyclase system, the influence of prolonged heavy metal treatment was investigated on cyclic AMP metabolism of this tissue. Although a 0.25 mg/kg dose of cadmium chloride given for 21 days did not alter fluoride-activated adenylate cyclase, it significantly enhanced the basal form of the enzyme by 46% (Table 3). When this dose was given for 45 days, both the basal and the fluoride-activated adenylate cyclase rose significantly above control values. Administration of 1.0 mg/kg dose of cadmium chloride for 21 days produced stimulation of hepatic adenylate cyclase activity, although greater enzymic increases were noted in rats given cadmium for 45 days. Table 3 also shows that cadmium treatment failed to alter the activity of hepatic phosphodiesterase. However, both doses of cadmium increased the endogenous levels of liver cyclic AMP regardless of whether the metal was given for 21 or 45 days.

E. Effect of Chronically Administered Cadmium on Hepatic Protein Kinase Activity

Cyclic AMP is believed to act within the cell through the activation of protein kinase(s) which phosphorylates other proteins having key roles in certain hormone-mediated responses. Since cadmium treatment enhanced both

TABLE 2

INFLUENCE OF CHRONICALLY ADMINISTERED CADMIUM ON HEPATIC GLUCONEOGENIC ENZYMES

Each value represents the mean ± s:e.m. of 5-6 animals in the group. Rats were administered cadmium chloride (0.25 or 1.0 mg/kg/day; i.p.) for 21 or 45 days. Enzyme activities are expressed as micromoles of substrate metabolized per milligram protein per hr. Data are also given in percentages (in parentheses) with values from control animals taken as 100%.

Treatment	Dose (mg/kg per day)	Enzymes			
		PC	PEPCK	FD-Pase	G6-Pase
	0.0 (Control)	219.8±2.6 (100)	15.0±0.8 (100)	4.3±0.1 (100)	2.7±0.2 (100)
Cadmium (21 days)	0.25	252.5±2.7 (115)	17.5±0.8 (117)	4.9±0.1 (114)	2.9±0.1 (107)
	1.0	280.6±5.9 (128)*	19.9±1.2 (133)*	5.6±0.2 (130)*	3.7±0.2 (137)*
	0.0 (Control)	226.0±21.0 (100)	15.0±0.0 (100)	4.7±0.2 (100)	2.8±0.3 (100)
Cadmium (45 days)	0.25	283.0±10.0 (125)*	23.0±3.0 (153)*	5.5±0.2 (117)	3.9±0.2 (139)*
	1.0	318.0±18.0 (141)*	24.0±1.0 (160)*	5.9±0.1 (126)*	4.7±0.2 (168)*

*Significantly different from control values at $p < 0.05$.

TABLE 3

EFFECTS OF CHRONIC CADMIUM ON HEPATIC ADENYLATE CYCLASE, PHOSPHODIESTERASE AND CYCLIC AMP

Each value represents the mean ± s.e.m. of 5 rats in the group. Animals were administered cadmium chloride (0.25 or 1.0 mg/kg/day; i.p.) for 21 or 45 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Treatment	Dose (mg/kg per day)	Adenylate Cyclase (pmol/mg protein/min)		PDE (nmol/mg protein/min)	Cyclic AMP (pmol/mg tissue)
		-F	+F		
	0.0	4.8±0.3 (100)	25.3±1.6 (100)	12.9±0.17 (100)	0.75±0.08 (100)
Cadmium (21 days)	0.25	7.0±0.8 (146)*	29.5±2.5 (117)	13.5±0.52 (105)	1.05±0.05 (140)*
	1.0	9.3±1.3 (194)*	49.3±3.3 (195)*	14.3±0.43 (111)	1.28±0.06 (171)*
	0.0	4.8±0.2 (100)	24.4±1.1 (100)	13.7±0.52 (100)	0.81±0.07 (100)
Cadmium (45 days)	0.25	7.2±0.2 (150)*	35.3±1.0 (145)*	13.2±0.57 (96)	1.18±0.06 (146)*
	1.0	11.0±0.5 (229)*	54.0±2.4 (221)*	13.9±0.53 (102)	1.48±0.07 (183)*

*Significantly different from control values at $p < 0.05$.

gluconeogenesis and cyclic AMP synthesis, it was of interest to examine if prolonged heavy metal treatment altered the activity of protein kinase as well. Data presented in Table 4 demonstrate that administration of cadmium (0.25 mg/kg) failed to significantly alter both the cyclic AMP-dependent and the independent form of protein kinase. In contrast, the 1.0 mg/kg dose of the metal, while slightly elevating the cyclic AMP-independent form, markedly reduced the cyclic AMP-dependent protein kinase to 76% of the control values.

F. Effects on Hepatic Protein Kinase Activity Ratio and Cyclic AMP Binding Activity

In the present study, although the low dose (0.25 mg/kg) cadmium treatment failed to alter the cyclic AMP binding capacity, the high dose (1 mg/kg) treatment significantly decreased the cyclic AMP-binding activity from 2.59±0.14 to 2.10±0.15 pmol cyclic AMP bound per mg of protein (Figure 4). Furthermore, although the kinase activity ratio, an indication of the relative activity of cyclic AMP-dependent and independent protein kinase, was not significantly altered by the low dose of cadmium, this ratio was lowered (35%) from 8.06±0.59 to 5.26±0.40 in livers of rats given the higher dose of the heavy metal.

2. CADMIUM-INDUCED METABOLIC ALTERATIONS FOLLOWING DISCONTINUATION OF THE HEAVY METAL TREATMENT

A. Effect of 14-Day Discontinuation of Cadmium Administration on Liver Glycogen, Blood Glucose and Serum Urea

Since cadmium has a long biological half-life, it was of interest to see if the metabolic consequences of cadmium toxicity were of a persistent nature. Data in Table 5 demonstrate that cadmium treatment (21 days) elevated blood glucose, serum urea, but lowered hepatic glycogen. Discontinuation of exposure to the heavy metal for 2 weeks in animals that had previously received cadmium for 21 days failed to restore the observed biochemical changes to levels noted in control animals. The metabolic alterations were not significantly different

TABLE 4

EFFECT OF CHRONIC CADMIUM ON HEPATIC PROTEIN KINASE ACTIVITY

Each value represents the mean ± s.e.m. of 4-5 animals in the group. Rats were administered cadmium chloride (0.25 or 1.0 mg/kg/day; i.p.) for 45 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Tissue	Cadmium Dose (mg/kg/day)	Kinase Activity (pmol ^{32}P incorporated/mg protein)	
		-cAMP	+cAMP
Liver	0.0 (Control)	43.77±3.91 (100)	352.95±13.95 (100)
	0.25	55.60±8.72 (127)	389.48±23.64 (110)
	1.00	51.01±5.44 (117)	267.07±15.81 (76)*

*Significantly different from control values at $p < 0.05$.

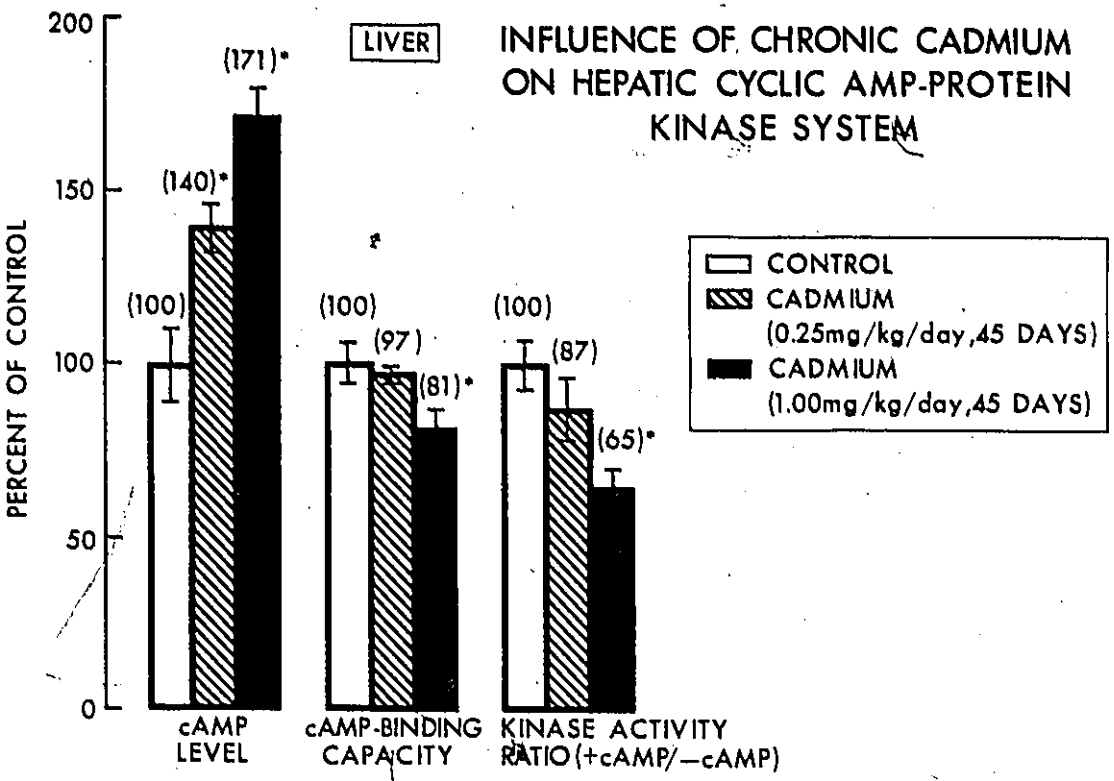

FIG. 4

Influence of chronically administered cadmium on hepatic cyclic AMP levels, cyclic AMP binding activity and protein kinase activity ratio. Each bar represents the mean ± s.e.m. of 4 rats in the group. Rats were administered cadmium chloride (0.25 or 1.0 mg/kg/day; i.p.) for 45 days. Data are expressed as percentages (in parentheses) with values of control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

TABLE 5

INFLUENCE OF 14 DAY DISCONTINUATION OF CADMIUM TREATMENT ON LIVER GLYCOGEN, BLOOD GLUCOSE AND SERUM UREA

Each value represents the mean ± s.e.m. of 5-6 animals in the group. Treated rats were administered cadmium chloride (1.0 mg/kg/day; i.p.) for 21 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 14 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Parameter Measured	Control	"Treated"	"Withdrawn"
Liver Glycogen (g/100 g)	2.04±0.3 (100)	1.10±0.1 (54)*	1.40±0.1 (69)*
Blood Glucose (mg/100 ml)	94±3.0 (100)	140±1.0 (149)*	149±9.0 (158)*
Serum Urea (mg/100 ml)	15.0±0.1 (100)	25.0±1.0 (167)*	27.6±2.1 (184)*

*Significantly different from control values taken at $p < 0.05$.

from those noted in animals treated with cadmium for 21 days.

B. Effect of 14-Day Withdrawal from Cadmium Treatment on Hepatic Gluconeogenic Enzymes

Administration of cadmium chloride (1 mg/kg) for 21 days enhanced the activities of all four gluconeogenic enzymes. Two weeks following the cessation of cadmium administration, the activities of PC, PEPCK and FD-Pase were still significantly elevated as compared to the control values; however, they were not significantly different from those noted in the "treated" group (Table 6). The activity of G6-Pase, on the other hand, was significantly greater in the "withdrawn" as compared to the "treated" group.

C. Effect of 14-Day Discontinuation on Hepatic Cyclic AMP

Since alterations of hepatic carbohydrate metabolism induced by the 3 week cadmium treatment were quite persistent, it was of interest to examine if cadmium-induced alterations of cyclic AMP metabolism also persisted after withdrawal for 14 days. Data presented in Table 7 demonstrate that cadmium-induced increases in the basal activity of adenylate cyclase as well as cyclic AMP levels remained significantly elevated as compared to control values; however, they were significantly lower than those seen for the "treated" group. The activity of fluoride-stimulated form of adenylate cyclase returned to a value which was not significantly different from that noted in control animals.

D. Influence of 28-Day Discontinuation on Metabolic Consequences of 45-Day Cadmium Treatment

Results presented in Figure 5 further illustrate the persistent nature of cadmium-induced metabolic alterations. Even 4 weeks following the cessation of cadmium administration, the metabolic changes induced by 45 day heavy metal treatment still persisted. In point of fact, the reduction in liver glycogen and the rise in blood urea, hepatic PEPCK, FD-Pase and G6-Pase appeared to be somewhat greater in the "withdrawn" group when compared with the "treated" animals. The remaining parameters either remained unchanged or were lower

TABLE 6

INFLUENCE OF 14 DAY DISCONTINUATION OF CADMIUM TREATMENT ON HEPATIC GLUCONEOGENIC ENZYMES

Each value represents the mean ± s.e.m. of 5-6 animals in the group. Rats were administered cadmium chloride (1.0 mg/kg/day; i.p.) for 21 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 14 days. Control rats received an equal volume of physiological saline. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Enzyme	Enzyme Activity (μmol/mg protein/hr)		
	Control	"Treated"	"Withdrawn"
PC	219.8±2.6 (100)	280.6±5.9 (128)*	301.1±4.8 (137)*
PEPCK	15.0±0.78 (100)	19.9±1.2 (133)*	21.1±1.6 (141)*
FD-Pase	4.3±0.1 (100)	5.6±0.2 (130)*	5.5±0.2 (128)*
G6-Pase	2.7±0.2 (100)	3.7±0.2 (137)*	4.4±0.3 (163)*†

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of "treated" group at $p < 0.05$.

TABLE 7

INFLUENCE OF 14 DAY DISCONTINUATION OF CADMIUM TREATMENT ON HEPATIC CYCLIC AMP METABOLISM

Each value represents the mean ± s.e.m. of 5-6 animals in the group. "Treated" rats received cadmium chloride (1.0 mg/kg/day, i.p.) for 21 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 14 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Parameter Measured		Control	"Treated"	"Withdrawn"
Adenylate Cyclase (pmol/mg protein/min)	(-F)	4.8±0.31 (100)	9.3±1.30 (194)*	6.7±0.81 (140)*†
	(+F)	25.3±1.62 (100)	49.3±3.33 (195)*	29.1±1.91 (115)†
PDE (nmol/mg protein/min)		12.9±0.17 (100)	14.3±0.43 (111)	13.0±0.35 (101)
Cyclic AMP (pmol/mg tissue)		0.75±0.08 (100)	1.28±0.06 (171)*	1.07±0.05 (143)*†

*Significantly different from control values at $p < 0.05$.

† Significantly different from values of "treated" group at $p < 0.05$.

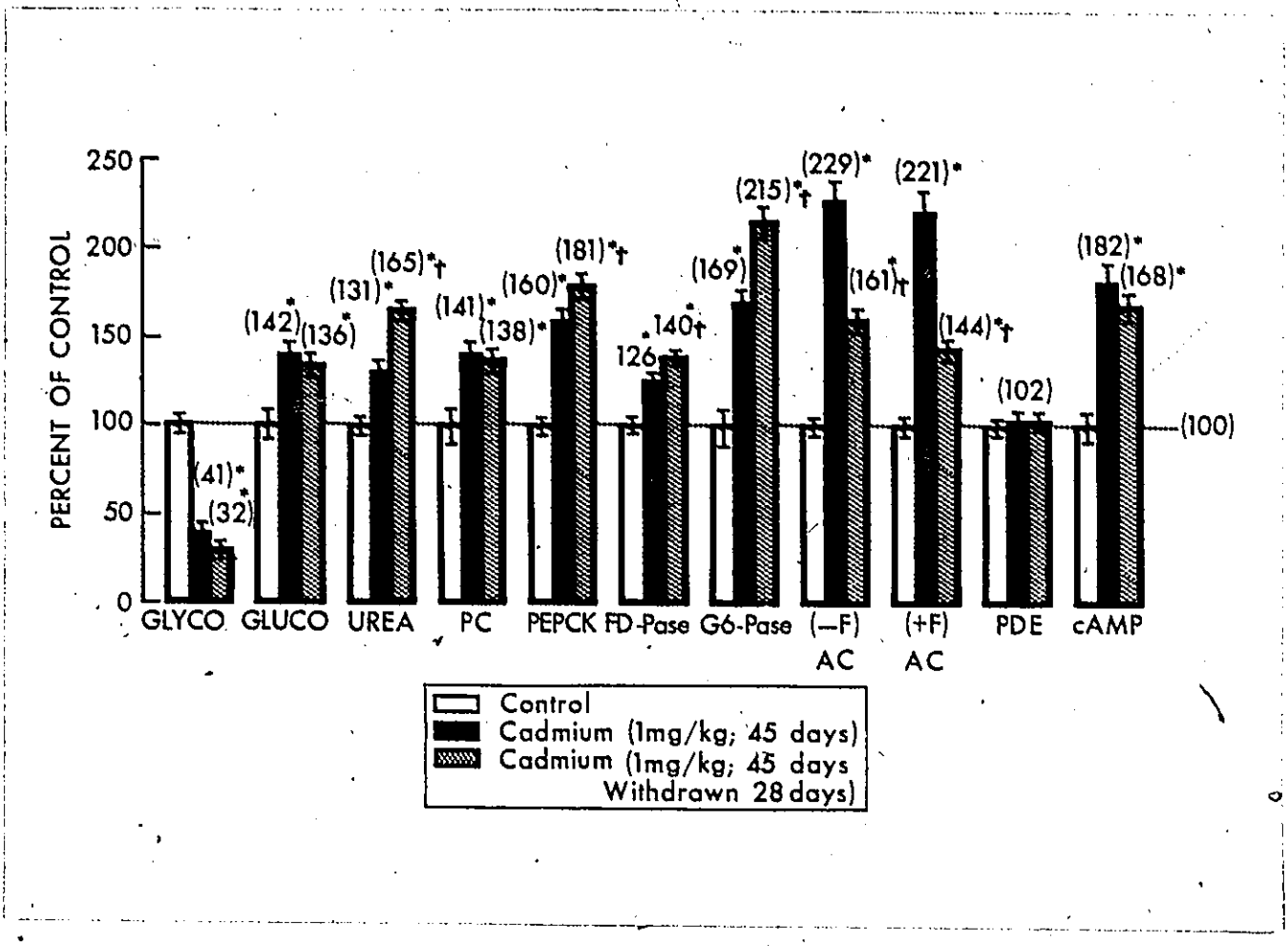

FIG. 5

Effect of 28 day discontinuation of cadmium administration on blood glucose, serum urea, hepatic gluconeogenic enzymes and cyclic AMP metabolism. Each bar represents the mean ± s.e.m. of 5-6 animals in the group. Rats in the "treated" group received cadmium chloride (1.0 mg/kg/day; i.p.) for 45 days. Half of these rats were then maintained for 28 days without additional treatment and constituted the "withdrawn" group. Data are expressed as percentages with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of "treated" group at $p < 0.05$.

than the values in the "treated" group. It is of interest that the glycosuria and proteinuria seen in cadmium-treated rats also persisted even when cadmium administration was discontinued for as long as 4 weeks.

3. EFFECTS OF CHRONIC CADMIUM TREATMENT ON RENAL CORTICAL GLUCONEOGENESIS AND CYCLIC AMP METABOLISM

A. Effect of 45-Day Cadmium Treatment and a 28-Day Treatment-Free State on Renal Gluconeogenic Enzymes

Evidence indicates that there is a close relationship between altered kidney and liver function and changes in the gluconeogenic capacity of these two tissues (217). We were therefore prompted to examine the influence of chronic cadmium treatment on renal gluconeogenic enzymes. Results presented in Table 8 demonstrate that administration of cadmium chloride (1 mg/kg) for 45 days caused a marked stimulation of the four key gluconeogenic enzymes. Furthermore, cessation of cadmium administration, like in the case of hepatic enzymes, failed to reverse the effects of chronic cadmium treatment. The activity of PC in the "withdrawn" group was significantly greater than that in the "treated" animals. However, the activities of PEPCK, FD-Pase and G6-Pase in the "withdrawn" group failed to differ significantly from those in the "treated" group.

B. Effect of Cadmium on Kidney Cortex Cyclic AMP Metabolism

In contrast to the hepatic tissue, cadmium caused a marked decrease in the concentration of renal cortical cyclic AMP. Treatment with 0.25 mg/kg dose of cadmium for 45 days lowered the cyclic AMP level from 1.46±0.14 to 0.97±0.04 pmoles per mg tissue (Figure 6). The higher dose of the heavy metal (1.0 mg/kg) further decreased the endogenous cyclic AMP concentration to 0.48±0.04 pmoles per mg tissue. Although the low dose did not alter the cyclic AMP binding activity of kidney cortex, the higher dose of cadmium significantly enhanced (13%) the cyclic AMP binding capacity from 2.45±0.05 to 2.76±0.08 pmoles

TABLE 8

EFFECT OF 28 DAY DISCONTINUATION OF CADMIUM TREATMENT AFTER 45 DAYS OF CADMIUM ADMINISTRATION ON RENAL GLUCONEOGENIC ENZYMES

Each value represents the mean ± s.e.m. of 4-5 animals in the group. Rats were administered cadmium chloride (1.0 mg/kg/day; i.p.) for 45 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 28 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Enzyme	Enzyme Activity (μmol/mg protein/hr)		
	Control	"Treated"	"Withdrawn"
PC	74±3 (100)	160±7 (216)*	196±9 (265)*†
PEPCK	25±2 (100)	34±1 (136)*	38±1 (152)*
FD-Pase	7.7±0.5 (100)	11.9±0.3 (155)*	9.9±0.1 (129)*
G6-Pase	10±1 (100)	13±0 (130)*	14±1 (140)*

*Significantly different from control values at $p<0.05$.

†Significantly different from values of "treated" group at $p<0.05$.

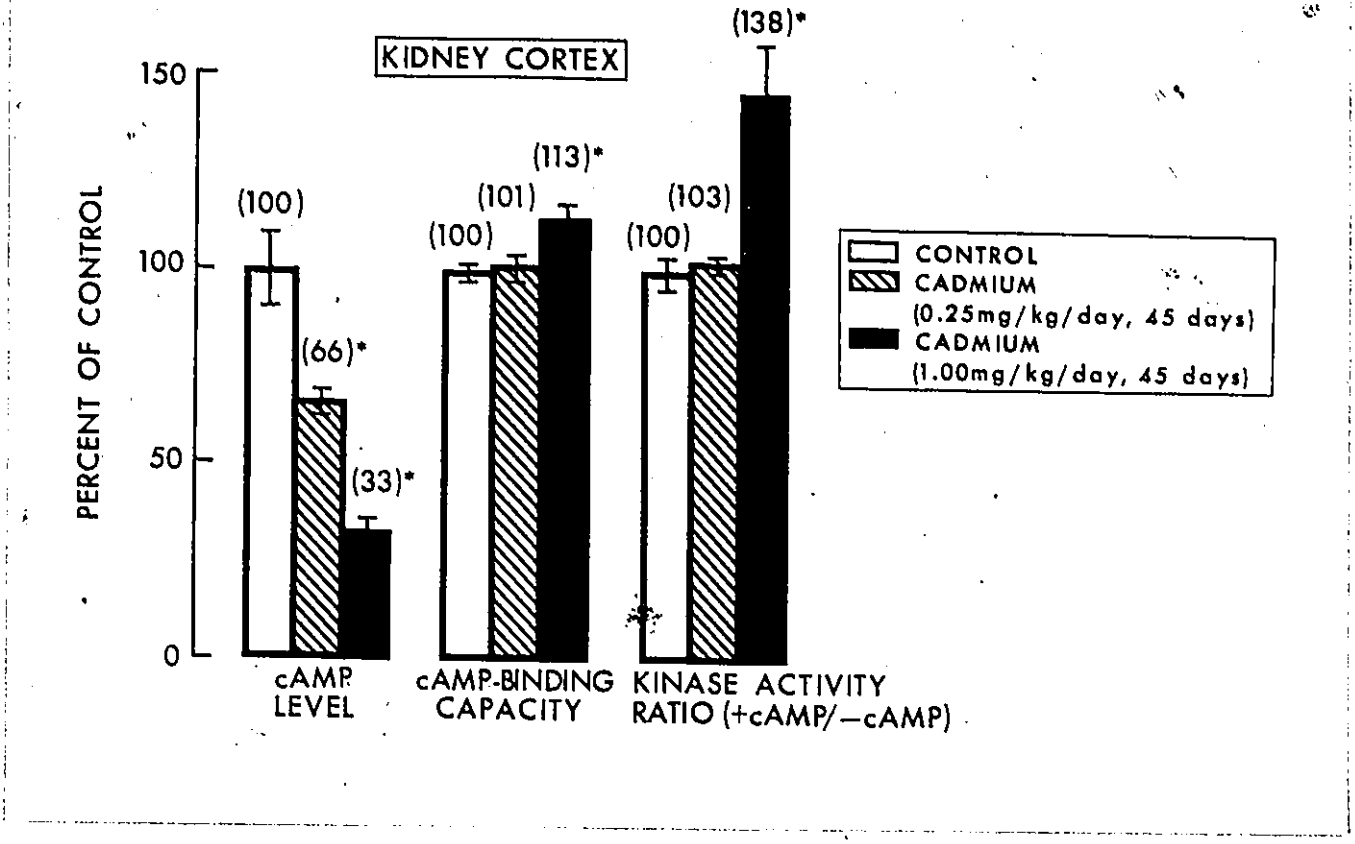

FIG. 6

Influence of chronically administered cadmium on renal cortical cyclic AMP levels, cyclic AMP binding activity and protein kinase ratio: Each bar represents the mean ± s.e.m. of 4 rats in the group. Rats were administered cadmium chloride (0.25 or 1.0 mg/kg/day, i.p.) for 45 days. Data are given in percentages with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

cyclic AMP bound per mg protein.

C. Effect of Chronic Cadmium on Renal Protein Kinase Activity

Whereas exposure to the 0.25 mg/kg dose of cadmium reduced the activities of cyclic AMP-independent and dependent forms of protein kinase by 15% and 13% of the control values respectively, treatment with the larger dose produced greater decreases in both the cyclic AMP-dependent and independent forms of renal cortical protein kinase (Table 9). It is of interest that the ratio of the two kinases was significantly enhanced only in the case of rats given 1 mg/kg dose of cadmium (Figure 6).

4. INFLUENCE OF CHRONIC CADMIUM TREATMENT ON TESTICULAR AND PROSTATIC METABOLISM

A. Influence of Chronic Cadmium Administration on Testicular and Prostatic Weights

Acute administration of cadmium has been found to damage rat testes, resulting in permanent sterility (140). Chronic treatment with cadmium also has been reported to reduce fertility of male rats (154). Data presented in Table 10 demonstrate that administration of 1 mg/kg dose of cadmium chloride for 45 days significantly impaired testicular and prostatic growth. After 45 days of heavy metal treatment, the testes weighed only 60% of the control values. Similarly, the mean prostatic weight of the cadmium-treated animals was 60% of the values seen for control rats. Following cessation of cadmium administration for 28 days in rats that had previously been exposed to this metal for 45 days, the testicular and prostatic weights showed the same relative degree of atrophy as seen in the 45-day "treated" group.

B. Influence of Cadmium on Cyclic AMP Metabolism and Protein Kinase Activity of Rat Testes

Since the biochemical basis of cadmium-induced testicular injury is still unknown, it was of interest to examine the influence of chronic cadmium treatment on testicular adenylate cyclase-cyclic AMP-protein kinase system. The results

TABLE 9

EFFECT OF CHRONIC CADMIUM ON RENAL PROTEIN KINASE ACTIVITY

Each value represents the mean ± s.e.m. of 4-5 animals in the group. Rats were administered cadmium chloride (0.25 or 1.0 mg/kg/day; i.p.) for 45 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Tissue	Cadmium Dose (mg/kg/day)	Kinase Activity (pmol ^{32}P incorporated/mg protein)	
		-cAMP	+cAMP
Kidney Cortex	0.0 (Control)	219.04±8.74 (100)	446.20±13.96 (100)
	0.25	186.18±10.65 (85)*	389.95±21.73 (87)*
	1.0	109.62±17.60 (50)*	308.28±28.26 (69)*

*Significantly different from control values at $p < 0.05$.

TABLE 10

EFFECT OF CHRONIC CADMIUM ADMINISTRATION ON TESTICULAR AND PROSTATIC WEIGHTS

Each value represents the mean ± s.e.m. of 6 rats in the group. Rats were administered cadmium chloride (1.0 mg/kg/day; i.p.) for 45 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 28 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

	Testes Wt. (g)	Prostate Wt. (mg)
Control	3.2±0.1 (100)	465±24 (100)
"Treated"	2.1±0.2 (66)*	279±27 (60)*
"Withdrawn"	2.0±0.1 (63)*	297±35 (64)*

*Significantly different from control values at $p < 0.05$.

in Figure 7 illustrate that daily exposure to cadmium for 45 days significantly increased the activity of testicular adenylate cyclase from 1.5±0.3 to 2.4±0.1 pmoles cyclic AMP formed/min/mg of tissue. Likewise, phosphodiesterase activity was enhanced from a control value of 0.29±0.03 to 0.40±0.01 μmoles cyclic AMP metabolized/hr/mg wet weight of testicular tissue. Following cadmium treatment, the endogenous levels of cyclic AMP remained essentially unchanged from a control value of 1.04 pmoles per mg of tissue. This relative lack of change in the intracellular concentration of cyclic AMP was also reflected in cyclic AMP binding studies. Testicular protein kinase from 45-day cadmium-injected rats showed no significant change in the affinity for cyclic AMP from a control value of 0.81±0.03 pmoles cyclic AMP bound/mg protein. While the activity of cyclic AMP-independent form of protein kinase was reduced slightly by the heavy metal from a control value of 3.37±0.10 to 2.69±0.26 nmoles ^{32}P incorporated/7 min/mg of protein, the cyclic AMP-dependent form of testicular protein kinase decreased significantly from a control value of 7.11±0.84 to 4.12±0.47 nmoles ^{32}P incorporated/7 min/mg of protein. Figure 7 also shows that even though testicular adenylate cyclase returned to control values in the "withdrawn" group, subsequent cyclic AMP metabolism was not fully restored following the cessation of cadmium treatment for 28 days.

C. Influence of Cadmium on Prostatic Cyclic AMP Metabolism

As the accessory reproductive tissues are dependent on testicular biosynthesis of androgens for maintenance of their glandular activity, the influence of this heavy metal also was investigated on cyclic AMP metabolism of the prostate gland. Figure 8 shows the influence of chronic exposure to cadmium on cyclic AMP metabolism in the rat prostate gland. After 45 days of daily cadmium treatment, adenylate cyclase activity was reduced by 45% ($p < 0.05$) from a control value of 1.35±0.15 to 0.74±0.14 pmoles of cyclic AMP formed per min per mg of tissue. In contrast, the activity of prostatic

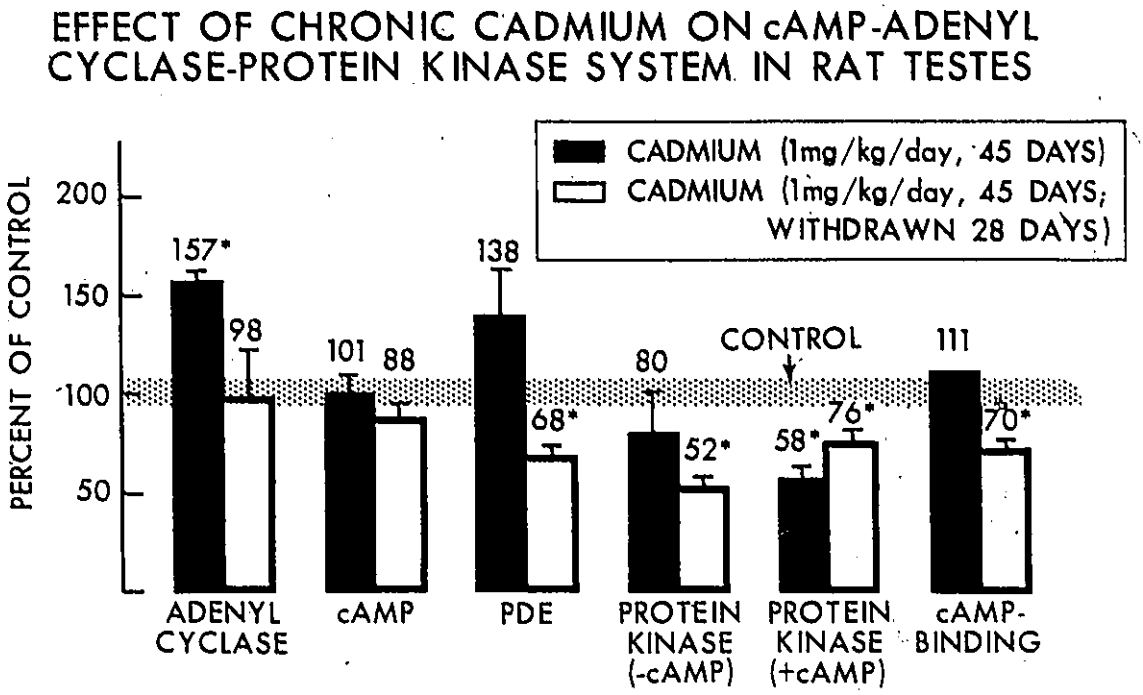

FIG. 7

The influence of cadmium on cyclic AMP metabolism and protein kinase activity of rat testes. Each bar represents the mean ± s.e.m. of 6 rats in the group. Rats were administered cadmium chloride (1.0 mg/kg/day; i.p.) for 45 days. Half of these rats, the "withdrawn" group, were maintained without additional treatment for 28 days. Data are given in percentages with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

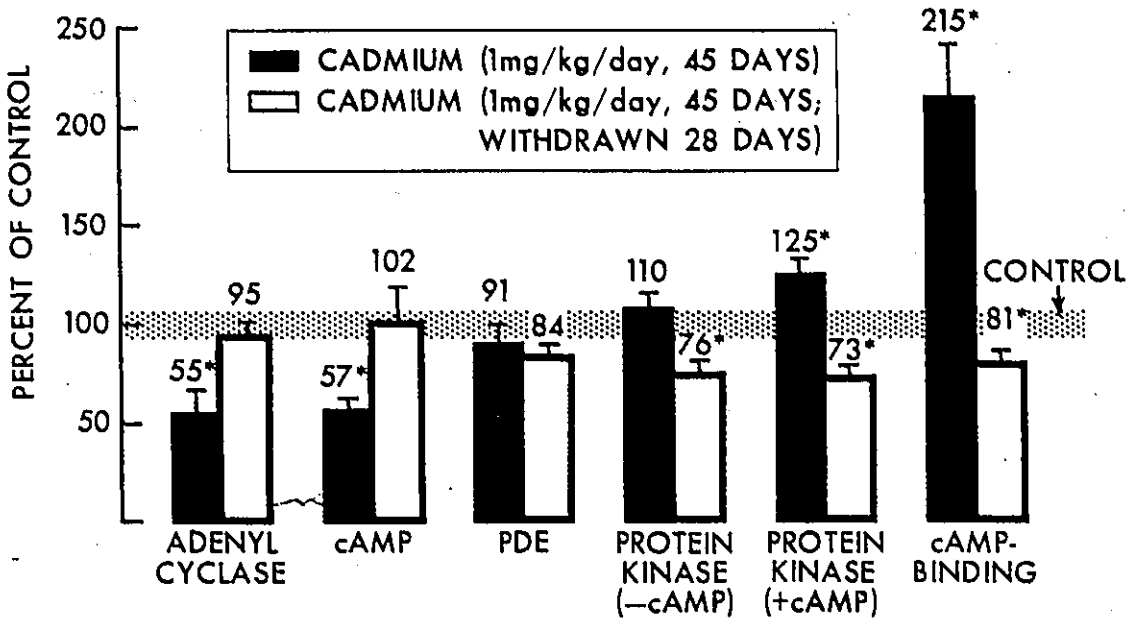

FIG. 8

Changes in prostatic cyclic AMP metabolism and protein kinase activity after chronic exposure to cadmium. Each bar represents the mean ± s.e.m. of 6 rats in the group. Data are given in percentages with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

phosphodiesterase was not significantly altered when compared with controls. The cyclic AMP levels were significantly decreased from a control value of 1.20±0.17 to 0.69±0.06 pmol per mg of tissue. The reduction in endogenous cyclic AMP concentration was reflected in a marked increase in the cyclic AMP binding capacity of prostatic protein kinase which rose from a control value of 0.31±0.01 to 0.66±0.08 pmoles cyclic AMP bound per mg of protein. While the cyclic AMP-independent protein kinase showed only a slight rise, the cyclic AMP-dependent form of the enzyme was significantly increased from 0.99±0.08 to 1.24±0.08 nmoles of ^{32}P incorporated/7 min/mg protein. After the 28-day withdrawal period in rats previously given cadmium for 45 days, cyclic AMP levels and adenylate cyclase activity returned to control values. However, the cyclic AMP binding by protein kinase as well as the activity of the cyclic AMP-dependent and the independent forms of prostatic protein kinase showed a significant drop compared to the "treated" values and were, in fact, even lower than those seen in the control group.

5. METABOLIC EFFECTS OF AN ACUTE DOSE OF CADMIUM

A. Effects of Acute Cadmium Treatment on Blood Glucose, Serum Urea, Hepatic Glycogen and Gluconeogenic Enzymes

To examine whether stimulation of hepatic gluconeogenesis and cyclic AMP synthesis could also be produced by administration of a single large dose of cadmium, rats were injected intraperitoneally with 60 mg of cadmium chloride per kg and killed 1 hr later. Whereas acute treatment with cadmium decreased hepatic glycogen levels, it produced a significant rise in the concentration of blood glucose and serum urea (Figure 9). However, acute cadmium treatment failed to significantly alter the activity of any of the four key gluconeogenic enzymes examined.

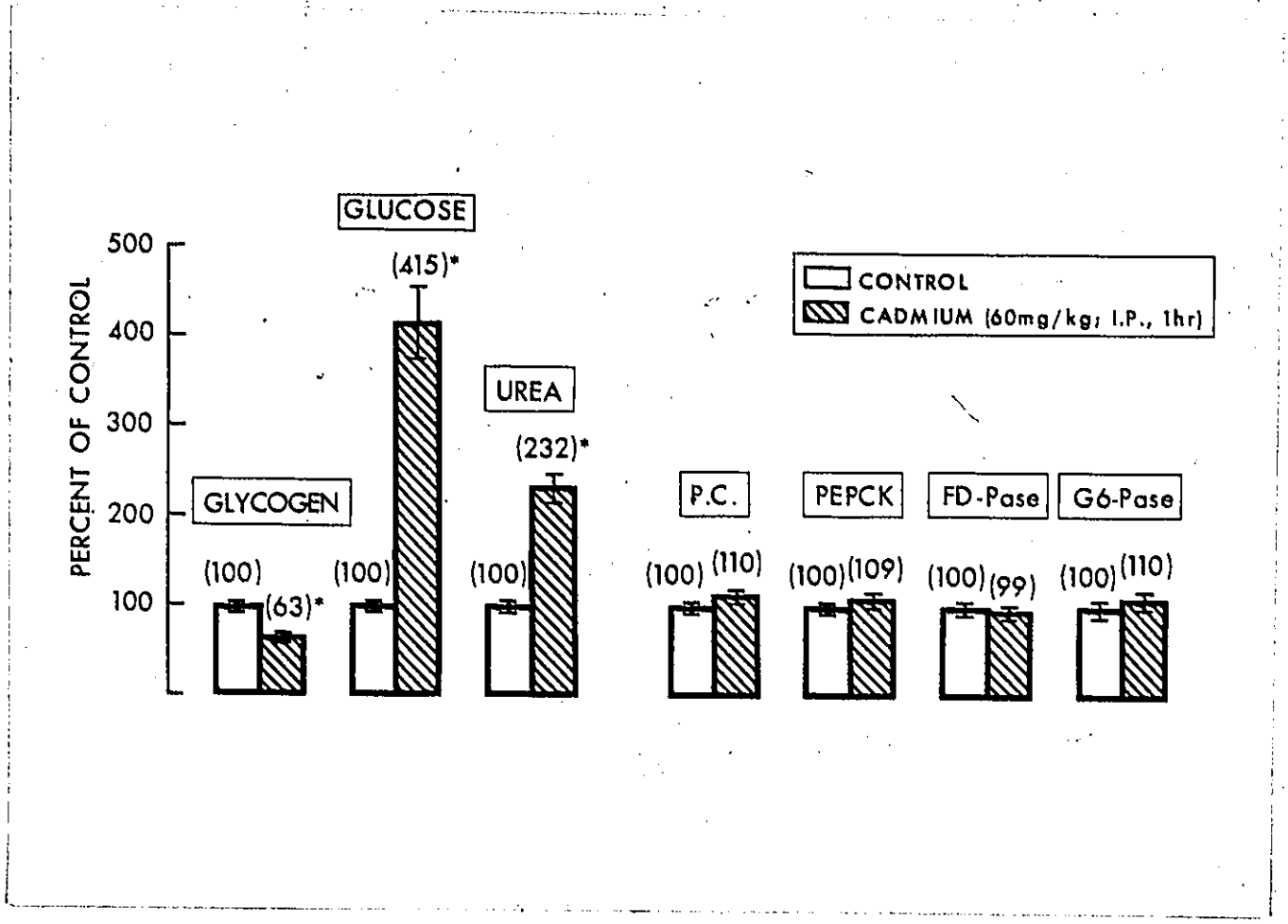

FIG. 9

Effect of acute cadmium treatment on blood glucose, serum urea, hepatic glycogen and gluconeogenic enzymes. Each bar represents the mean ± s.e.m. of 4-5 animals in the group. Rats were administered cadmium chloride (60 mg/kg; i.p.) and killed 1 hr later. Data are expressed in percentages with control values taken as 100%.

*Significantly different from control values at $p < 0.05$.

B. Effect of Acute Cadmium Treatment on Hepatic Cyclic AMP Metabolism

Although the four key gluconeogenic enzymes remained unchanged in animals acutely exposed to cadmium, this treatment did increase both the basal and the fluoride-activated form(s) of adenylate cyclase. Additionally, administration of an acute dose of this metal elevated cyclic AMP levels, but decreased the activity of hepatic phosphodiesterase (Table 11).

6. PROTECTIVE EFFECT OF SELENIUM ON CERTAIN HEPATOTOXIC MANIFESTATIONS OF SUBACUTE CADMIUM ADMINISTRATION

Since selenium has been reported to prevent testicular necrosis caused by cadmium treatment (147), it was of interest to examine if selenium could also afford protection against cadmium-induced elevation of hepatic gluconeogenic enzymes. A 7-day multiple dosing schedule of cadmium administration was selected because it was found to induce certain changes in carbohydrate metabolism resembling those produced by chronic cadmium exposure.

A. Effect of Subacute Administration of Cadmium on Hepatic Gluconeogenic Enzymes: Prevention by Selenium

Subacute effects of cadmium, selenium and selenium plus cadmium on various key hepatic gluconeogenic enzymes are presented in Figure 10. Administration of cadmium (1 mg of $CdCl_2$ per kg) twice a day for 7 days significantly elevated the activities of PC to 135%, PEPCK to 142%, FD-Pase to 142% and G6-Pase to 138% of the control values. In contrast, exposure to selenium (1 mg of SeO_2 per kg) twice a day for the same period of time failed to produce any significant change in hepatic enzyme activities examined. However, when the same dose of selenium and cadmium were administered simultaneously, the activities of all four gluconeogenic enzymes were significantly lower than those noted for rats given cadmium alone, and were in the same range as control values.

TABLE 11

EFFECT OF ACUTE CADMIUM TREATMENT ON HEPATIC CYCLIC AMP METABOLISM

Each value represents the mean ± s.e.m. of 4-5 animals in the group. Rats were administered cadmium chloride (60 mg/kg; i.p.) and killed 1 hr later. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

Parameter Measured		Control	Cadmium-Treated
Adenylate Cyclase (pmol/mg protein/min)	(-F)	4.7±0.3 (100)	7.5±0.4 (160)*
	(+F)	22.4±1.1 (100)	31.5±1.2 (141)*
PDE (nmol/mg protein/min)		13.3±0.18 (100)	9.5±0.13 (71)*
Cyclic AMP (pmol/mg)		0.82±0.08 (100)	1.69±0.11 (206)*

*Significantly different from control values at $p < 0.05$.

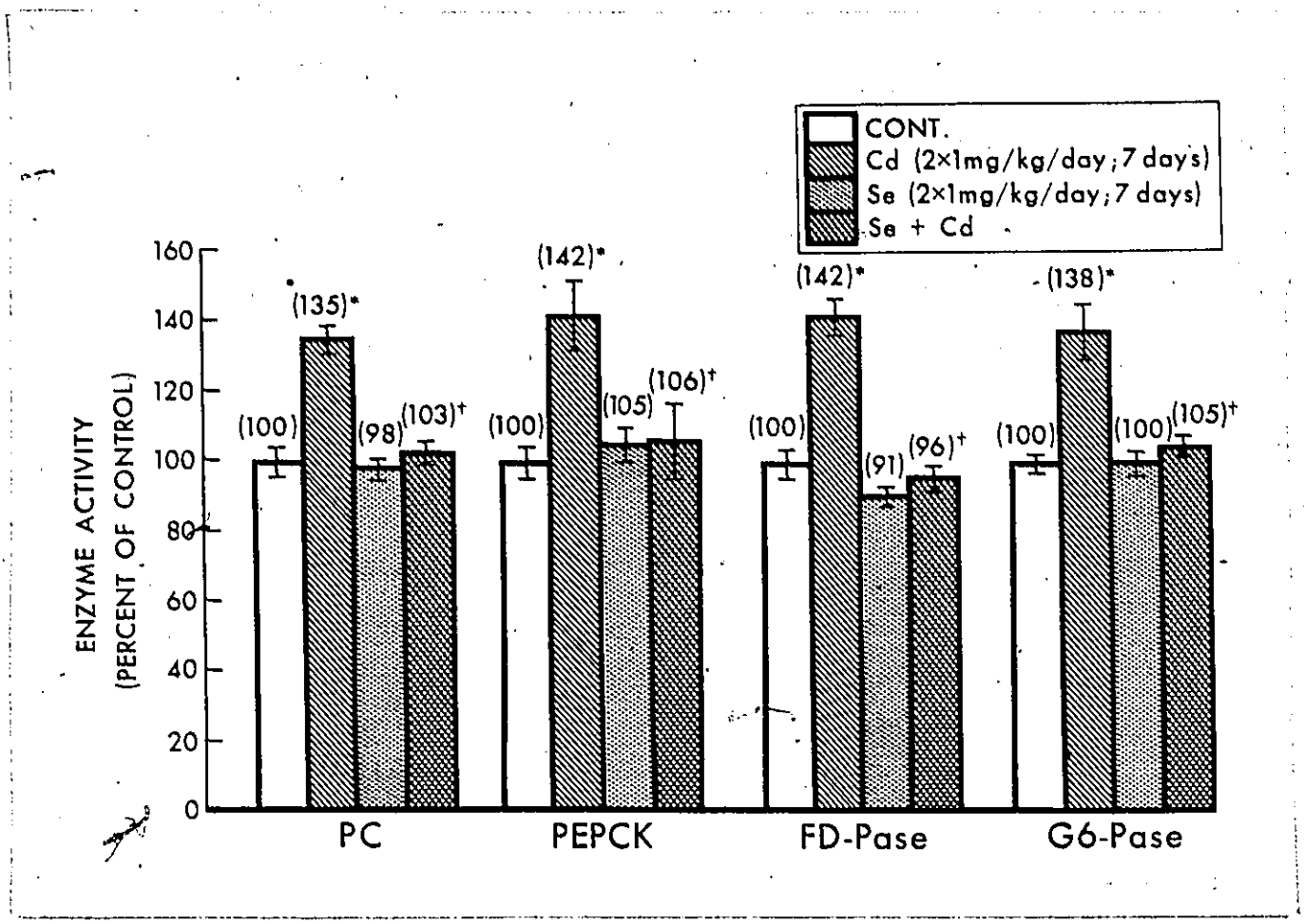

FIG. 10

Protective effect of selenium on cadmium-induced alterations in the activity of hepatic gluconeogenic enzymes. Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg, s.c.), selenium dioxide (1.0 mg/kg, s.c.) or both twice daily for 7 days. Enzyme activities were calculated as micromoles of substrate metabolized per milligram of protein per hr. Data are given in percentages (in parentheses) with values for control or cadmium-treated animals taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated rats at $p < 0.05$.

B. Effects of Subacute Exposure to Cadmium, Selenium or Both on Hepatic Cyclic AMP Levels

Since selenium effectively prevented the cadmium effects on gluconeogenic enzymes, the question whether selenium also prevented the cadmium-induced elevation of hepatic cyclic AMP content was examined. Table 12 shows that subacute exposure to cadmium resulted in a marked increase in the concentration of endogenous cyclic AMP in hepatic tissue. Treatment with selenium alone failed to significantly alter hepatic cyclic AMP; however, when selenium was administered concomitantly with cadmium, elevation in the cyclic nucleotide level was similar to that noted in cadmium-treated rats.

7. EFFECTS OF SUBACUTE CADMIUM ADMINISTRATION ON GLUCOSE TOLERANCE AND INSULIN SECRETION IN INTACT RATS

A. Influence of Cadmium on Glucose Tolerance

Since chronic, subacute as well as acute cadmium treatment induced hyperglycemia, it was of interest to examine if subacute cadmium treatment altered the ability of intact rats to handle a glucose load. Results in Figure 11 demonstrate the effects of subacute exposure to cadmium on resting blood glucose levels and glucose tolerance in intact rats. Blood glucose concentrations were determined immediately before and 15, 30 and 60 min after the glucose load (2.0 g/kg). Rats pretreated with cadmium displayed hyperglycemia and reduced glucose tolerance as reflected by significant elevation of blood glucose values over those attained by control rats at 15, 30 and 60 min after the glucose load.

B. Effects of a Glucose Load on Immuno Reactive Insulin of Rats Exposed to Cadmium

Since cadmium treatment induced glucose intolerance in rats, we were prompted to investigate the effects of cadmium on insulin secretory response. Figure 12 demonstrates that immediately prior to glucose administration, serum IRI concentration of rats pretreated with cadmium was markedly depressed.

TABLE 12

EFFECTS OF SUBACUTE EXPOSURE TO CADMIUM, SELENIUM OR BOTH ON HEPATIC CYCLIC AMP LEVELS

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium (1.0 mg of $CdCl_2$ per kg, s.c.), selenium (1.0 mg of SeO_2 per kg, s.c.) or both twice daily for 7 consecutive days. Data are also given in percentages with values from control animals taken as 100%.

Treatment	Dose (mg/kg/day)	Cyclic AMP Level (pmol/mg tissue)	% Control
Control	0.0	0.79±0.15	100
Cadmium	2x1.0	1.90±0.11	241*
Selenium	2x1.0	0.82±0.26	104
Cadmium + Selenium	2x1.0 2x1.0	1.73±0.21	219*

*Significantly different from control values at $p < 0.05$.

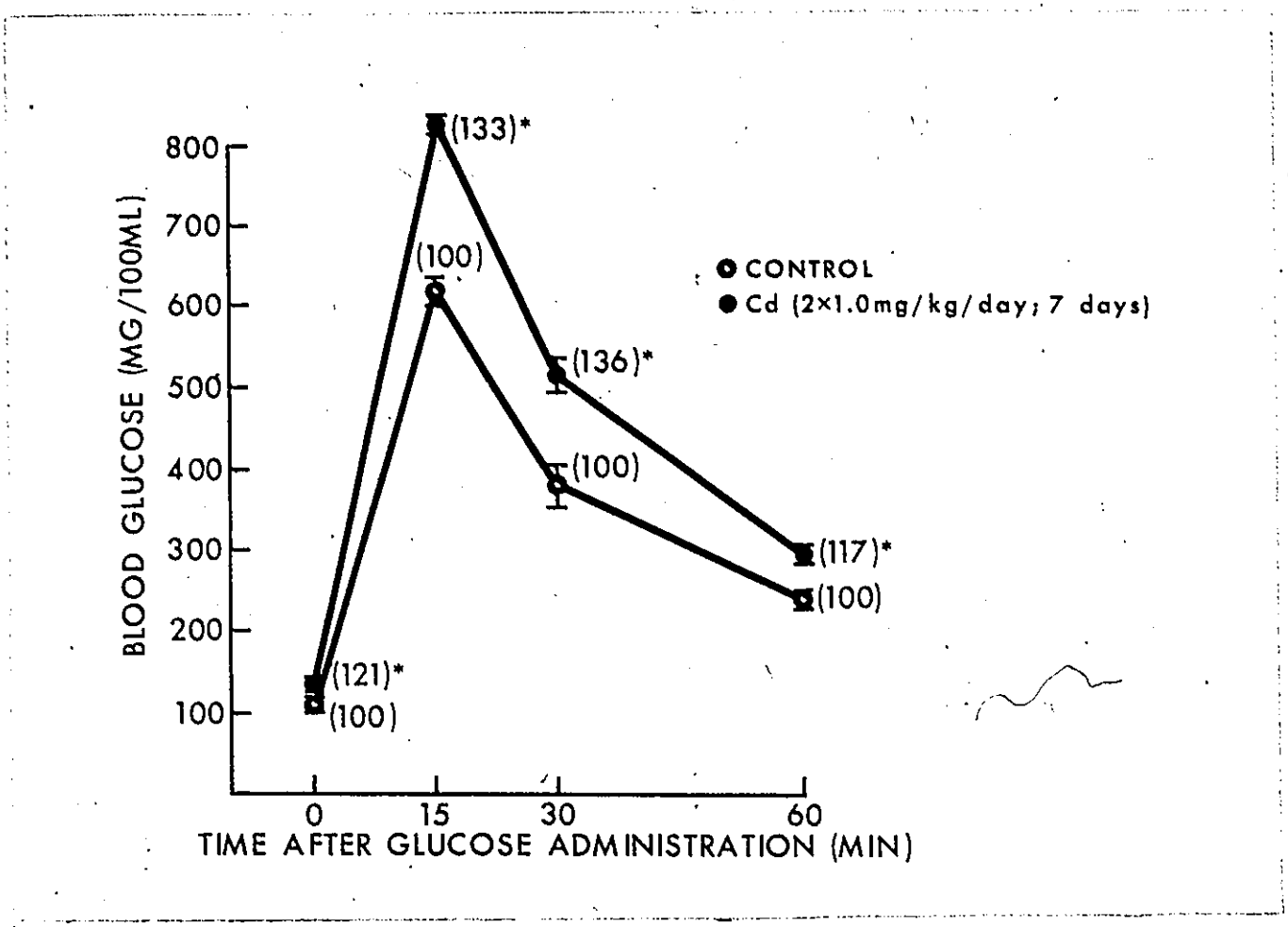

FIG. 11

Influence of subacute exposure to cadmium on glucose tolerance. Each point represents the mean ± s.e.m. of 4-5 rats in the group. Rats were pretreated with cadmium chloride (1 mg/kg s.c.) twice daily for 7 consecutive days. Glucose tolerance test was initiated 24 hr after the administration of the final dose of cadmium by giving a glucose load (2.0 g/kg i.p.). Data are also given as percentages (in parentheses) with values from control animals taken as 100%.

*Significantly different from control values at $p < 0.05$.

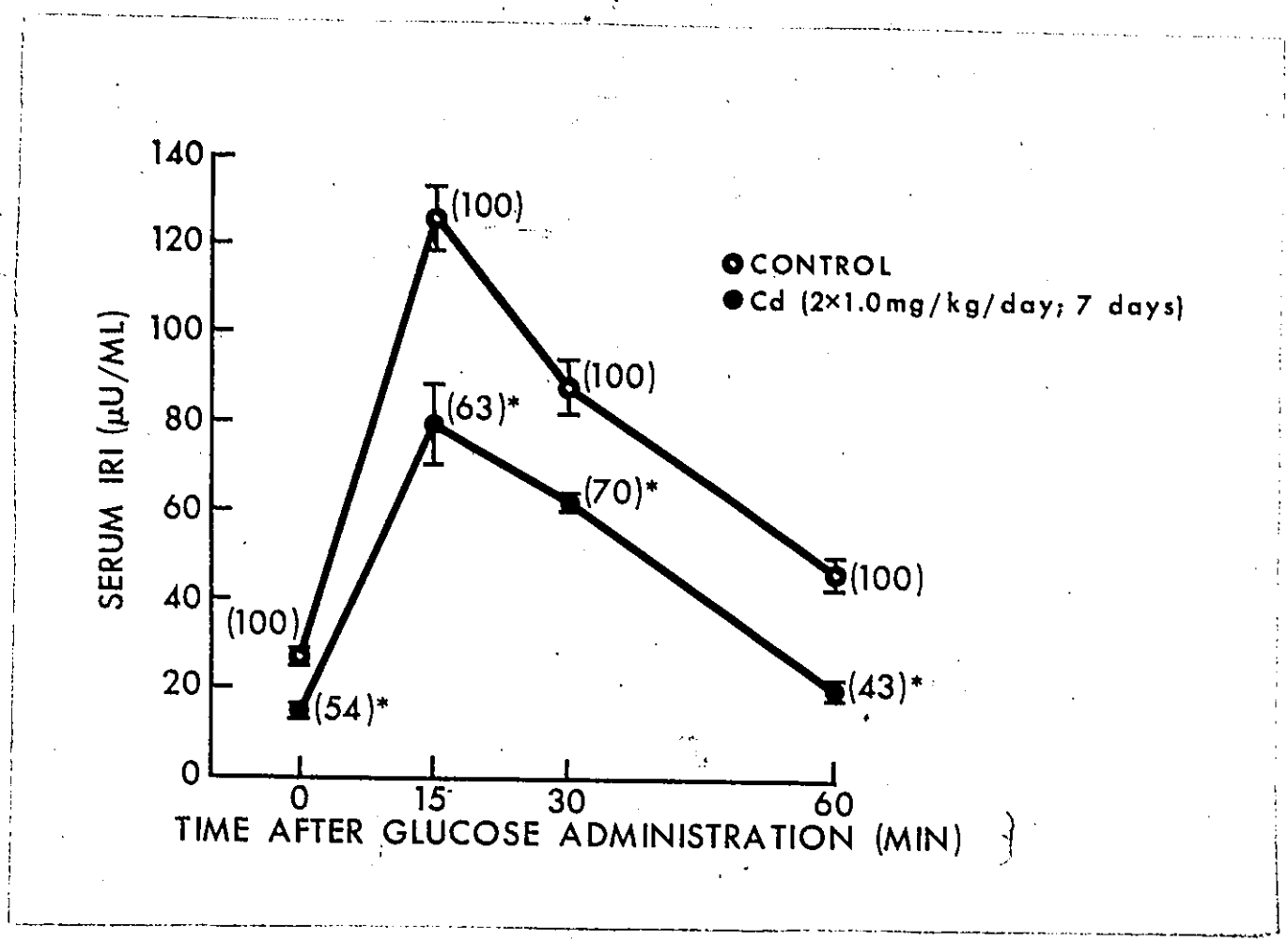

FIG. 12

Effect of a glucose load on immuno reactive insulin of rats exposed to cadmium. Each point represents the mean ± s.e.m. of 4-5 rats in the group. Rats were pretreated with cadmium chloride (1 mg/kg s.c.) twice daily for 7 consecutive days. Glucose (2.0 g/kg i.p.) was administered 24 hr after the final dose of cadmium. Data are also given as percentages (in parentheses) with values from the control animals taken as 100%.

*Significantly different from control values at $p < 0.05$.

Glucose administration (2 g/kg) produced prompt elevation in serum IRI concentration which like the glucose level, peaked at 15 min interval. In the control group, glucose load evoked a normal elevation in serum IRI level; however, in rats pretreated with cadmium, significant suppression of glucose stimulated serum IRI increase was noted at 15, 30 and 60 min intervals.

C. Influence of Cadmium on Phentolamine-Stimulated Increase in Serum IRI Levels

Since cadmium has been reported to release catecholamines from adrenals (289), and catecholamines can influence insulin secretion as well as glucose levels, the question whether the impaired pancreatic function was due to increased catecholamine release or due to some other effect of cadmium on the pancreas was investigated. Administration of 20 mg/kg of phentolamine, an alpha blocking agent, caused an increase of 266% in IRI levels of normal rats, whereas the same dose caused only an 83% rise in cadmium-exposed animals (Figure 13). A similar trend was observed when rats were given lower doses of phentolamine (10 and 4 mg/kg).

8. EFFECT OF SELENIUM ON CADMIUM INDUCED ALTERATIONS IN PANCREATIC FUNCTION

A. Effects of Selenium on Cadmium-Induced Loss of Glucose Tolerance

Results in Figure 14 demonstrate the effects of subacute exposure to cadmium, selenium and selenium plus cadmium on resting blood glucose levels and glucose tolerance in intact rats. Blood glucose concentrations were determined immediately before and 15, 30 and 60 min after the glucose load (2.0 g/kg). In contrast to cadmium, selenium pretreatment failed to alter significantly both the resting glucose level or the normal response to a glucose load. Simultaneous administration of selenium with cadmium resulted in elevation of resting blood glucose, but the increase was significantly lower than that observed in the group exposed to cadmium alone. Similarly, simultaneous

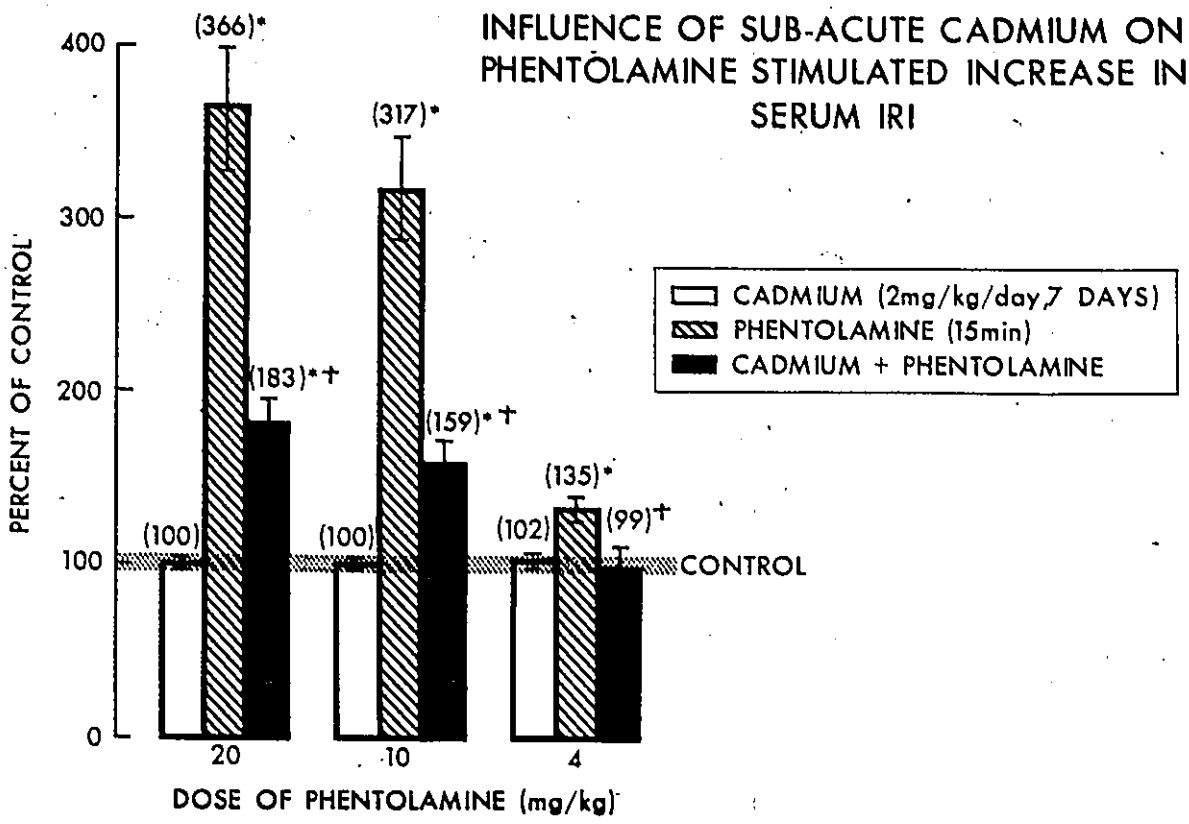

FIG. 13

Influence of cadmium on phentolamine-stimulated increase in serum IRI levels. Each bar represents the mean ± s.e.m. of 5-6 animals in the group. Animals were administered cadmium chloride (1.0 mg/kg, s.c.) twice daily for 7 days. Phentolamine mesylate (4, 10 or 20 mg/kg) was administered 24 hr after the final injection of cadmium and serum IRI levels were determined 15 min later. Data are expressed as percentages with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of control rats administered phentolamine at $p < 0.05$.

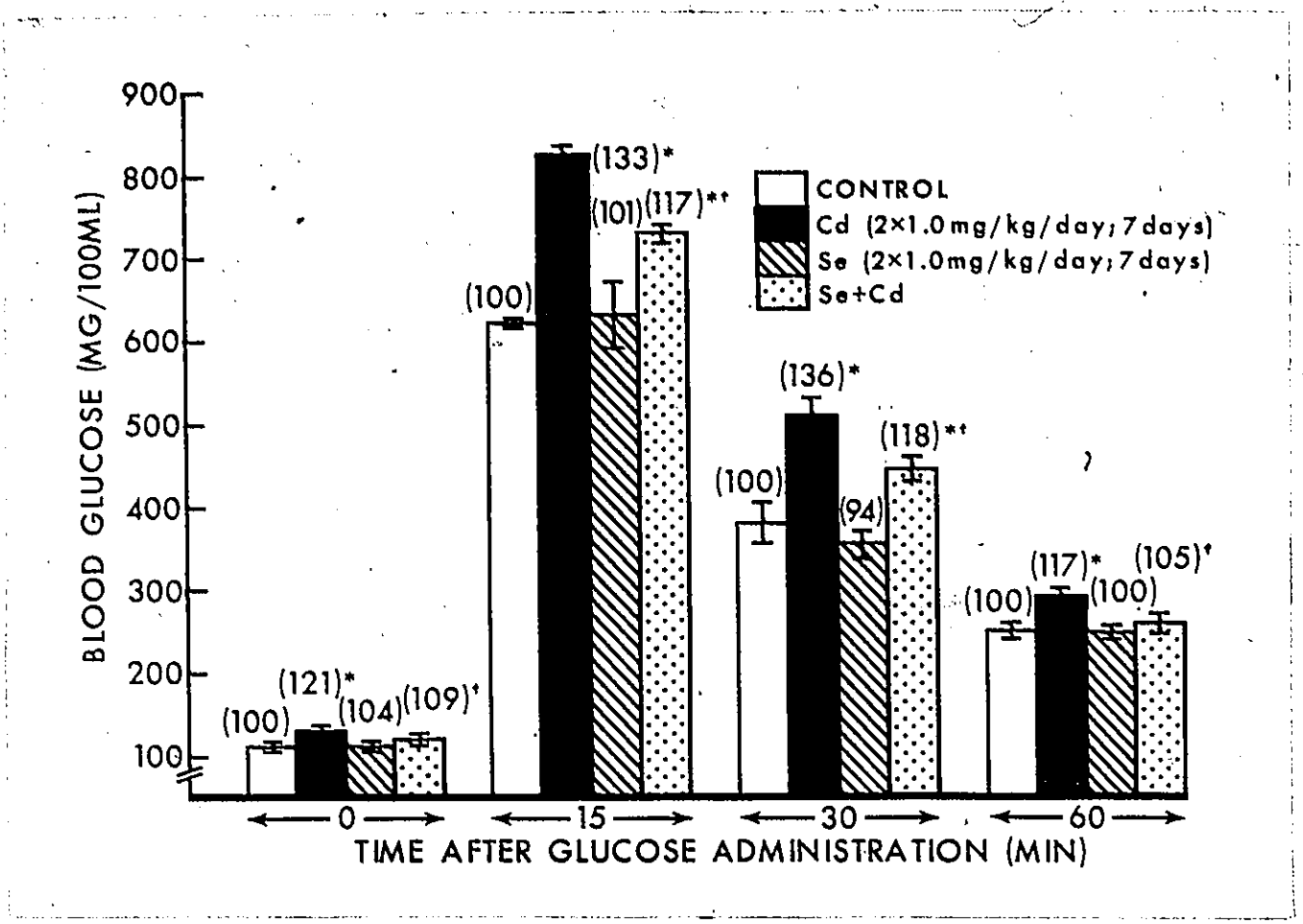

FIG. 14

Effects of selenium on cadmium-induced loss of glucose tolerance. Each column represents the mean ± s.e.m. of 4-5 rats in the group. Rats were pretreated with cadmium (1.0 mg of $CdCl_2$ per kg s.c.), selenium (1.0 mg of SeO_2 per kg s.c.) or both twice a day for 7 consecutive days. Glucose tolerance test was initiated 24 hr after the administration of the final dose(s) of cadmium and/or selenium by giving a glucose load (2.0 g/kg i.p.). Data are also given above each column as percentages of the values from the respective control groups.

*Significantly different from control values at $p < 0.05$.

†Significantly different from the values of cadmium-pretreated rats at $p < 0.05$.

administration of selenium markedly improved the cadmium-induced glucose intolerance as judged from significantly lower blood glucose levels than those of cadmium-treated rats at various time intervals. It may be noted however, that in rats receiving both cadmium and selenium, glucose levels at 15 and 30 min intervals were significantly higher than the respective control values.

B. Effect of Selenium on Cadmium-Induced Suppression of IRI Release in Response To a Glucose Load

Since selenium partially ameliorated the cadmium-induced glucose intolerance, it was of interest to study if selenium also improved the cadmium-induced suppression of pancreatic function. Figure 15 shows the effect of cadmium, selenium and selenium plus cadmium on serum IRI levels of rats challenged with a glucose load. Immediately prior to glucose administration, serum IRI concentration of rats pretreated with selenium remained within the normal range whereas that of rats exposed to cadmium was markedly depressed. Although rats exposed to cadmium and selenium simultaneously also displayed lower than normal serum IRI levels, these were significantly higher than those observed in rats pretreated with cadmium alone. Although in animals pretreated with cadmium a significant suppression of glucose-stimulated serum IRI increase was noted, the rats pretreated with selenium alone displayed normal elevation in serum IRI levels. In animals receiving selenium and cadmium simultaneously, glucose load elicited consistently greater increases in serum IRI concentration than it did in the group pretreated with cadmium alone; however, increases in serum IRI levels at 15 and 60 min intervals were still lower than those of control rats.

C. Effects of Selenium on Insulinogenic Indices of Normal and Cadmium-Treated Rats Following Glucose Load

Insulinogenic index, the term which expresses the ratio of serum IRI (microunits of IRI per ml of serum) to blood glucose concentration (milligrams of glucose/ml of blood), is believed to reflect pancreatic secretory activity

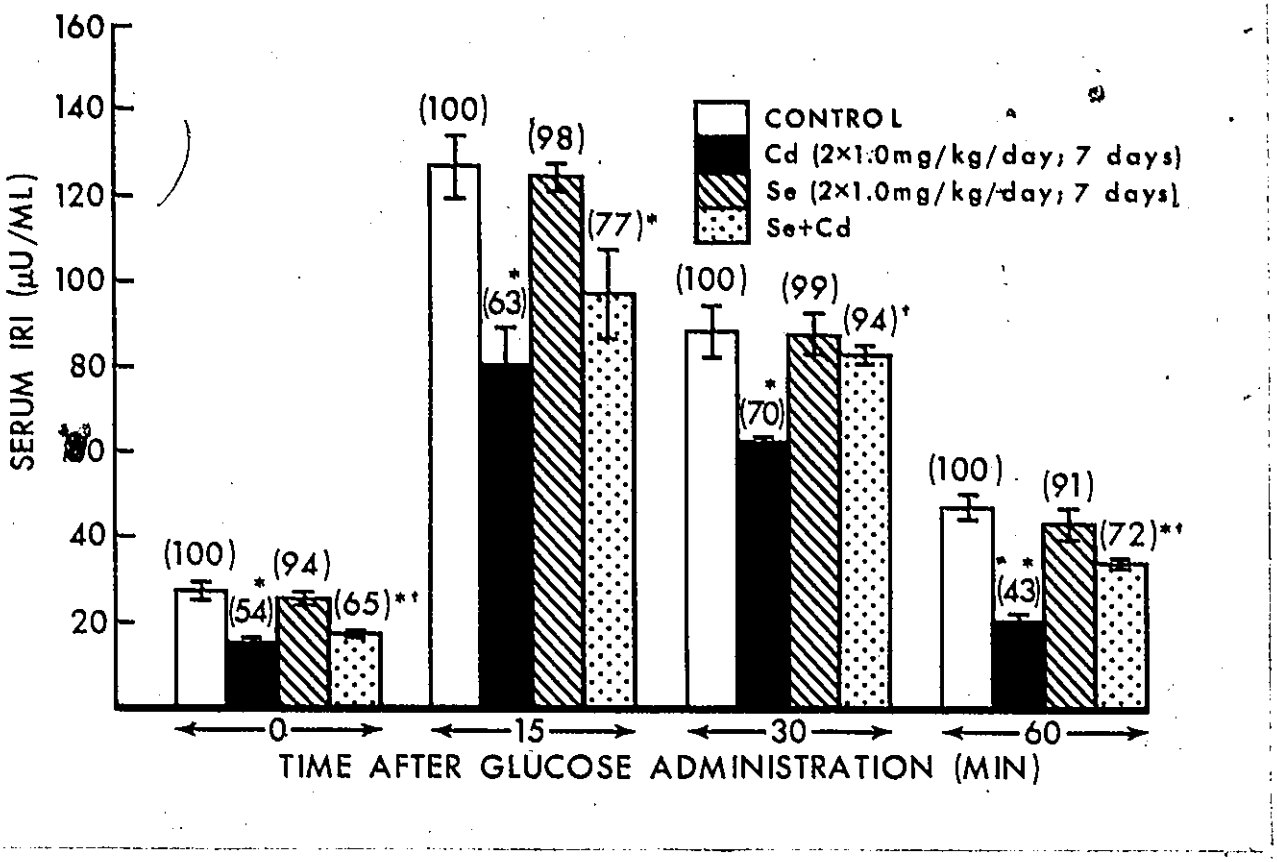

FIG. 15

Effects of selenium on cadmium-induced suppression of immunoreactive insulin (IRI) release in response to a glucose load. Each column represents the mean ± s.e.m. of 4-5 rats in the group. Rats were pretreated with cadmium (1.0 mg of $CdCl_2$/kg s.c.), selenium (1.0 mg of SeO_2/kg s.c.) or both twice a day for 7 consecutive days. Glucose load (2.0 g/kg i.p.) was administered 24 hr after the administration of the final dose(s) of cadmium and/or selenium. Data are also given above each column as percentages of the values from the respective control groups.

*Significantly different from control values at $p < 0.05$.

†Significantly different from the values of cadmium-pretreated rats at $p < 0.05$.

(291). When the data were expressed in terms of insulinogenic indices (Table 13), a definite trend seemed to emerge. Immediately before and 15, 30 and 60 min after the glucose load, selenium failed to affect significantly the insulinogenic index. In contrast, cadmium pretreatment resulted in markedly reduced insulinogenic indices at every time interval tested, suggesting that cadmium produces a decrease in pancreatic secretory activity. Although the group receiving both cadmium and selenium also had significantly reduced insulinogenic indices, the decreases were less pronounced than those seen in rats pretreated with cadmium alone, and the insulinogenic indices at various time intervals were significantly greater than those of cadmium-treated rats.

D. Effects of Subacute Cadmium and/or Selenium on Estimated Total Insulin Released During 1 hr Following Glucose Administration

Data in Table 14 show the total amount of IRI released in various groups during the 1 hr period after glucose load, as calculated by integrating area under the appropriate curve. It can be seen that the total amount of IRI released by cadmium intoxicated animals was significantly lower than in control animals. Selenium treatment, by itself, did not significantly alter the amount of IRI released. However, in rats receiving both selenium and cadmium simultaneously, significantly more IRI was released than in rats receiving cadmium alone.

9. PREVENTION BY ZINC OF CADMIUM-INDUCED ALTERATIONS IN PANCREATIC FUNCTION

A. Effects of Zinc on Cadmium-Induced Glucose Intolerance

Many of the manifestations of acute cadmium toxicity in experimental animals can be eliminated by prior or simultaneous injection of zinc salts. Thus, zinc administration has prevented cadmium-induced testicular damage (135), the toxaemia-like condition in pregnant rats (292) and the teratogenic effects of cadmium (293). It was therefore of interest to examine if zinc could also alter the response of pancreatic tissue to toxic effects of cadmium.

TABLE 13

EFFECT OF SELENIUM ON THE INSULINOGENIC INDICES OF NORMAL AND CADMIUM-TREATED RATS FOLLOWING GLUCOSE LOAD

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were pretreated with cadmium (1.0 mg of $CdCl_2$ per kg, s.c.), selenium (1.0 mg of SeO_2 per kg, s.c.) or both twice a day for 7 days. Glucose (2.0 g/kg) was administered 24 hr after the final dose(s) of cadmium and/or selenium. Insulinogenic index represents a ratio expressed as serum IRI (microunits per milliliter)/blood glucose (milligrams per 100 ml). Data are also given in percentages with values from control (in parentheses) or cadmium-treated animals [in brackets] taken as 100%.

Treatment	Dose (mg/kg/day)	Insulinogenic Index at Time After Glucose Administration			
		0 min	15 min	30 min	60 min
Control	0.0	0.25±0.001 (100)	0.21±0.010 (100)	0.24±0.029 (100)	0.21±0.006 (100)
Cadmium	2x1	0.11±0.003 (44)* [100]	0.10±0.009 (48)* [100]	0.12±0.011 (50)* [100]	0.07±0.004 (33)* [100]
Selenium	2x1	0.23±0.003 (92)	0.21±0.018 (100)	0.26±0.017 (108)	0.19±0.009 (91)
Cadmium + Selenium	2x1 2x1	0.15±0.006 (60)* [136]†	0.15±0.006 (71)* [150]†	0.14±0.013 (58)* [117]†	0.13±0.001 (62)* [186]†

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated rats at $p < 0.05$.

TABLE 14

EFFECTS OF SUBACUTE CADMIUM AND/OR SELENIUM ON INTEGRATED IRI LEVELS OVER A 60 MIN PERIOD AFTER ADMINISTRATION OF A GLUCOSE LOAD

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg, s.c.), selenium dioxide (1.0 mg/kg, s.c.) or both, twice daily for 7 days. Data are also given in percentages (in parentheses) with values from control animals taken as 100%.

Treatment	Dose (mg/kg/day)	Estimated Total Insulin Released (min x μU/ml)	Percent of Control
Control	0.0	3167±205	(100)
Cadmium	2x1.0	2129±146	(67)*
Selenium	2x1.0	3126±138	(99)†
Selenium + Cadmium	2x1.0 2x1.0	2916±149	(92)†

*Significantly different from control values at p 0.05.

†Significantly different from values of cadmium-treated group at p <0.05.

Blood glucose concentrations were determined immediately before, as well as 15, 30 and 60 min after the glucose load (2.0 g/kg i.p.) (Figure 16). Although zinc failed to alter the resting blood glucose level, exposure to cadmium significantly elevated it to 122% of the control value. Simultaneous administration of zinc and cadmium resulted in a blood glucose level that was significantly lower than that observed in the group exposed to cadmium alone. Peak blood glucose values in response to the glucose load were attained at 15 min in all groups examined; however, this value in cadmium-pretreated rats was significantly greater (38%) than that of control animals. Rats pretreated with zinc as well as cadmium attained blood glucose level that was in the same range as that of control animals. Similarly, at 30 and 60 min intervals, cadmium-pretreated rats attained significantly higher blood glucose levels than those of zinc or zinc plus cadmium pretreated rats; the results from the latter two groups not being significantly different from those of the control rats.

B. Effects of Glucose Load on Serum Levels of Immuno Reactive Insulin of Rats Exposed to Cadmium and/or Zinc

Since zinc, when administered concurrently with cadmium, prevented cadmium-induced glucose intolerance, it was of interest to examine if the cadmium-induced pancreatic suppression also was prevented by zinc. Figure 17 demonstrates that immediately before glucose administration, serum concentration of IRI of rats pretreated with zinc remained within the normal range. In contrast, the serum IRI level in rats exposed to cadmium was considerably depressed. Rats exposed to cadmium and zinc simultaneously on the other hand, displayed normal serum IRI content. Glucose administration (2 g/kg) produced prompt elevation in serum IRI concentration, which like the glucose level, peaked at the 15 min time point. In the zinc group, glucose load evoked a normal elevation in serum IRI level; however, in rats pretreated with cadmium, stat-

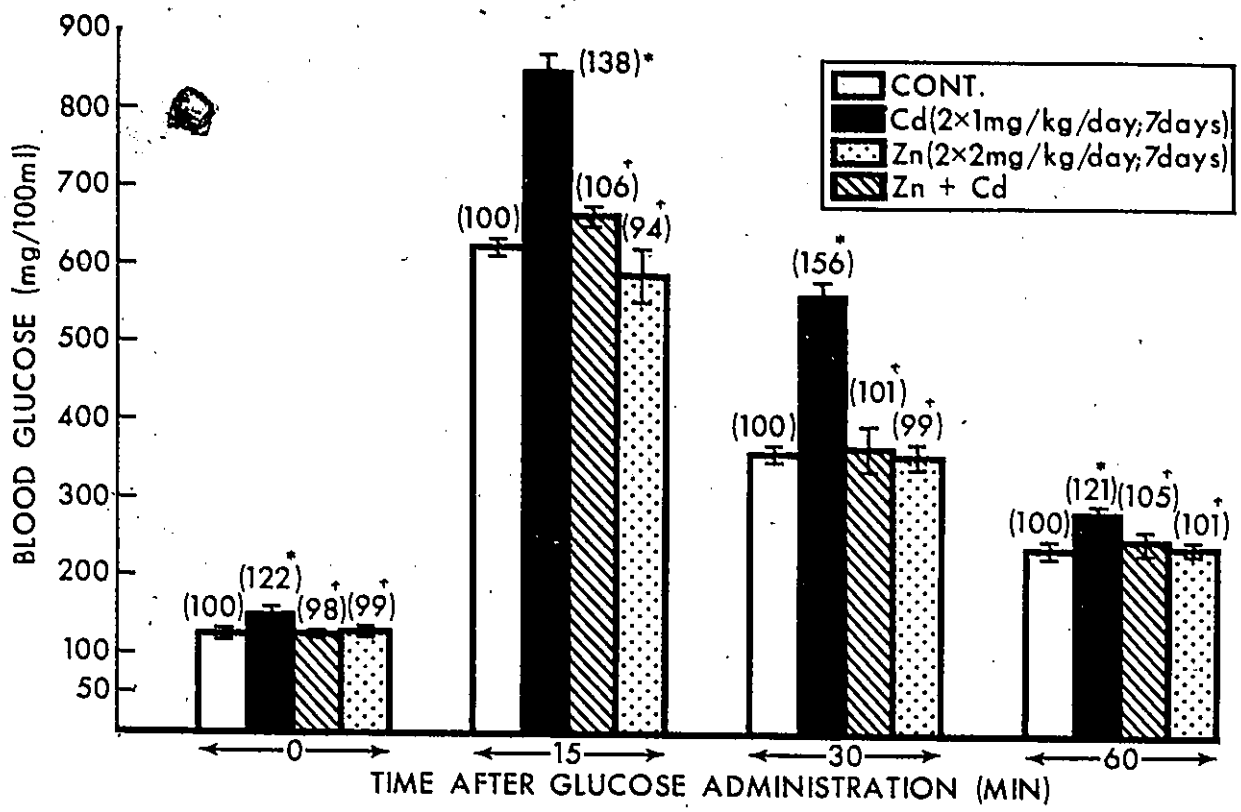

FIG. 16

Effects of zinc on cadmium-induced glucose intolerance. Each bar represents the mean ± s.e.m. of 5-6 animals in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s,c.) or both twice daily for 7 days. Glucose tolerance test was initiated 24 hr after the administration of the final dose(s) of cadmium and/or zinc by giving a glucose load (2.0 g/kg; i.p.). Data are also expressed in percentages (in parentheses) with values from the respective control groups taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-pretreated rats at $p < 0.05$.

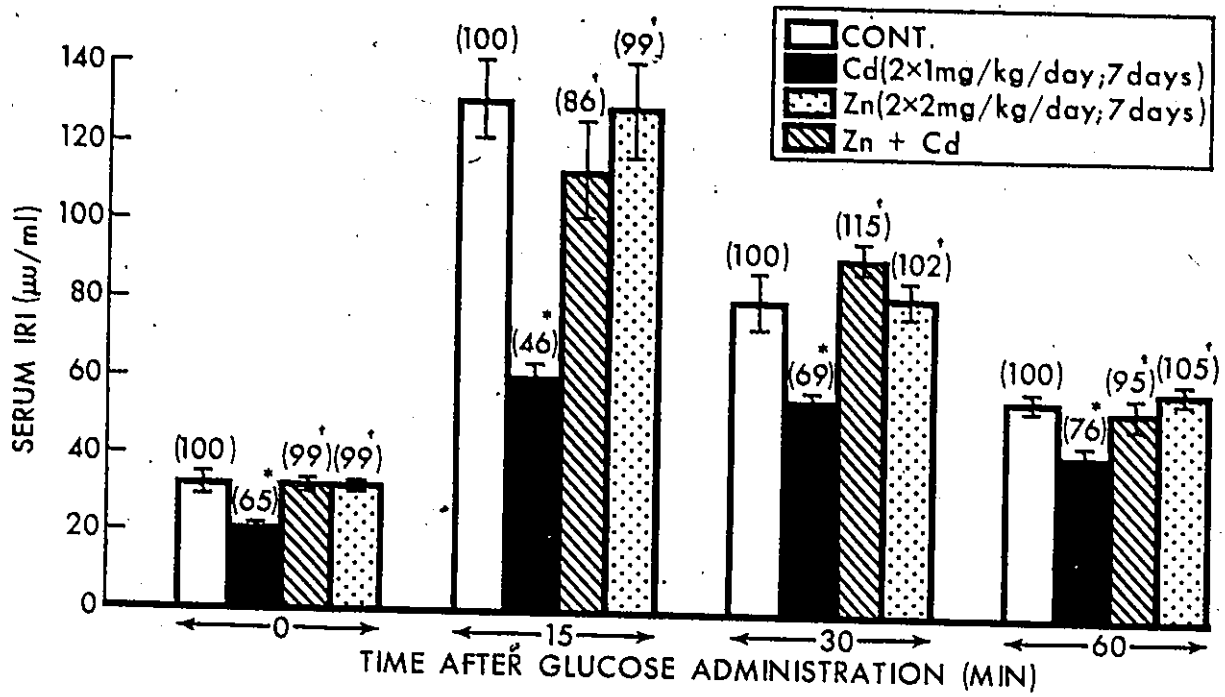

FIG. 17

Effect of cadmium, zinc and zinc + cadmium on immunoreactive insulin (IRI) release in response to a glucose load. Each bar represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s.c.) or both twice daily for 7 days. Glucose (2.0 g/kg; i.p.) was administered 24 hr after the final dose(s) of cadmium and/or zinc. Each point represents the mean ± s.e.m. of 5-6 animals. Data are also expressed in percentages (in parentheses) with values from the respective control groups taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-pretreated rats at $p < 0.05$.

istically significant suppression of glucose-stimulated serum IRI increases was noted at 15, 30 and 60 min. In animals receiving zinc plus cadmium simultaneously, glucose load elicited consistently greater increases in serum IRI concentrations than it did in the group pretreated with cadmium alone, and was not significantly different from the control values.

C. Effects of Subacute Cadmium and/or Zinc on Estimated Total Insulin Released Over 1 hr Period Following Administration of a Glucose Load

Table 15 shows the total amount of IRI released in various groups in the 1 hr period after glucose load, as calculated by integrating the area under the appropriate curve. It can be seen that the total amount of IRI released by cadmium intoxicated animals was significantly lower than in control animals. Zinc treatment, by itself, did not significantly alter the amount of IRI released. However, in rats receiving both zinc and cadmium, significantly more IRI was released than in rats receiving cadmium alone.

10. PREVENTION BY ZINC OF CADMIUM-INDUCED ALTERATIONS IN HEPATIC AND TESTICULAR METABOLISM

A. Protective Effect of Zinc on Cadmium-Induced Alterations in Hepatic Glycogen, Gluconeogenic Enzymes and Serum Urea

Since zinc could prevent certain effects of cadmium on glucose homeostasis, we were prompted to examine if this element could also protect against the effects of cadmium on hepatic gluconeogenesis. Administration of cadmium significantly elevated the activities of PC, PEPCK, FD-Pase and G6-Pase (Table 16). On the other hand, exposure to zinc failed to produce any significant change in the activities of these four enzymes. When zinc and cadmium were administered concurrently, the activities of all four gluconeogenic enzymes were significantly lower than those noted for rats given cadmium alone, and were in the same range as control values. In contrast to the gluconeogenic enzymes, cadmium treatment resulted in a reduction of hepatic glycogen level. Although zinc by itself

TABLE 15

EFFECTS OF SUBACUTE CADMIUM AND/OR ZINC ON INTEGRATED IRI LEVELS OVER A 60 MIN PERIOD AFTER ADMINISTRATION OF A GLUCOSE LOAD

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s.c.) or both twice daily for 7 consecutive days. Data are also given in percentages (in parentheses) with values from control animals taken as 100%.

Treatment	Dose (mg/kg/day)	Estimated Total Insulin Released (min x μU/ml)	Percent of Control
Control	0.0	2938±266	(100)
Cadmium	2x1.0	1691±93	(58*)
Zinc	2x2.0	3010±201	(102†)
Zinc + Cadmium	2x2.0 2x1.0	2903±220	(99†)

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated group at $p < 0.05$.

TABLE 16

PROTECTIVE EFFECT OF ZINC ON CADMIUM-INDUCED ALTERATIONS IN HEPATIC GLUCONEOGENIC ENZYMES AND GLYCOGEN, CYCLIC AMP AND SERUM UREA LEVELS

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s.c.) or both twice daily for 7 days. Data are also given in percentages with values for control (in parentheses) or cadmium treated (in brackets) taken as 100%.

Parameters Examined	Treatment			
	Control	Cadmium	Zinc	Zinc + Cadmium
PC	306±5.7 (100)	430±13.7 (141)* [100]	316±4.8 (103) [73]†	305±8.8 (100) [71]†
PEPCK	9.8±0.61 (100)	18.1±2.04 (185)* [100]	9.05±0.69 (92) [50]†	10.6±1.09 (108) [59]†
FD-Pase	6.05±0.31 (100)	7.86±0.50 (130)* [100]	6.49±0.17 (107) [83]†	6.24±0.29 (103) [79]†
G6-Pase	2.32±0.13 (100)	2.90±0.11 (125)* [100]	2.35±0.26 (101) [81]†	2.30±0.05 (99) [79]†
Glycogen	2.18±0.2 (100)	1.43±0.09 (66)* [100]	1.95±0.16 (89) [136]†	2.23±0.26 (102) [156]†
Urea	17.6±0.50 (100)	28.4±1.15 (161)* [100]	18.4±0.51 (105) [65]†	18.4±0.51 (105) [65]†

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated rats at $p < 0.05$.

was without any appreciable effect when given in combination with cadmium, it prevented the cadmium-induced fall in liver glycogen. Cadmium treatment also increased serum urea by 61% and this rise was effectively prevented by simultaneous administration of zinc, although zinc alone was without any significant effect.

B. Protection by Zinc Against Cadmium-Induced Elevation in Hepatic Cyclic AMP Levels

Subacute exposure to cadmium resulted in a marked increase in hepatic cyclic AMP concentration (Table 17). Treatment with zinc alone failed to alter hepatic cyclic AMP levels significantly. However, when zinc was administered at the same time as cadmium, the observed elevation in hepatic cyclic AMP level was prevented and the values remained in the range of control rats.

C. Effects of Cadmium and Zinc on Adenylate Cyclcase Activity and its Responsiveness to Hormonal Stimulation

Results presented in Figure 18 demonstrate that cadmium-induced elevation of hepatic cyclic AMP level was accompanied by enhancement in the activity of the cyclic AMP synthesizing enzyme, adenylate cyclase. Epinephrine, fluoride and glucagon were all able to stimulate adenylate cyclase activity in both normal and cadmium-exposed animals; however, in cadmium-treated rats, glucagon and epinephrine stimulated the enzymatic activity to levels higher than in the corresponding controls whereas fluoride did not. Furthermore, although zinc by itself did not alter the sensitivity of adenylate cyclase to epinephrine, glucagon or fluoride, administration of this trace element concurrently with cadmium significantly prevented the cadmium-stimulated increases in the activity of both the basal as well as the hormone-stimulated forms of the hepatic enzyme.

D. Influence of Zinc on Cadmium-Induced Alterations in Testicular Cyclic AMP and Prostaglandin F Levels

Since zinc has been reported to protect animals against cadmium-induced gonadal necrosis (135), it was of interest to examine if it also exerted

TABLE 17

PROTECTIVE EFFECT OF ZINC AGAINST THE CADMIUM-INDUCED INCREASE IN HEPATIC CYCLIC AMP LEVELS

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s.c.) or both twice daily for 7 days. Data are also expressed in percentages with values from control animals taken as 100%.

Treatment	Dose (mg/kg/day)	Cyclic AMP Level (pmol/mg tissue)	Percent of Control
Control	0.0	0.74±0.01	100
Cadmium	2x1.0	1.36±0.06	184*
Zinc	2x2.0	0.80±0.03	108
Zinc + Cadmium	2x2.0 2x1.0	0.79±0.02	107

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated group at $p < 0.05$.

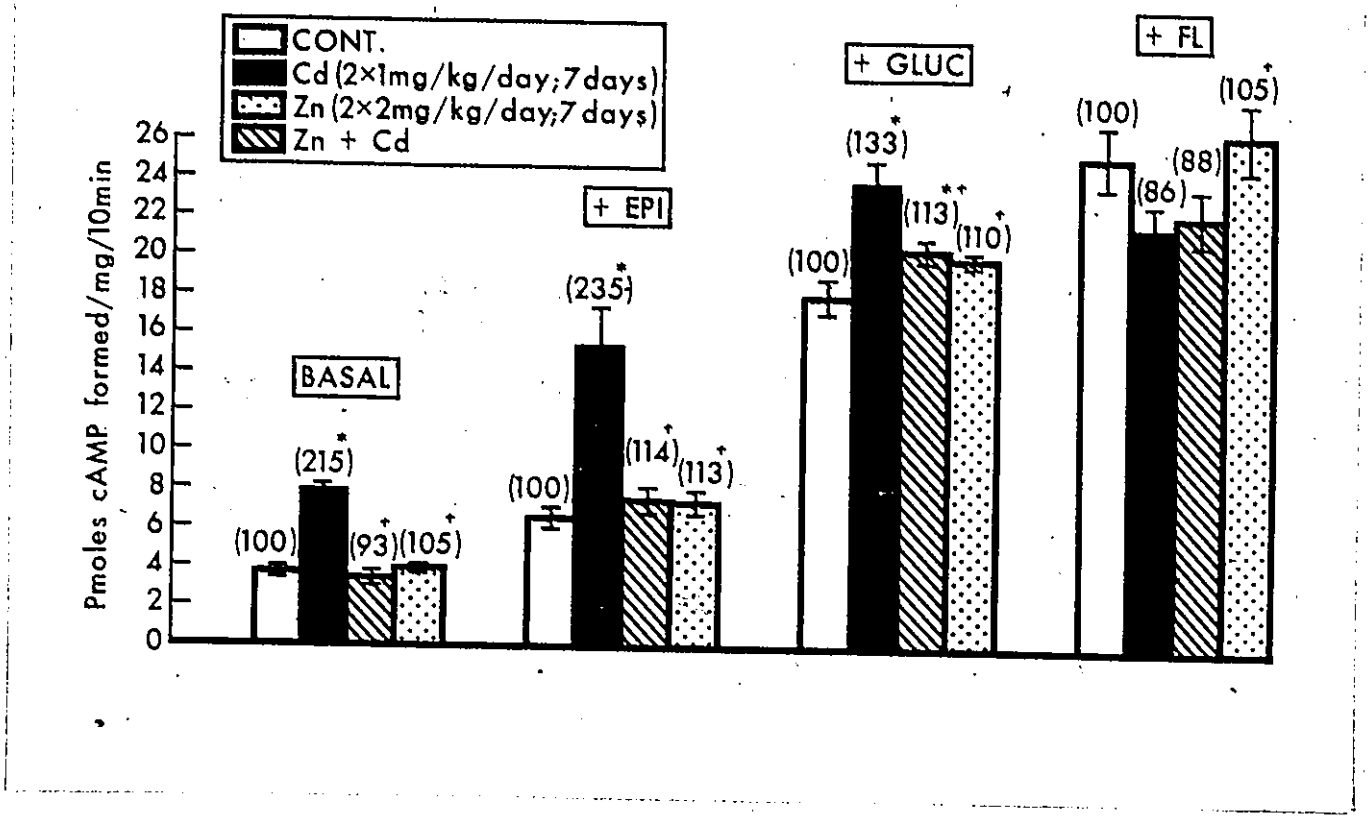

FIG. 18

Zinc protection of cadmium-induced alterations in the responsiveness of hepatic adenylate cyclase to epinephrine (EPI), glucagon (GLUC) or fluoride (FL). Each bar represents the mean ± s.e.m. of 5-6 animals in the group. Rats were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg; s.c.) or both twice daily for 7 days. Data are also expressed in percentages (in parentheses) with values from the respective control groups taken as 100%.

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated rats at $p < 0.05$.

protective effects on the testicular cyclic AMP and prostaglandin F levels. Data in Table 18 demonstrate that although cadmium-induced drop in cyclic AMP concentration was not statistically significant, the total testicular cyclic AMP content was markedly reduced by cadmium treatment. Zinc, on the other hand, failed to significantly alter the concentration as well as content of testicular cyclic AMP. However, when zinc was administered concurrently with cadmium, a significant increase was noted in testicular cyclic AMP concentration as well as content. Although administration of cadmium and/or zinc failed to significantly alter the concentration of testicular PGF, cadmium treatment reduced the total PGF content of testes. Similarly, although zinc treatment alone failed to alter testicular PGF when administered concurrently with cadmium, it prevented the cadmium-induced depression of testicular PGF content.

11. LONG TERM EFFECTS OF ORALLY ADMINISTERED CADMIUM ON NEONATAL RATS

Since it is the long term exposure to small amounts of ingested cadmium that presents a potential health hazard, it was of interest to examine whether orally administered cadmium also produced various toxic manifestations produced by parenterally administered cadmium. In order to achieve appropriate monitoring over the amount of cadmium administered as well as simulating a more physiological mode of cadmium exposure, new born rats were administered cadmium (0.1 or 1 μg/g) orally as cadmium chloride solution by intubation.

A. Growth of Young Rats Following Daily Oral Doses of Cadmium Chloride

Body weight changes (growth) of new born rats exposed to two different doses of cadmium are shown in Figure 19. There was no significant difference in body weight gain of those young rats receiving daily oral doses of 0.1 μg/g cadmium (as cadmium chloride) daily over the period of 43 days. However, young rats receiving higher doses of cadmium (1.0 μg/g) displayed a

TABLE 18

INFLUENCE OF ZINC AND CADMIUM-INDUCED ALTERATIONS IN TESTICULAR CYCLIC AMP AND PROSTAGLANDIN F LEVELS

Each value represents the mean ± s.e.m. of 5-6 rats in the group. Animals were administered cadmium chloride (1.0 mg/kg; s.c.), zinc chloride (2.0 mg/kg, s.c.) or both twice daily for 7 days. Data are also given in percentages (in parentheses) with values from control animals taken as 100%.

Treatment	Dose (mg/kg/day)	Cyclic AMP Levels		Prostaglandin F Levels	
		pmol/mg	nmol/testes	p gm/mg	n gm/testes
Control	0.0	2.87±0.10 (100)	7.58±0.40 (100)	5.40±0.55 (100)	14.19±1.46 (100)
Cadmium	2x1.0	2.35±0.27 (82)	3.95±0.83 (52)*	4.84±0.46 (90)	9.65±1.36 (68)*
Zinc	2x2.0	3.15±0.14 (110)	8.32±0.62 (110)	5.95±1.04 (110)	15.51±2.7 (109)
Cadmium + Zinc	2x1.0 2x2.0	3.92±0.30 (137)*	10.12±0.76 (134)*	4.51±0.89 (84)	14.71±3.66 (104)

*Significantly different from control values at $p < 0.05$.

†Significantly different from values of cadmium-treated rats at $p < 0.05$.

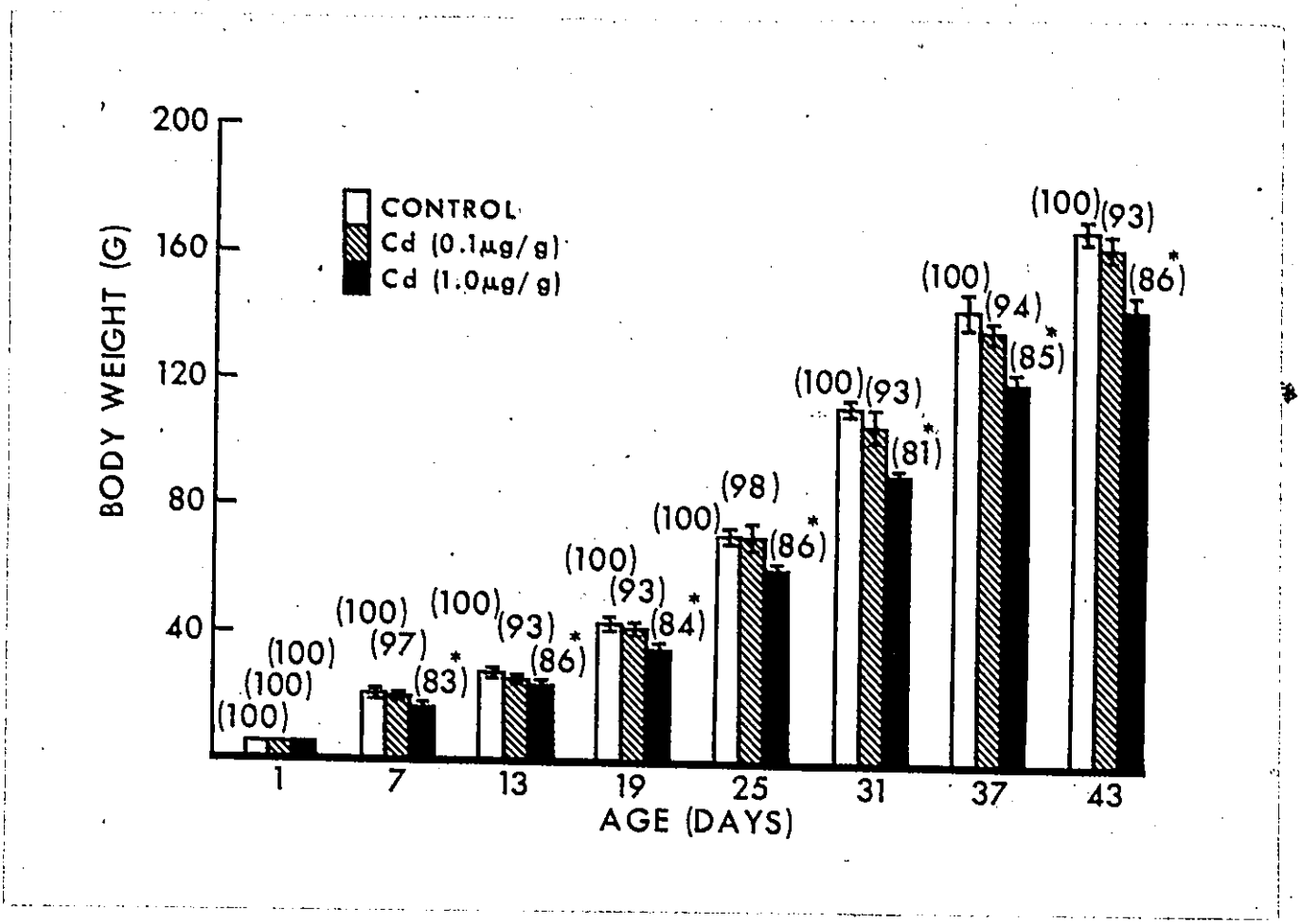

FIG. 19

Influence of neonatal exposure to oral doses of cadmium chloride on body weight changes (growth). Each column represents the mean ± s.e.m. of 5-6 values in the group. One day old rats were administered water (control) or cadmium (0.1 or 1.0 µg/g) as a cadmium chloride solution for 43 days.

*Significantly different from control values at $p < 0.05$.

slight but significant depression in growth relative to the control group. Figure 20 shows a photograph comparing the appearance of a normal 25 day old rat with age matched rats exposed to either 0.1 or 1.0 µg/g cadmium orally. There appeared to be no noticeable difference in the appearance of rats neonatally exposed to 0.1 µg/g cadmium. However, animals exposed to the higher dose of cadmium (1.0 µg/g), as indicated in the lower panel, seemed to be smaller in size as compared to control or low dose exposed rats.

B. Effects of Chronic Cadmium Ingestion on Testicular Cyclic AMP and Prostaglandin F Levels

Since short term parenteral administration of cadmium altered both the testicular cyclic AMP and prostaglandin F content, it was of interest to examine if oral long term cadmium treatment also could manifest such changes. Data in Table 19 demonstrate that daily administration of low (0.1 µg/g) or high dose (1.0 µg/g) of cadmium for 45 days caused a significant drop in endogenous cyclic AMP levels of the testes, whether expressed as pmol/mg tissue or nmol/organ. Similarly, a marked fall in the testicular prostaglandin F concentration or content was observed upon oral cadmium treatment.

C. Effects of Cadmium Ingestion on Blood Glucose, Serum Urea and Hepatic Glycogen Levels

Data presented in Table 20 demonstrate that although the low dose (0.1 µg/g) of oral cadmium for 45 days caused significant elevation in blood glucose. The increment of blood sugar was also significantly greater than control values in rats exposed to the higher level of cadmium (1.0 µg/g). In contrast, both doses of cadmium markedly reduced hepatic glycogen content. Similarly, serum urea levels were significantly increased in rats exposed to either dose of cadmium. Furthermore, the alterations in glucose, glycogen and urea were of a greater magnitude in animals exposed to the higher dose of the heavy metal.

D. Effects of Cadmium Ingestion on Hepatic Gluconeogenesis

Since like parenteral administration, cadmium ingestion elevated blood

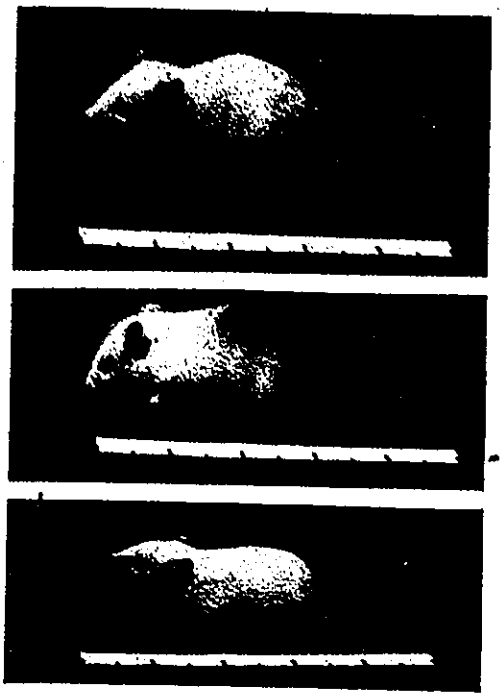

FIG. 20

Photograph comparing the appearance of normal 25 day old rat (top panel) with age-matched rats exposed to 0.1 µg/g cadmium (middle panel) or 1.0 µg/g cadmium (bottom panel).

TABLE 19

INFLUENCE OF CHRONIC CADMIUM EXPOSURE ON TESTICULAR CYCLIC AMP AND PROSTAGLANDIN F LEVELS

Each value represents the mean ± s.e.m. of 5-6 animals in the group. Rats were administered cadmium (0.1 or 1.0 µg/g) orally in the form of cadmium chloride solution (0.005 ml/g), for 45 days. Data are also given in percentages (in parentheses) with control values taken as 100%.

Treatment	Dose of Cadmium (µg/g)	Cyclic AMP Levels		Prostaglandin F Levels	
		pmol/mg	nmol/testes	p gm/mg	n gm/testes
Control	0.0	3.65±0.08 (100)	10.23±0.27 (100)	3.35±0.19 (100)	9.72±0.55 (100)
Low Dose	0.1	3.18±0.10 (87)*	8.04±0.22 (79)*	2.25±0.09 (67)*	5.67±0.21 (58)*
High Dose	1.0	2.94±0.11 (81)*	7.47±0.27 (73)*	2.08±0.06 (62)*	5.05±0.31 (52)*

*Significantly different from control values at $p < 0.05$.

TABLE 20

EFFECTS OF ORAL CADMIUM ADMINISTRATION ON BLOOD GLUCOSE, SERUM UREA AND HEPATIC GLYCOGEN LEVELS

Each value represents the mean ± s.e.m. of 5-6 animals in the group. Rats were administered cadmium (0.1 or 1.0 µg/g/day) by intubation in the form of cadmium chloride solution for 45 days. Data are also given in percentages (in parentheses) with control values taken as 100%.

Treatment	Control	Cadmium (0.1 µg/g)	Cadmium (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)*

*Significantly different from control values at $p < 0.05$.

glucose and urea and lowered hepatic glycogen content, we were prompted to examine if orally administered cadmium also enhanced the activities of various hepatic gluconeogenic enzymes. Data in Figure 21 demonstrate that long term oral administration of the low dose of cadmium (0.1 µg/g/day) did indeed elevate the activities of PC, PEPCK, FD-Pase and G6-Pase. Likewise, the 45-day high dose of cadmium (1.0 µg/g/day) 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 the low doses of the heavy metal.

E. Effect of Long Term Oral Cadmium Administration on Hepatic Adenylate Cyclase Activity

As the alterations of hepatic carbohydrate metabolism after subacute cadmium-exposure were accompanied by changes in hepatic cyclic AMP metabolism, it was of interest to examine whether a similar relationship existed between these two parameters following chronic oral exposure to the heavy metal. Daily administration of cadmium at a dose of 0.1 or 1.0 µg/g by intubation for 45 days elevated hepatic cyclic AMP levels by 32% and 57%, respectively. This increase of hepatic cyclic AMP was accompanied by enhancement in the activity of the cyclic AMP synthesizing enzyme, adenylate cyclase. Epinephrine, fluoride as well as glucagon were all able to stimulate adenylate cyclase activity in normal and cadmium-exposed animals; however, in the latter group, both glucagon and epinephrine stimulated enzymatic activity to levels higher than those seen in corresponding controls (Figure 22). In contrast, the sensitivity of hepatic adenylate cyclase to fluoride stimulation remained similar in both control and cadmium exposed animals.

F. Effect of Chronic Cadmium Ingestion on Serum Insulin and Pancreatic Acid Ethanol-Extractable Insulin Concentration

As already shown in Table 20, chronic exposure to oral cadmium also induced hyperglycemia. It was thus of interest to investigate if serum IRI

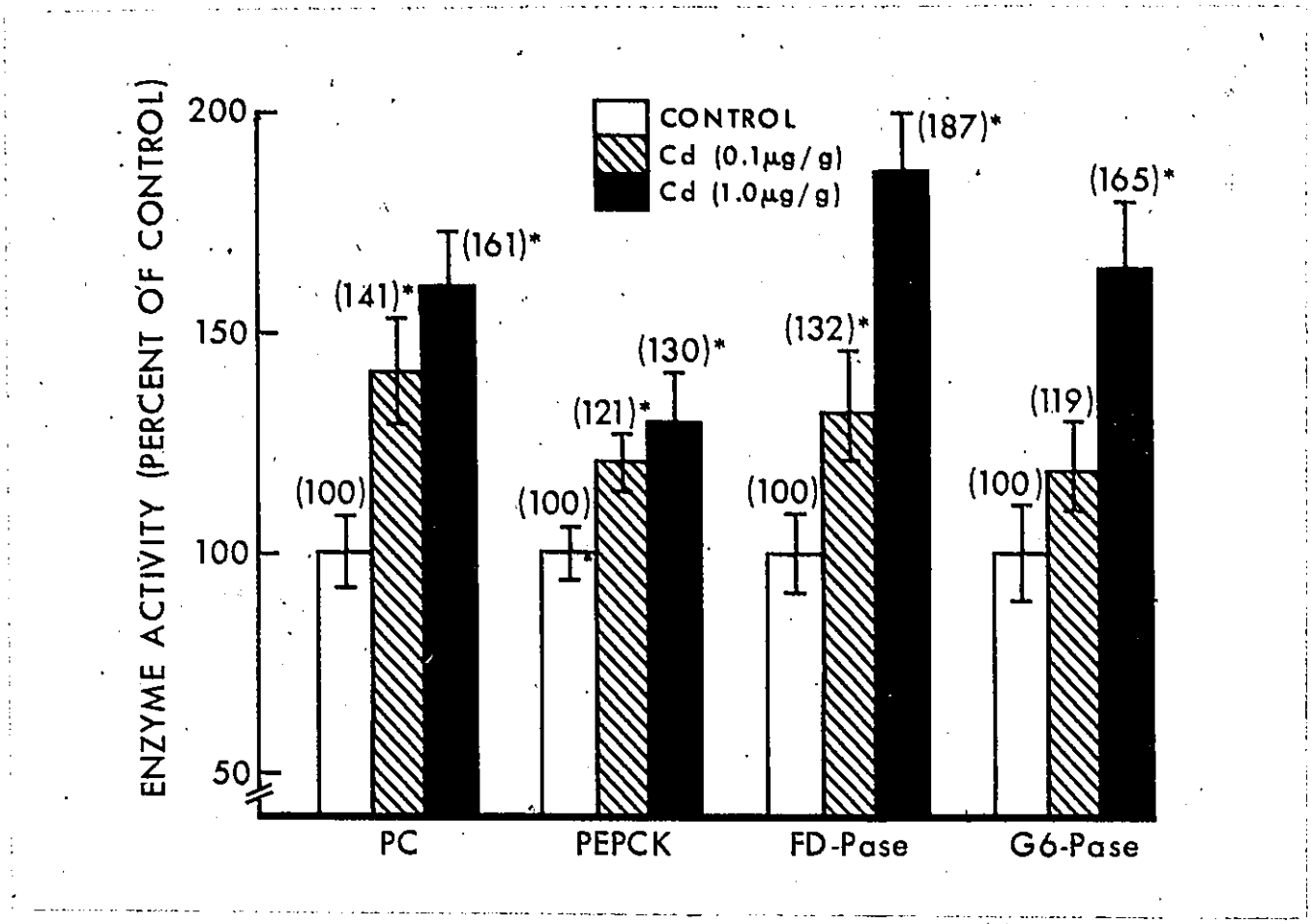

FIG. 21

Stimulatory effect of orally administered cadmium on hepatic gluconeogenic enzymes. Each column represents the mean ± s.e.m. of 5-6 values in the group. Neonatal rats were administered water (control) or cadmium (0.1 or 1.0 µg/g) as cadmium chloride solution for 45 days. Data are given in percentages (in parentheses) with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

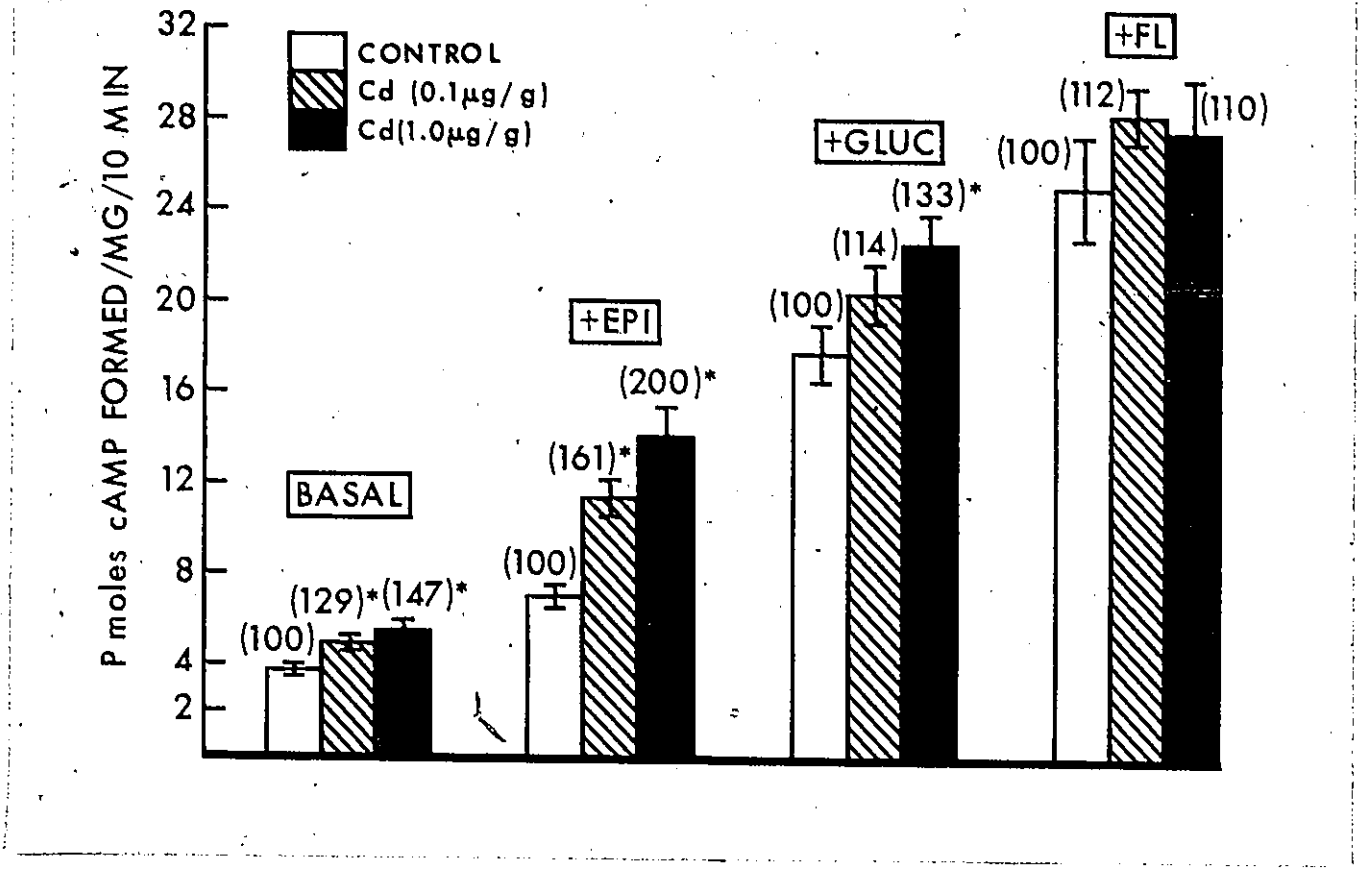

FIG. 22

Influence of chronic oral cadmium treatment on the responsiveness of hepatic adenylate cyclase to epinephrine (EPI), Glucagon (GLUC) or fluoride (FL). Each column represents the mean ± s.e.m. of 5-6 animals in the group. Neonatal rats were administered water (control) or cadmium (0.1 or 1.0 µg/g) as cadmium chloride solution by intubation daily for 45 days. Data are also given in percentages (in parentheses) with values from control rats taken as 100%.

*Significantly different from control values at $p < 0.05$.

levels also were altered by oral cadmium treatment. Data in Table 21 demonstrate that administration of cadmium (0.1 µg/g/day) for 45 days failed to significantly alter the basal serum IRI level. However, the maintenance of normal IRI levels in face of hyperglycemia suggested a possible decrease in the ratio of blood glucose to serum insulin indicating in a suppression of pancreatic insulin secretory activity. We therefore examined the influence of cadmium exposure on pancreatic IRI content. Although cadmium treatment failed to exert any significant effect on pancreatic weight, the IRI concentration of the pancreatic tissue was markedly reduced in rats exposed to this heavy metal.

G. Influence of Long Term Cadmium Ingestion on Insulin Secretion from Isolated Rat Islets

Subacute cadmium treatment was found to induce glucose intolerance (Figure 14) and impair the glucose-stimulated increase of serum IRI levels (Figure 15). Furthermore, since long term exposure to oral cadmium reduced pancreatic insulin as well as the insulinogenic index, the question whether cadmium-induced impairment of pancreatic function could be localized to the level of pancreatic islets also was investigated. Data in Table 22 illustrate the effect of oral cadmium treatment on IRI release from isolated islets in response to varying glucose concentrations. In the presence of 50 mg% glucose, the rate of IRI release from islets of control animals was not significantly different from that noted in rats exposed to low dose of cadmium (0.1 µg/g/day) for 45 days. However, in the presence of 150 mg% 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 cadmium-exposed rats. Similarly, in the presence of 300 mg% glucose, whereas the rate of IRI release from control islets increased by over 250%, this increment in the rate of IRI release from islets of low dose cadmium exposed animals was only 113%.

TABLE 21

SERUM INSULIN LEVELS AND ACID ETHANOL-EXTRACTABLE INSULIN CONTENT OF PANCREAS FROM CADMIUM-EXPOSED RATS

Each value represents the mean ± s.e.m. of 6 rats in the group. Rats were administered cadmium (0.1 µg/g/day) by intubation in the form of cadmium chloride solution for 45 days. Data are also given in percentages (in parentheses) with control values taken as 100%.

Treatment	Serum Insulin (µU/ml)	Pancreatic Insulin (U/g)
Control	38±2.9 (100)	4.27±0.49 (100)
Cd (0.1 µg/g/day) for 45 days	41±3.7 (108)	2.54±0.56 (60)*

*Significantly different from control values at $p < 0.05$.

TABLE 22

EFFECTS OF ORAL CADMIUM ADMINISTRATION ON INSULIN RELEASE FROM ISOLATED RAT ISLETS

Each value represents the mean ± s.e.m. of n incubations from 4 rats in the group; each n represents one batch of 5 islets. Rats were administered cadmium (0.1 or 1.0 µg/g/day) by intubation in the form of cadmium chloride solution for 45 days. Data are expressed as µunits of IRI released by 5 islets over the 90 min incubation period, as described under experimental procedures. Data are also given in percentages (in parentheses) with control values taken as 100%.

Treatment	Amount of Cadmium (µg/g)	Rate of Insulin Release in Presence of Varying Glucose Concentrations (µU/5 Islets/90 min)		
		Glucose (mg%)		
		50	150	300
Control	0.0	128±13 (n=20) (100)	270±14 (n=20) (100)	450±27 (n=20) (100)
Low Dose	0.1	112±15 (n=20) (88)	131±14 (n=20) (49)*	239±13 (n=20) (53)*
Control	0.0	135±14 (n=20) (100)	287±32 (n=19) (100)	549±53 (n=20) (100)
High Dose	1.0	132±20 (n=18) (98)	161±20 (n=18) (56)*	217±32 (n=18) (40)*

*Significantly different from control values at $p < 0.05$.

It is of interest that the islets obtained from rats exposed to the higher amount of cadmium (1.0 µg/g/day) for 45 days also elicited a similar suppression of IRI release in response to different glucose concentrations.

H. Influence of Long Term Cadmium Ingestion on Ultrastructure of Beta Cells in Isolated Islets

The ultrastructure of the beta cells in isolated islets from control rats appeared normal in comparison to the islets fixed in situ, as described by Munger (294) and Lacy (295). The beta cells in the isolated islets were limited by a continuous plasma membrane and contained round or oval nuclei (N), surrounded by double nuclear membranes. The cytoplasm contained an abundance of rough endoplasmic reticulum composed, in lamellar arrangement, of two membranes with ribonucleoprotein granules attached to their outer surfaces (Figure 23). Golgi complex was comprised of smooth membranous sacs that were usually found in perinuclear position. The remainder of the cytoplasm contained sparcely dispersed mitochondria and numerous secretory granules. The specific granules of the beta cells were individually enclosed by a definite agranular limiting membrane. Between the granule and the limiting membrane, there was frequently a clear space devoid of any detectable density. The granule itself usually occupied an eccentric position with respect to the limiting membrane, and was presumed to represent the storage form of insulin (295). The mitochondria of adult beta cells were frequently rod-shaped in profile and possessed numerous parallel cristae.

Similarly, beta cells of islets from rats exposed to cadmium (0.1 µg/g/day) for 45 days also were examined electron microscopically at ultrastructural level. Preliminary results indicated in Figure 24 demonstrate some degenerative changes in the beta cells, possibly explaining insular origin of marked hyperglycemic action of this heavy metal. The beta cells of cadmium-exposed rats appeared normal except for the frequent occurrence in the cytoplasm of circular

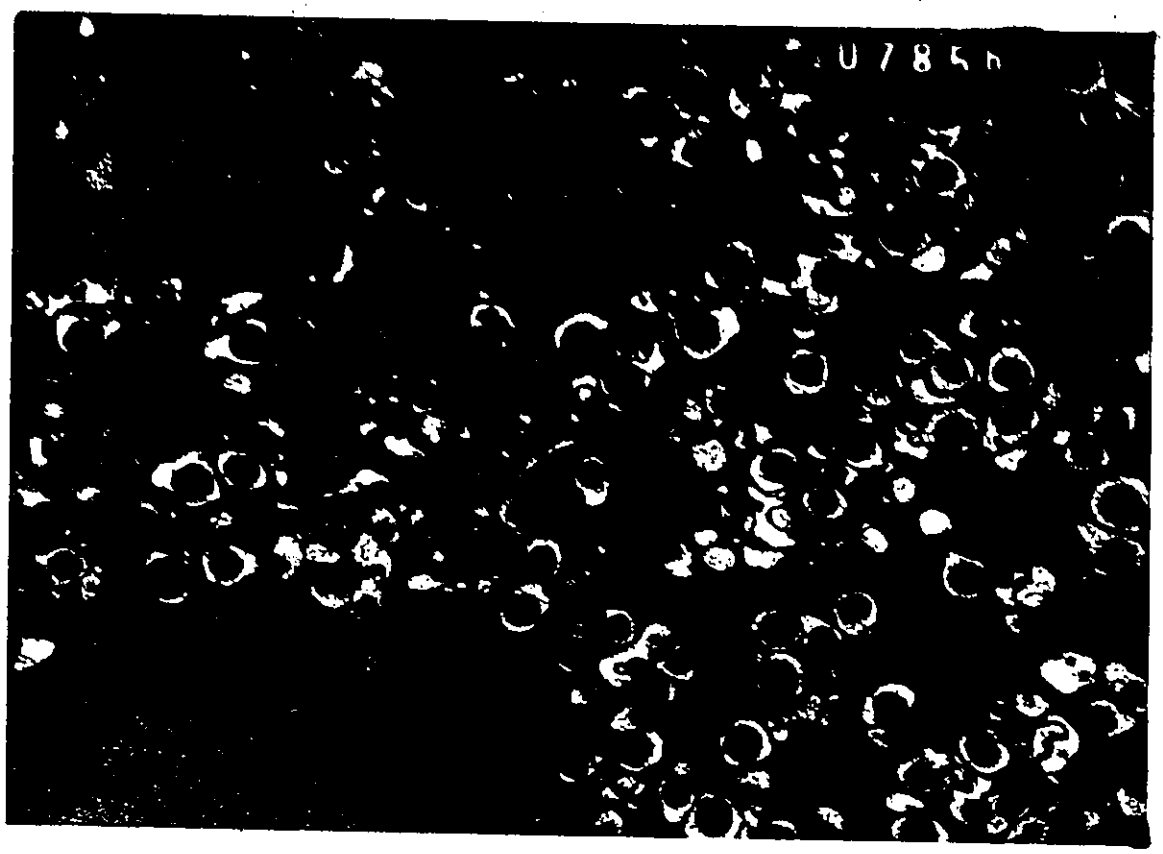

FIG. 23

Electron micrograph of a portion of a normal beta cell from isolated islet of 45-day old control rats. Beta cell nucleus (N) showed some condensation at the nuclear membrane (NM). Also present in the cytoplasm were rough endoplasmic reticulum (REM), secretory granules (SG), and mitochondria (MT). Glutraldehyde osmium fixation x 10,000.
(Prepared through the kind courtesy of Professor J. Metuzals, Department of Anatomy, University of Ottawa.)

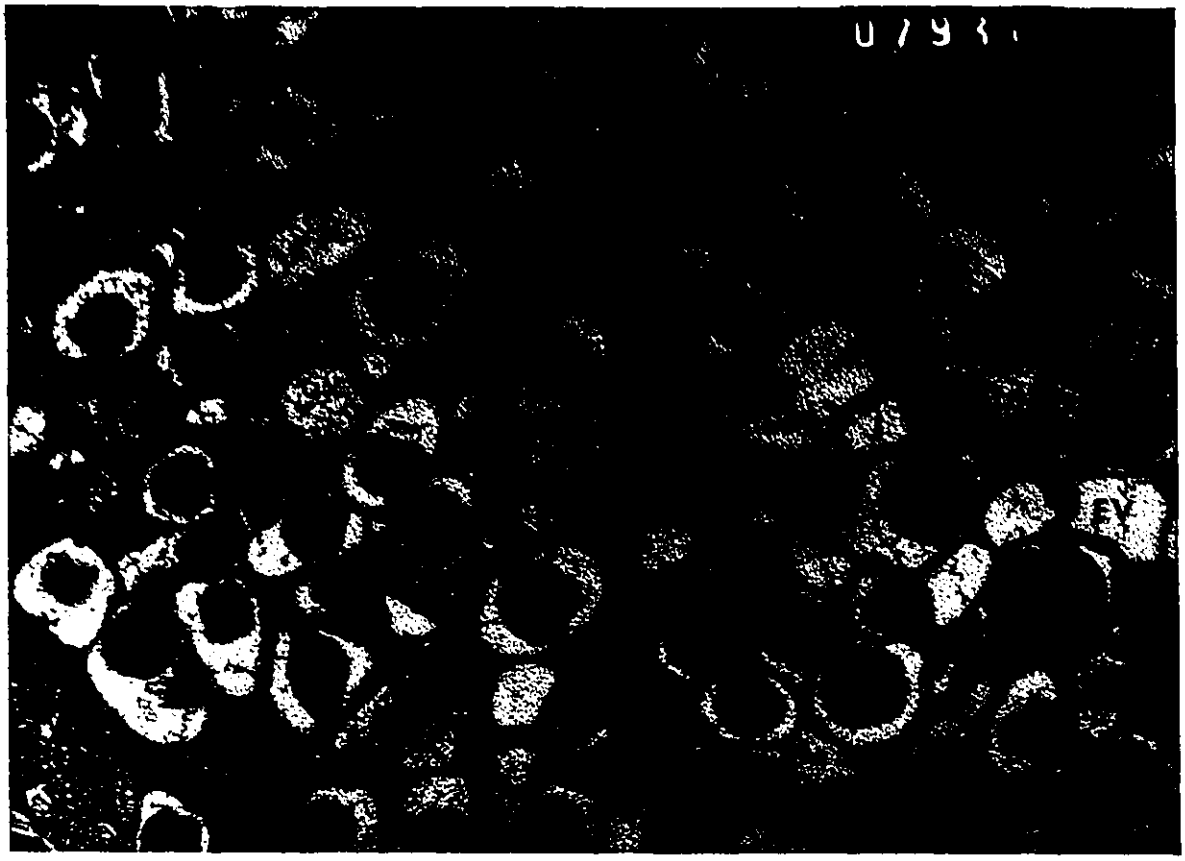

FIG. 24

Electron micrograph of a portion of a beta cell from islets isolated from cadmium-exposed rats. Neonatal rats were administered cadmium (0.1 µg/g) as a cadmium chloride solution through intubation daily for 45 days. The beta cells of islets from cadmium-exposed rats appeared normal except for the occurrence in the cytoplasm of circular membranes (SM) or rod-like structures (RS), enclosed by smooth limiting membrane as well as more frequent occurrence of "empty vacuoles" (EV). Glutraldehyde osmium fixation x 17,000. (Prepared through the kind courtesy of Professor J. Metuzals, Department of Anatomy, University of Ottawa.)

membranes (SM) or rod-like structures (RS) enclosed by smooth limiting membrane. These structures may represent a form of degenerating mitochondria. However, the possibility also exists that these may actually be lysosomes (Figure 24). Another interesting feature of the beta cells from cadmium-exposed rats was the more frequent occurrence of "empty vacuoles" (EV) devoid of any detectable density (Figure 24) which may represent degranulation of the secretory beta granules or saccules.

V. DISCUSSION

1. RESPONSE OF HEPATIC AND RENAL CARBOHYDRATE METABOLISM TO CADMIUM TREATMENT

The increasing environmental levels of cadmium, its cumulative nature and its toxicological potential, all warrant studies on the metabolic effects of this heavy metal. The results demonstrate that cadmium intoxication disturbs glucose homeostasis, in that parenteral administration of cadmium to rats reduces liver glycogen levels and increases the concentration of blood glucose. Moreover, chronic treatment with cadmium markedly elevates the gluconeogenic potential of liver, as indicated by augmentation in the activities of hepatic PC, PEPCK, FD-Pase and G6-Pase, the four key, rate-limiting enzymes involved in the formation of glucose from non-carbohydrate precursors. The observed enzymic increases as well as the changes in blood glucose and hepatic glycogen persisted even after discontinuation of cadmium administration for 4 weeks. Recently, prolonged cadmium treatment was found to lower the levels of hepatic glycogen through stimulation of the glycogenolytic enzyme, phosphorylase a (82,121). Our data are in accord with these findings and since the elevation in blood glucose and the depression of hepatic glycogen were apparent as early as 1 hr after the administration of cadmium, it is possible that the observed hyperglycemic response might be due, at least in part, to liver glycogenolysis. It is of interest that cadmium also causes the release of adrenal catecholamines (289). Since epinephrine enhances glycogenolysis, it is possible that glycogenolytic effect of cadmium is partly a reflection of catecholamine release from the adrenal medulla.

Evidence indicates that there is a close relationship between altered kidney and liver function and changes in the gluconeogenic capacity of these two tissues (217,221). It has been suggested that glycosuria is a consequence of lowered renal threshold to glucose (119). However, the mechanisms involved in the maintenance of normal or elevated blood glucose levels despite the

increased loss of glucose through the urine have not yet been fully clarified. Changes in gluconeogenesis are important to metabolic adaptations in man and other mammals, particularly in response to changing physiologic and pathologic conditions (179). Our study shows that although acute exposure to cadmium failed to stimulate gluconeogenic enzymes, exposure to the heavy metal for 45 days resulted in significant increases in the activities of the gluconeogenic enzymes, both in liver as well as in the kidney cortex.

It seems unlikely that the gluconeogenic process plays any significant role in the initial elevation of blood glucose seen at 1 hr after acute cadmium administration, but it is conceivable that the enhanced capacity of hepatic and renal cortical tissues to transform non-carbohydrate substrates into glucose contributes to the maintenance of elevated blood glucose levels following chronic exposure to this heavy metal. Our results also show that acute or chronic administration of cadmium produces a rise in the concentration of serum urea. Since urea represents the chief metabolic product of protein and amino acid catabolism (220), the present data seem to be consistent with the suggestion that cadmium alters protein metabolism. Indeed, administration of cadmium to rabbits has been reported to enhance production of the hepatic soluble protein metallothionein, retard growth rate and produce serum protein dyscrasias (82, 83). The observed elevation of serum urea in rats chronically exposed to cadmium could also be related to enhanced glucose synthesis from proteins and amino acids.

2. INFLUENCE OF CADMIUM TREATMENT ON CYCLIC AMP METABOLISM

Chronic cadmium treatment was shown to alter glucose homeostasis by enhancing the conversion of glycogen into glucose through activation of phosphorylase a (82), as well as by elevating the glucose-synthesizing capacity of the liver and kidney cortex by stimulating the activities of the four key

gluconeogenic enzymes. Likewise, administration of cyclic AMP not only stimulated glycogenolysis by activating phosphorylase a, but also enhanced the process of gluconeogenesis by stimulating the various gluconeogenic enzymes (179). Recent investigations on the mechanism by which cyclic AMP exerts its diverse effects seem to have concentrated on the ability of this nucleotide to stimulate protein kinase activity (239,249,298,290). Cyclic AMP acts as a mediator in the glycogenolytic effect of epinephrine in liver and this effect has been shown to be mediated by activation of phosphorylase kinase. Chambaut et al. (298) proposed that in liver, like several other tissues, cyclic AMP acts on protein kinase by binding to a receptor subunit leading to dissociation of the enzyme into active catalytic subunit and cyclic AMP-receptor complex. It has been presumed that alterations in the level of cyclic AMP result in a concomitant change in the activity of cyclic AMP-independent or the active form of the enzyme. The change in kinase activity consequently alters the rate of phosphorylation of various proteins such as histones, ribosomes and enzymes.

The present study demonstrates that chronic exposure to cadmium significantly elevates the cyclic AMP levels of hepatic tissue. The increase in the endogenous cyclic AMP concentration is also reflected by a decrease in the cyclic AMP-binding capacity of hepatic protein kinase. Although the low dose of cadmium failed to produce any significant change in the activities of either cyclic AMP-dependent or independent forms of the enzyme, treatment with the higher dose markedly reduced kinase activity, assayed in the presence of cyclic AMP. Furthermore, the kinase activity ratio, an indication of the relative activity of cyclic AMP-dependent and independent protein kinase, was also reduced by this dose of the heavy metal. It seems probable that the cadmium-induced alterations in hepatic carbohydrate metabolism might be related to modulation of the cyclic AMP-protein kinase system. In sharp contrast to

this tissue, cadmium treatment led to a significant decrease in the cyclic AMP levels of the renal cortex, which was accompanied by an increase in the cyclic AMP binding capacity of protein kinase. While the 0.25 mg/kg dose failed to appreciably change the kidney cortex protein kinase ratio, treatment with the higher dose of cadmium increased it. It is difficult at present to reconcile these findings since both cadmium as well as cyclic AMP have been found to produce marked enhancement of renal gluconeogenic capacity (265). However, the possibility exists that the observed decrease in kidney cortex cyclic AMP may be due to enhanced breakdown by phosphodiesterase and/or increased urinary excretion of this nucleotide due to tubular damage. Indeed, chronic exposure to cadmium in man and experimental animals has been found to produce renal tubular syndrome which often includes proteinuria, glycosuria, aminoaciduria, hypercalciuria, impaired concentrating ability and altered acid excretion. Furthermore, although the classic observations of Sutherland and co-workers provided strong evidence for the role of cyclic AMP in the action of epinephrine on hepatic glycogenolysis, recent observations (272,273) suggest the existence of an additional mechanism(s) for catecholamine action on liver carbohydrate metabolism. These recent data would seem to support the view that epinephrine stimulates hepatic glycogenolysis and gluconeogenesis partly by a mechanism which does not involve a rise in intracellular levels of cyclic AMP (273).

Cyclic AMP, which mediates the action of a number of hormones, has also been shown to control the regulation of a variety of cellular functions. There is evidence indicating that cyclic AMP may be involved in the regulation of cellular function of male and female reproductive accessory organs (299). Since testes are very sensitive to the toxic effects of cadmium, it was of interest to examine the influence of chronic cadmium treatment on testicular adenylate cyclase-cyclic AMP-protein kinase system. Furthermore, as the accessory reproductive tissues are dependent on testicular biosynthesis of

androgens for maintenance of their glandular activity, the influence of this heavy metal was investigated on cyclic AMP metabolism of the prostate gland. The present study shows that chronic treatment with cadmium produced a significant increase in testicular adenylate cyclase activity without any change in the endogenous levels of cyclic AMP. The lack of any significant alteration in testicular cyclic nucleotide may have been due to the concomitant increase in its metabolism by phosphodiesterase. This was also reflected in the unaltered binding capacity of testicular protein kinase preparations obtained from cadmium-treated rats. However, the activities of both the cyclic AMP-dependent and the -independent forms of protein kinase were reduced following heavy metal administration. It is of interest that cessation of cadmium administration for 28 days following a 45-day period of daily cadmium exposure failed to fully restore the testicular cyclic AMP metabolism. Cyclic AMP has been implicated as an intracellular "second messenger" in the actions of gonadotropins on testosterone synthesis in mammalian testes (300). In addition, cyclic AMP has been found to enhance the phosphorylation of certain histones in several body tissues including the testes and the prostate glands (301,302). The decrease in the activities of both forms of protein kinase may exert a pronounced effect on the control of DNA replication and transcription. Lee and Dixon (154) studied the effect of cadmium on spermatogonia and found that the rate of incorporation of thymidine into DNA was markedly decreased following a single dose of 1 mg/kg of the heavy metal, suggesting that cadmium inhibited the testicular DNA synthesis. In addition, recovery from infertility following exposure to a single dose of cadmium was prolonged indicating that both the replicating and the differentiating stem cell populations may have been affected by this heavy metal. In the prostate, daily injections of 1 mg/kg of cadmium over a 45-day interval markedly reduced tissue levels of cyclic AMP. This response could be attributed to decreased adenylate cyclase since degradation of cyclic AMP by phosphodiesterase to 5'-AMP was not significantly altered.

Cadmium caused a marked increase in the availability of cyclic AMP binding sites to protein kinase preparations of the prostate gland. However, the increase in protein kinase binding capacity probably represents a shift in the equilibrium between free and bound cyclic AMP in response to a reduction in the endogenous pool of cyclic AMP as reflected by an increase in the total number of free receptor sites present in the tissue. Despite the low levels of prostate cyclic AMP, the activities of both the cyclic AMP-dependent and the independent forms of protein kinase were increased. This response is of particular interest in light of the fact that the prostate gland underwent a mild atrophy in response to cadmium treatment. However, a similar response involving both cyclic AMP-dependent and independent protein kinase has been reported in the atrophied prostate gland following castration (303). Discontinuation of treatment for 28 days in rats previously exposed to cadmium reduced the heavy metal induced alterations in prostatic cyclic AMP. However, in the case of cyclic AMP binding by protein kinase, as well as the activities of both the cyclic AMP-dependent and the independent forms of the enzyme, the increases in the "treated" group were reduced to even lower than the control values following heavy metal withdrawal. It seems that organ atrophy and impaired cyclic AMP metabolism are important cadmium-induced biochemical alterations in the rat prostate gland, although the possibility that these changes might reflect impaired testicular testosterone biosynthesis and subsequent prostatic regression cannot be excluded. This may be particularly important in the light of recent evidence which indicates that cyclic AMP plays an intermediary role in androgenic responses of the prostate gland (304). However, the degree of prostatic atrophy seen following cadmium treatment was not as marked as that observed after hypophysectomy or castration suggesting that some testosterone stimulation of secondary reproductive organs was still occurring.

Cadmium is known to either activate or inhibit a large number of enzyme systems *in vivo* and *in vitro* (25). Even though cadmium affects cyclic AMP metabolism, the exact biochemical consequences of cadmium-induced alterations in cyclic AMP levels and protein kinase activity of rat testes and prostate glands are difficult to assess. Lee and Dixon (154) found that cadmium treatment inhibited the thymidine uptake into spermatogonial cells of the testes and suggested that cadmium might interact with both phosphate and base sites of DNA and thereby affect the unwinding and rewinding of DNA which is essential for replication and transcription. It is conceivable that cadmium-induced alterations in cyclic AMP metabolism might regulate gene expression through cyclic AMP-dependent phosphorylation of nucleoproteins by protein kinase (301), in a manner similar to that shown by Langan (249) for the action of glucagon on cyclic AMP metabolism and on the subsequent biosynthesis of hepatic proteins.

3. THE INHIBITORY EFFECT OF CADMIUM ON PANCREATIC SECRETORY ACTIVITY: GLUCOSE HOMEOSTASIS

The pancreas has been found to accumulate fairly high concentrations of cadmium, surpassed only by those of kidneys and liver. This is of particular interest since reports indicate that cadmium has injurious effects on the cellular architecture as well as on the functional capacity of the pancreatic tissue. Voinar (124) was the first to demonstrate that administration of cadmium (i.v.) into rabbits produced hyperglycemia and decreased the hypoglycemic and convulsant effects of insulin. Furthermore, Barbieri *et al.* (125) observed that cadmium treatment induces alterations of pancreatic function. Upon histological examination, they found evidence of an increased ratio of alpha to beta cells. This shift in the cell type ratio was considered to be indicative of diminished insulin-secretive activity. In concordance with this report, Havu (126) found that in fish (Corpus Scorpius), intramuscular injection of cadmium resulted in accumulation of this heavy metal in the islet

tissue. Furthermore, it was found that like alloxan, cadmium accumulation was associated with necrotic lesions of beta cells, hyperglycemia and glycosuria. Although all these reports implicated altered insulin secretory activity, it was not until 1973 that Ghafghazi and Mennear (127) demonstrated that administration of a single dose of cadmium (6 mg/kg, i.p.) produced glucose intolerance in intact mice, that was associated with a decreased pancreatic secretory activity as evidenced by decreased insulinogenic indices in cadmium-treated mice.

As indicated earlier, chronic (45 days) administration of cadmium (0.25 or 1.0 mg/kg/day) was found to result in enhancement of hepatic gluconeogenic enzymes, cyclic AMP levels and blood glucose concentration. It has been observed that insulin deficiency caused either by insulin antiserum or alloxan treatment also increases blood glucose, cyclic AMP as well as the rate of gluconeogenesis in the liver (179,250,255). These observations, together with the facts that pancreatic tissue has an extremely high affinity for cadmium (77,82) and that decreased pancreatic function has been reported in man and mouse (127,133), suggested that metabolic alterations caused by exposure to cadmium might be due, at least in part, to lack of insulin. To examine this possibility, a 7-day multiple dosing schedule of cadmium administration was selected which resulted in certain changes in carbohydrate metabolism resembling those produced by the chronic (45 day) mode of cadmium administration. This subacute exposure of rats to cadmium (1 mg/kg, twice daily for 7 days), like the chronic treatment, produced hyperglycemia, augmentation of the activities of PC, PEPCK, FD-Pase and G6-Pase as well as an elevation of hepatic cyclic AMP levels. Results obtained in these experiments also demonstrated that hyperglycemia in rats produced by subacute cadmium treatment was indeed accompanied by hypoinsulinemia. In addition, cadmium-induced suppression of pancreatic function was further unveiled upon administration of a glucose load;

in contrast to control animals, cadmium-exposed rats demonstrated a marked glucose intolerance that was associated with a significant decrease in the glucose-stimulated insulin release. Consequently, when the data were expressed in terms of insulinogenic indices, a marked reduction in this ratio was noted in cadmium pretreated rats, suggesting that cadmium produces a decrease in pancreatic secretory activity. Similarly, calculation of the total amount of insulin released in the 1 hr period following glucose load demonstrated marked suppression of insulin release in cadmium-intoxicated animals as compared to controls. These observations agree, at least in part, with those of Ghafghazi and Mennear (127) who found that subacute administration of cadmium (4.0 mg/kg/day; 15 days) significantly decreased the resting IRI concentrations of mice. Although no glucose intolerance was observed by these investigators, it was pointed out that due to cadmium-induced renal damage, enhanced urinary glucose excretion could have masked the glucose intolerance. Indeed, Ithakissios and co-workers (128) reported that although chronic cadmium administration failed to produce persistent hyperglycemia during glucose tolerance test, animals excreted significantly more glucose in their urine than did the control animals.

Although cadmium suppresses pancreatic function, the precise biochemical mechanism of this action remains unknown. Since cadmium has been reported to release catecholamines from adrenals (289), and catecholamines can influence insulin secretion as well as glucose levels, it was of interest to examine if suppressed pancreatic secretory activity was due to increased catecholamine release or due to some other effect on the pancreas. Administration of 20 mg/kg of phentolamine, an α-adrenergic blocking agent, caused an increase of 266% in IRI levels of normal rats, whereas the same dose caused only an 83% rise in cadmium-exposed animals. A similar trend was observed when rats were given a lower dose of phentolamine (10 mg/kg). Pancreatic beta cells contain α-receptors, the stimulation of which suppresses insulin

release. On the other hand, pancreatic beta cells also contain β-receptors which when stimulated, increase the release of this hormone. Phentolamine stimulates the release of insulin by blocking the α-suppressive effect and unmasking the β-stimulatory effect of the circulating catecholamines. Our results demonstrate that the normal sharp increase in serum IRI levels in response to phentolamine is substantially inhibited in cadmium-exposed rats. This indicates that suppressed pancreatic function could not be due to the increased catecholamine secretion alone, because the unmasking of β-receptors would lead to insulin secretory response of a greater magnitude, when circulatory levels of catecholamines are enhanced. Our data are in accord with those of Ghafghazi and Mennear (127) who demonstrated that adrenalectomy did not prevent the cadmium-induced glucose intolerance. More recently, Ghafghazi and Mennear (129) provided further evidence to demonstrate that cadmium has direct inhibitory effect on the ability of pancreas to secrete insulin. They reported that perfusion of the isolated rat pancreas with cadmium (1×10^{-3} and 5×10^{-4} M) inhibited the insulin secretory response to glucose (300 mg%), indicating that cadmium may be inflicting damage directly at the level of pancreatic insulin secretory mechanism(s).

4. EFFECTS OF CHRONIC ORAL CADMIUM ADMINISTRATION ON NEW-BORN RATS

The present study demonstrated that parenteral administration of cadmium induced biochemical or functional lesions in several organ systems, including the hepatic, renal, pancreatic and testicular tissues. However, chronic cadmium poisoning in man is usually the result of inhalation or ingestion, presenting a rather slow but continuous exposure. Furthermore, Murthy and Rhea (58) have reported that human milk contains, on the average, 0.019 ppm of cadmium. It was therefore of interest to examine if chronic exposure of new-born rats to oral cadmium would produce the functional or biochemical lesions which were

observed following the parenteral administration of cadmium. In order to stimulate a more physiological mode of cadmium exposure as well as monitoring the amount of cadmium administered, new-born rats were given cadmium (0.1 or 1.0 µg/g) by intubation as a cadmium chloride solution (approximately 0.005 l/g). Administration of a low dose of cadmium (0.1 µg/g) for 45 days failed to appreciably alter the growth rate of new-born rats; however, the higher dose (1.0 µg/g) resulted in a slight but significant suppression of body weight gain. Like chronic injection of cadmium, oral administration for 45 days resulted in hyperglycemia, uremia and diminution of hepatic glycogen. Furthermore, orally administered cadmium elevated the activities of hepatic gluconeogenic enzymes, cyclic AMP levels as well as the adenylate cyclase activity. The responsiveness of hepatic adenylate cyclase to stimulation by epinephrine and glucagon also was similarly enhanced by oral cadmium.

In contrast to the parenteral mode of cadmium exposure, oral administration of cadmium (0.1 µg/g/day) for 45 days failed to significantly alter the basal IRI levels. However, the maintenance of IRI levels to basal values in light of the observed hyperglycemia could be indicative of suppressed pancreatic function. Indeed, determination of pancreatic IRI content revealed that in the cadmium-exposed rats, there was a marked reduction of endogenous IRI levels. This means that the cadmium-induced suppression of pancreatic function may be due to a reduction in the synthesis and/or release of insulin. In order to assess the responsiveness of the islets of Langerhans to glucose, islets isolated from the pancreas of control as well as cadmium-exposed animals were incubated in vitro and their rate of insulin release was determined in the presence of varying concentrations of glucose. In presence of 50 mg% glucose, there was no significant difference in the rate of IRI release. However, in presence of higher glucose concentrations (150 mg% and 300 mg%), the islets from cadmium-exposed rats released significantly less IRI than that from control

animals. These experiments indicate that long-term exposure to oral cadmium may indeed inflict lesions at the level of pancreatic islets and more specifically, at the level of the pancreatic beta cells. Recently, Ithakissios et al. (130) studied the effect of multiple injection of cadmium on insulin secretion from perfused rat pancreas. They found that whereas administration of 0.25 mg/kg dose of cadmium every second day for 70 doses failed to significantly alter the amount of insulin secreted from perfused pancreas, 70 doses of 0.5 mg/kg caused a marked inhibition in the amount of insulin secreted in response to glucose stimulation. It is of interest that earlier studies had presented evidence indicating that cadmium possessed a marked pancreotoxic potential. Barbieri et al. (125) reported that cadmium administration resulted in accumulation of this metal in the rabbit pancreas and that this accumulation was accompanied by a decrease in the ratio of beta to alpha cells. In addition, Havu (126) observed necrotic lesions of pancreatic beta cells in fish, Cottus scorpius, injected with cadmium. Our 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. (125). The observation that ethanol-extractable insulin content of the pancreas was reduced following chronic cadmium exposure lends additional support to this hypothesis. However, impaired insulin synthesis could also result in lowered pancreatic insulin content.

In order to confirm structural viability of the beta cells following the isolation of the islets, electron microscopic examination was carried out. The beta cells from control islets appeared normal and contained intact organelles including numerous secretory granules. However, preliminary examination of beta cells from cadmium-exposed rats revealed some degenerative changes. The beta cells from cadmium-exposed rats appeared normal except for the frequent

occurrence in the cytoplasm of structures resembling a form of degenerating mitochondria. Furthermore, there was a more frequent occurrence of "empty vacuoles" that appeared to be secretory saccules devoid of the dense granules that are supposed to contain insulin. These observations may possibly provide further evidence for insular origin of marked hyperglycemic effect of cadmium. Insulin release is always thought of as being strictly a calcium-dependent phenomenon (305). At a critical cytosolic calcium concentration, an interaction between calcium ion and the microtubular-microfilamentous system is presumed to result in emiocytosis of the insulin secretory granules (305,306). Elevation of cytosolic calcium can be achieved by uptake of extracellular calcium and/or translocation of intracellular calcium from vacuolar system (306). Recently, Ghafghazi and Mennear (129) demonstrated that perfusion of isolated rat pancreas with cadmium inhibited insulin 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 and Mennear (129) hypothesized that cadmium-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 was obtained in the same laboratory when Esposito-Avella (307) demonstrated that incubation of isolated mouse islets with cadmium (1×10^{-5} and 1×10^{-7} M) and glucose (300 mg%) reduced the uptake of ^{45}calcium. Mitochondria constitute the major source of intracellular calcium available for translocation into the cytosol. Thus, if following chronic cadmium exposure, pancreatic damage extends to the level of the beta cell mitochondria, it remains possible that cadmium may not only be interfering with calcium uptake, but that it may also be interfering with the translocation of intracellular calcium.

5. PROTECTIVE EFFECT OF SELENIUM ON CERTAIN HEPATOTOXIC AND PANCREOTOXIC MANIFESTATIONS OF SUBACUTE CADMIUM ADMINISTRATION

The biological actions of metals, both essential and non-essential, are often heavily conditioned by metal ion antagonism, i.e. one metal may induce a biological effect by altering the requirements for another metal(s) (25). The work of Parizek (135,292) first demonstrated the effectiveness of zinc in preventing the cadmium-induced testicular damage. Since then, several other manifestations of cadmium toxicity have also been reported to be either prevented or reversed by the administration of zinc, including hypertension (161), teratogenic effect (293) and carcinogenic effect (146). Furthermore, it has now been recognized that cadmium effects can also be profoundly altered or abolished by several other agents including selenium (147), copper (308), calcium (65) or iron (309). The biochemical basis of such antagonistic effects are still largely unexplored, as are their roles in manifestations of toxicity of heavy metals in man (25).

Mason and Young (147) have shown that selenium is approximately 100 times as effective as zinc in preventing testicular injury produced by cadmium. Since cadmium administration altered pancreatic function and carbohydrate metabolism in the rat, it was of interest to assess the metabolic effects of selenium and to investigate if selenium afforded any protection to the various metabolic and functional alterations caused by cadmium. Selenium, when given subacutely to rats, failed to alter either the gluconeogenic potential or the cyclic AMP levels of hepatic tissue. Schearer (310) similarly reported that selenium-administration does not cause changes in the concentrations of glycolytic or citric acid cycle intermediates in rat liver. However, our experiments demonstrate that when selenium is administered simultaneously with cadmium, it completely prevents the cadmium-induced activation of the four key gluconeogenic enzymes. Similarly, although selenium treatment by

itself failed to change the resting blood glucose or serum IRI levels, when administered concurrently with cadmium, it significantly improves the cadmium-induced suppression of serum IRI and the elevation of blood glucose. Nevertheless, simultaneous administration of these two elements failed to significantly reduce the cadmium-induced elevation of hepatic cyclic AMP levels. Pretreatment with selenium and cadmium also significantly ameliorated the glucose tolerance and insulinogenic indices, indicating improved pancreatic secretory activity. The mechanism by which selenium affords partial protection to cadmium-induced pancreatic damage and altered hepatic metabolism is, at present, obscure. Cadmium has high affinity for sulfhydryl (SH) groups. Also, SH inhibitors including alloxan and cadmium chloride have been reported to induce selective necrosis in the beta cell region (126). Therefore, as has been postulated by Gunn et al. (153) for the testes, it is possible that selenium temporarily complexes with cadmium preventing sufficient free cadmium from reacting with pancreatic SH groups. Scott (311) reported that chicks fed selenium-free diet showed severe degeneration and fibrosis of the pancreas even when diet was supplemented with all nutrients known to be required. This observation points to another possibility that cadmium might in some manner be interfering with the availability of selenium, essential for pancreatic integrity, which is partially overcome by administration of exogenous selenium. Still another mechanism of the protective effect of selenium can be derived from the observation that SH-inhibitors, including alloxan and cadmium chloride reduce the amount of pancreatic zinc (126). Zinc deficiency has been reported to markedly suppress pancreatic function (312) and selenium, either by direct competition or indirectly by protecting SH groups from free cadmium might spare zinc which is essential for normal pancreatic function.

6. PREVENTION BY ZINC OF CADMIUM-INDUCED ALTERATIONS IN PANCREATIC AND HEPATIC FUNCTIONS

Cadmium is closely related to zinc and is found wherever zinc is found in nature. Although cadmium and zinc have similar chemical properties, each element affects the mammalian organism diversely and is handled differently by it. Zinc is an essential trace element necessary for the maintenance of normal biochemical functions and whose absorption and turnover are under homeostatic regulatory mechanisms (24). Cadmium, on the other hand, is a highly toxic, non-essential element that does not obey homeostatic control and accumulates in the organism with age (46,313).

Since Parizek (135) demonstrated that cadmium-induced testicular degeneration could be prevented by zinc, it was of interest to examine the subacute effects of zinc as well as its ability to prevent the cadmium-induced alterations in pancreatic and hepatic functions. The results reported in this study demonstrate that the toxic effects of cadmium on hepatic metabolism and pancreatic function can be effectively prevented by simultaneous administration of zinc. In rats, subacute cadmium treatment resulted in suppression of pancreatic function as demonstrated by marked reduction of glucose tolerance associated with a decrease in the glucose-stimulated insulin release. The mechanism by which cadmium suppresses the insulin secretory response is not clear.

Considerable attention has been directed to the possible importance of zinc in the aetiology of diabetes. There appears to be significantly less zinc concentrated in the diabetic pancreas in comparison to the normal tissue (314). Furthermore, several investigators have reported decreased glucose tolerance and glucose-stimulated insulin release in zinc-deficient rats (24, 312). This impairment in zinc-deficient animals appears to be similar to that reported in the present study for cadmium-intoxicated rats and is of particular interest in view of the present finding that the pancreotoxic

effects of cadmium were prevented by simultaneous administration of zinc. This suggests that cadmium, as an "antimetabolite" of zinc may be suppressing the pancreatic function either by replacing functional zinc or by altering the availability of the essential trace metal; the effect(s) being overcome by the exogenously administered zinc.

There is evidence that SH groups play an important role in the mechanism(s) leading to the release of stored insulin from pancreatic beta cells (126, 315-317). These observations are of interest since like alloxan (a potent diabetogenic agent), cadmium is also a potent SH inhibitor (126). The exact mechanism by which the interaction with pancreatic SH groups results in altered insulin secretory activity is not known. However, studies indicate that both the mono- and dithiol-inhibitors (including cadmium) are potent zinc releasers in sculpin islets (126). Since zinc-thiol interactions are known to occur in biological systems, it is possible that exogenous zinc might be affording protection against cadmium-induced pancreatic damage by interacting with SH groups (and thus preventing SH-cadmium interaction and the consequent loss of zinc). As pointed out earlier, it is of interest that administration of selenium simultaneously with cadmium also partially prevented the cadmium-induced pancreatic damage. It was suggested that selenium might afford protection to the pancreas by preventing the cadmium-SH interactions and consequently sparing cadmium-induced zinc loss. This hypothesis may represent a common mechanism by which two diverse elements, zinc and selenium, might act to prevent the cadmium-induced pancreatic damage.

Insulin reduces the blood glucose level not only by increasing the membranal transport of sugars, but also by enhancing the conversion of glucose into glycogen and triglycerides and decreasing the hepatic glucose production (318). Conversely, insulin deficiency caused either by insulin anti-serum or alloxan treatment increases blood glucose, cyclic AMP as well as glucose

synthesis in the liver and reduces its glycogen content (179,250,255). Since these observations appear to be similar to those noted in cadmium-intoxicated animals, it seems likely that the effects of cadmium treatment may be due, at least in part, to a lack of insulin. Furthermore, the ability of zinc to prevent various cadmium-induced hepatotoxic effects may be related indirectly to its protective effect against cadmium-induced insulin diminution.

Cadmium treatment resulted in increased serum urea level. Since cadmium is known to cause kidney damage (82), the high blood urea level could be due, at least in part, to renal damage. Simultaneous administration of zinc also prevented cadmium-induced uremia.

It has been postulated that cadmium is rendered innocuous by its rapid incorporation into a protein called metallothionein whose synthesis is stimulated by cadmium (319). Recent studies also suggest that zinc may stimulate the formation of a metallothionein-like protein, which in addition to concentrating zinc, may also sequester cadmium (320). This may therefore represent another detoxifying mechanism.

Cyclic AMP is believed to play an important role in processes of gluconeogenesis as well as glycogenolysis (226,321). In the present study, cadmium caused a significant elevation in hepatic cyclic AMP content. Since administration of cyclic AMP has also been found to increase blood glucose and urea levels, enhance synthesis of glucose from non-carbohydrate precursors and lower hepatic glycogen concentration (234,250,251,266), it would appear that the cadmium-induced changes in hepatic carbohydrate metabolism resemble those produced by cyclic AMP. Furthermore, since insulin deficiency has been found to elevate the hepatic cyclic AMP concentration (179), the observed increase in cyclic nucleotide levels may be related to the cadmium-induced insulin deficiency.

The cadmium-induced elevation of hepatic cyclic AMP was accompanied by stimulation of the basal, epinephrine as well as glucagon-stimulated form(s) of adenylate cyclase. However, the responsiveness of the enzyme to fluoride remained unaltered. This is concordant with the view that fluoride ion acts through some direct action on the catalytic component and not through the hormone-discriminators (322). Intoxication with cadmium increased the responsiveness of hepatic adenylate cyclase to epinephrine by a greater degree than to glucagon. This may be relevant to the recent finding that the state of diabetes increases the epinephrine-sensitive adenylate cyclase activity of rat liver (323). Possibly, the protective effect of zinc against cadmium-induced changes in hormone-sensitivity of hepatic adenylate cyclase may be secondary to its prevention of pancreatic damage by cadmium.

In conclusion, exposure to cadmium produces marked alterations in carbohydrate metabolism of kidney cortex and hepatic tissue. Glucose homeostasis is a complex phenomenon involving interplay between several hormones and various metabolic pathways. The present investigation demonstrates that cadmium administration causes disturbances in glucose homeostasis. In order to help visualize the possible sites of cadmium action, a general scheme is presented in Figure 25 showing some of the possible control points which may be affected by cadmium exposure. Glucagon, epinephrine and cortisol represent three of the five major hormones known to raise blood glucose level which may include synthesis of new glucose from non-carbohydrate precursors (gluconeogenesis) or breakdown of liver glycogen (glycogenolysis). Both of these processes involved in glucose production are enhanced in animals treated with cadmium as is the release of adrenal medullary hormone, epinephrine. It remains possible that release of glucagon or cortisol may similarly be altered by cadmium. Cyclic AMP is known to promote hepatic glucose production through enhanced substrate uptake, gluconeogenesis and glycogenolysis. Cadmium also enhances cyclic AMP formation. Following cadmium treatment, the responsiveness

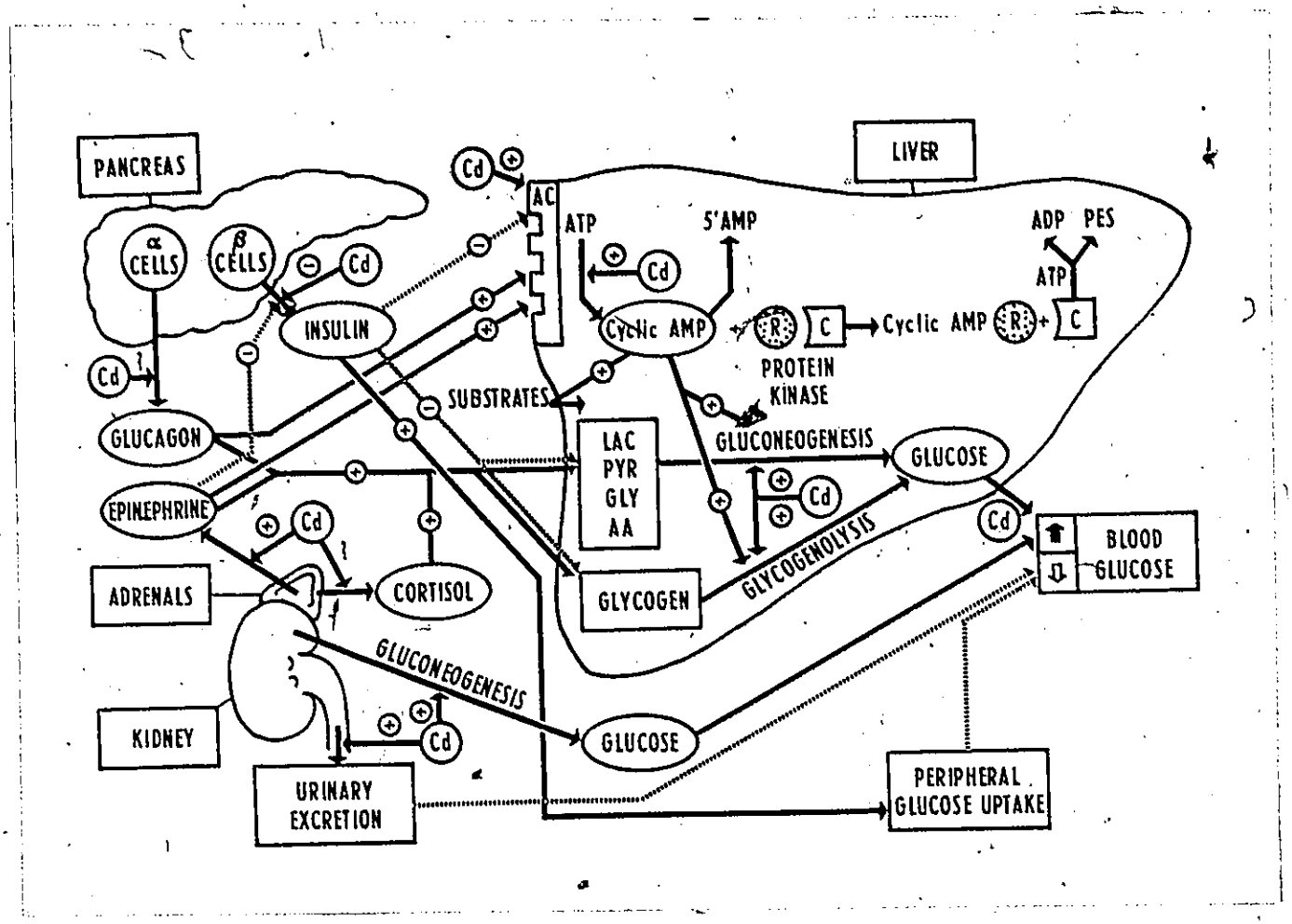

FIG. 25

Hormonal interplay in glucose homeostasis; possible sites of cadmium action. Stimulation of an enzyme, pathway or release process is indicated by a (+) symbol whereas the inhibition by a (-) symbol. The scheme represents some of the possible control points where cadmium may be exerting its effect(s) to produce disturbances in glucose homeostasis.

of adenylate cyclase to epinephrine and glucagon is also enhanced. Insulin is the only hormone that acts to lower blood glucose levels. This hormone not only promotes the utilization of glucose by facilitating its entry into cells, but it also stimulates the polymerization of glucose into glycogen. Furthermore, insulin inhibits some of the effects of glucagon or epinephrine on glucose production as well as cyclic AMP synthesis. One of the important effects of cadmium seems to be its ability to depress pancreatic insulin production and/or release. Renal tissue also plays an important role in glucose excretion and hence may act to reduce blood sugar level; however, the gluconeogenic potential of renal cortex is also enhanced following cadmium exposure.

The present investigation demonstrates that cadmium possesses a wide toxicological potential as it can produce biochemical or functional lesions in hepatic, renal, pancreatic, prostatic as well as testicular tissues. Exposure to cadmium disturbs glucose homeostasis in that this metal reduces liver glycogen levels, increases the concentration of urinary and blood glucose and enhances the gluconeogenic potential of liver and kidney cortex (Figure 25). The alterations in hepatic carbohydrate metabolism resemble those produced by exogenously administered cyclic AMP and may indeed be associated with and probably triggered by stimulation of cyclic AMP synthesis seen in response to cadmium treatment. It is also of interest that cadmium-induced alterations in glucose homeostasis resemble those caused either by insulin anti-serum or alloxan treatment. The diabetogenic effects of cadmium may therefore be related to abnormal insulin secretory response observed in cadmium-treated rats, as reflected by glucose intolerance and suppression of glucose- or phentolamine-stimulated insulin release. It may be of significance that similar hepatotoxic and pancreotoxic effects were observed in neonatal rats chronically exposed to relatively low levels of cadmium via the oral route. Although the exact mechanism(s) underlying cadmium toxicity is still obscure, it is likely that

pancreatic or hepatic sulfhydryl groups may represent a possible site sensitive to cadmium action. Furthermore, the ability of zinc and selenium to afford protection against cadmium-induced lesions in both hepatic and pancreatic functions indicates that interactions between these metals may underlie the observed hepatotoxic and pancreotoxic effects of cadmium in mammals. It is apparent that a clearer understanding of trace element metabolism will be fruitful not only to our understanding of heavy metal toxicities, but also to the development of adequete prophylactic and detoxification measures against the inadvertent exposure to heavy metal pollutants.

VI. SUMMARY

VI. SUMMARY

There is sufficient evidence to indicate that cadmium is a highly toxic pollutant, posing a potential hazard to animal species. Within the past two decades, industrial production and use of cadmium has increased substantially (93). The worrisome void in our knowledge of long-range consequences of cadmium exposure has stimulated considerable interest in the study of metabolism, toxicity and interaction of cadmium with other compounds. The purpose of the present investigation was to examine the effects of cadmium exposure on carbohydrate metabolism and to elucidate the mechanism(s) by which this environmental pollutant alters glucose homeostasis. In addition, the possible protective effects of selenium and zinc on cadmium-induced biochemical and functional lesions were investigated.

Data in the present study demonstrate that daily intraperitoneal administration of cadmium chloride (0.25 or 1.0 mg/kg) for 21 or 45 days increased the activities of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-diphosphatase (FD-Pase) and glucose 6-phosphatase (G6-Pase) in rat liver. Furthermore, chronic cadmium treatment caused time- and dose-dependent increases in blood glucose and urea concentration and a decrease in the levels of hepatic glycogen. Moreover, chronic cadmium treatment markedly elevated cyclic AMP levels and the activity of hepatic adenylate cyclase. The observed increase in endogenous cyclic AMP content was also reflected by a decrease in the cyclic AMP binding capacity of hepatic protein kinase and a reduction in the kinase activity ratio. These results indicate that the hyperglycemia induced by chronic cadmium treatment is accompanied by enhanced capacity of the hepatic tissue to synthesize glucose and that the altered state of hepatic carbohydrate metabolism in cadmium intoxicated rats may be related to changes in hepatic cyclic AMP system. Discontinuation of

cadmium administration for 14 days in rats pretreated with cadmium chloride (1.0 mg/kg) for 21 days, failed to reverse the observed changes in hepatic cyclic AMP or carbohydrate metabolism. A similar persistence of metabolic alterations was noted in rats treated with cadmium for 45 days and subsequently maintained without additional treatment for 28 days.

Although liver is the major site of glucose synthesis, renal gluconeogenesis is also known to contribute to glucose production. Evidence has also been presented to demonstrate that daily intraperitoneal injection of cadmium chloride (1.0 mg/kg) for 45 days enhanced renal gluconeogenesis as evidenced by significant increase in kidney cortex PC, PEPCK, FD-Pase and G6-Pase. Furthermore, as in the case of liver, discontinuation of heavy metal treatment for 28 days failed to restore the observed biochemical alterations. In contrast to the hepatic tissue, cadmium treatment led to a significant decrease in the cyclic AMP levels of renal cortex which was accompanied by an increase in the cyclic AMP binding capacity of protein kinase. Although the lower dose (0.25 mg/kg) failed to appreciably change kidney cortex protein kinase ratio, treatment with the higher dose (1.0 mg/kg) of cadmium increased it significantly. It is difficult at present to reconcile these findings since both cadmium as well as cyclic AMP have been found to produce marked enhancement in renal gluconeogenic capacity. However, the possibility exists that the observed decrease in kidney cortex cyclic AMP may be due to enhanced breakdown by phosphodiesterase and/or increased urinary excretion of this nucleotide due to tubular damage.

Daily intraperitoneal injection of cadmium chloride (1.0 mg/kg) for 45 days also was found to exert gonadotoxic effects, as reflected by decreased prostatic and testicular weights of mature rats. In the prostate gland, chronic treatment with cadmium reduced cyclic AMP synthesis. Cyclic AMP binding to prostatic protein kinase was increased following cadmium administration, as

was the activity of the cyclic AMP-dependent form of protein kinase. In contrast, testicular adenylate cyclase was stimulated by cadmium treatment. However, the endogenous cyclic AMP levels remained unaffected since the increase in testicular adenylate cyclase was offset by a concomitant increase in the activity of phosphodiesterase. Although the activities of the cyclic AMP-dependent and the independent forms of testicular protein kinase were significantly depressed; the binding of cyclic AMP to protein kinase from testes of cadmium-treated rats was not affected. Discontinuation of treatment for 28 days resulted in a reversal of several of the cadmium-induced changes in prostatic cyclic AMP metabolism. However, the weight of the prostate glands remained essentially in the same range as that seen in the 45-day "treated group." In the case of testes, cessation of cadmium treatment restored adenylate cyclase and protein kinase (the cyclic AMP-dependent form) activities back to normal. However, endogenous cyclic AMP levels, the cyclic AMP binding capacity of protein kinase, as well as testicular phosphodiesterase and the cyclic AMP-independent form of protein kinse were still significantly reduced in the "withdrawn group." Data suggest that cyclic AMP metabolism in both the primary and the secondary reproductive organs of the male rat is altered following chronic cadmium treatment and that the metabolic changes persist even 28 days following the termination of daily exposure to the heavy metal.

Treatment with an acute dose of cadmium chloride (60 mg/kg) decreased hepatic glycogen content 1 hr after injection. In addition, acute cadmium exposure increased blood glucose, serum urea and hepatic cyclic AMP synthesis. However, the gluconeogenic enzymes remained relatively unaffected in these animals. The results indicate that although the gluconeogenic process may not play a significant role in the initial elevation of blood glucose seen at 1 hr after cadmium administration, the enhanced capacity of hepatic and renal cortical tissues to synthesize glucose does contribute to the maintenance of elevated blood glucose levels during chronic exposure to the heavy metal.

In contrast to the acute effects, subacute exposure to cadmium ($CdCl_2$, 1 mg/kg, twice daily for 7 days) was found to induce changes in hepatic metabolism that were qualitatively similar to those resulting from chronic cadmium treatment. Data demonstrate that hepatic cyclic AMP level was enhanced, as was the activity of basal, epinephrine as well as glucagon-stimulated form(s) of adenylate cyclase. Evidence also has been presented indicating that subacute cadmium exposure reduced testicular cyclic AMP and prostaglandin F levels. Whereas cadmium treatment ($CdCl_2$, 1 mg/kg, twice daily for 7 days) increased blood glucose concentration, the serum levels of insulin were suppressed. Furthermore, administration of a glucose load to cadmium pretreated animals resulted in decreased glucose tolerance that was associated with a marked suppression in the glucose-stimulated insulin release. Results demonstrate that the normal sharp increase in serum insulin levels in response to phentolamine (an α-adrenergic blocking agent) was also substantially inhibited in cadmium-exposed rats, indicating that suppressed pancreatic function could not be due to increased adrenal catecholamine secretion alone. These results demonstrate that cadmium has diabetogenic effects and that the observed alterations in hepatic carbohydrate and cyclic AMP metabolism may, at least in part, be due to the relative lack of insulin.

In contrast to cadmium effects, administration of selenium (SeO_2; 1 mg/kg, twice daily for 7 days) failed to alter appreciably the activities of gluconeogenic enzymes, hepatic cyclic AMP, blood glucose or serum insulin levels, glucose tolerance or the pancreatic secretory activity. However, administration of selenium concurrently with cadmium completely prevented the cadmium-induced increases in hepatic gluconeogenic enzymes. Furthermore, treatment with selenium ameliorated the cadmium-induced hyperglycemia, hypoinsulinemia, glucose intolerance and the suppression of pancreatic secretory activity, whereas it failed to alter significantly the cadmium-induced elevation of

hepatic cyclic AMP levels. Data provide evidence which suggests that although selenium alone is without any appreciable effect, when administered concurrently with cadmium, it prevents to varying degrees, several of the cadmium-induced metabolic and functional changes.

Similarly, evidence has been presented which shows that subacute administration of zinc ($ZnCl_2$, 2 mg/kg, twice daily for 7 days) failed to alter activities of hepatic gluconeogenic enzymes, cyclic AMP synthesis, as well as glucose clearance and insulin release in response to a glucose load. Testicular cyclic AMP and prostaglandin F levels remained unaltered as well. However, zinc, when administered along with cadmium, effectively prevented the cadmium-induced lesions in hepatic, testicular as well as pancreatic functions.

In order to simulate a more physiological mode of cadmium exposure, new-born rats were given cadmium (0.1 or 1.0 µg/g) as cadmium chloride solution by intubation. Data demonstrate that administration of a low dose of cadmium (0.1 µg/g) for 45 days failed to appreciably alter the growth rate of new-born rats; however, the higher dose (1.0 µg/g) resulted in a slight but significant suppression of body weight gain. Like chronic injection of cadmium, oral administration for 45 days resulted in hyperglycemia, uremia and diminution of hepatic glycogen. Furthermore, orally administered cadmium elevated the activities of hepatic gluconeogenic enzymes, cyclic AMP levels and enhanced the adenylate cyclase activity as well as its responsiveness to stimulation by epinephrine and glucagon. However, a marked fall in testicular cyclic AMP and prostaglandin F levels was observed in new-born rats receiving oral cadmium for 45 days. As expected, the various biochemical alterations observed with the higher dose of cadmium (1.0 µg/g) were of a greater magnitude than those noted with the lower dose of the heavy metal (0.1 µg/g).

Data also demonstrate that although oral administration of cadmium (0.1 µg/g/day) for 45 days failed to alter significantly the basal serum insulin levels, pancreatic insulin content was markedly reduced indicating

that insulin synthesis may be impaired. Furthermore, when the islets isolated from cadmium-exposed rats were incubated in presence of varying glucose concentrations, a marked reduction was observed in glucose-stimulated insulin release. These data indicate that cadmium suppresses the synthesis and/or release of insulin and the damage may be localized at the level of pancreatic islets.

The present investigation demonstrates that cadmium treatment increases the concentration of blood and urinary glucose and reduces liver glycogen levels. Furthermore, this pollutant also enhances the potential of hepatic and renal tissues to synthesize glucose. Since these alterations in carbohydrate metabolism resemble those produced by cyclic AMP, it is possible that the cadmium-induced metabolic changes may be associated with and probably triggered by stimulation of cyclic AMP synthesis. Furthermore, cadmium was shown to suppress pancreatic insulin synthesis and/or release. Since cadmium-induced metabolic alterations resemble those seen during experimental or clinical diabetes, the results indicate that the diabetogenic effects of cadmium may, at least in part, be a consequence of the relative lack of insulin. Neonatal exposure to relatively low levels of cadmium via the oral route also was found to exert similar effects on the hepatic, gonadal as well as pancreatic tissues. Furthermore, several of the cadmium-induced lesions could be prevented or modified by concurrent treatment with either zinc or selenium. The present investigation indicates that a clearer understanding of trace element interactions will be fruitful to our understanding of cadmium toxicity and also to the development of adequate prophylactic and detoxification measures against the inadvertent exposure to heavy metal pollutants.

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PUBLICATIONS

(a) Review Articles and Symposia Participation

1. R.L. Singhal, Z. Merali and P.D. Hrdina. Aspects of the biochemical toxicology of cadmium. In "American Society of Pharmacology and Experimental Therapeutics Symposium on Biochemical Aspects of Toxic Agents." (Ed. G. Plaa), University Of Montreal, Montreal, Que. Fed. Proc. 35, 75-80 (1976).

2. R.L. Singhal, Z. Merali, J.A. Thomas and G.R. Knotts. Cadmium pollution and its impact on body metabolism. Contemporary Education, Environmental Health Today and Tomorrow, Published by the School of Education, Indiana State University, Terre Haute, Ind. 66, 100-103 (1975).

3. Z. Merali, R.L. Singhal, P.D. Hrdina and G.M. Ling. Changes in brain cyclic AMP metabolism and acetylcholine and dopamine during narcotic dependence and withdrawal. Proceedings of the International Narcotic Research Club Conference on the Opiate Narcotics: Neurochemical Mechanisms in Analgesia and Dependence, Virginia, Pergamon Press, N.Y. Life Sci. 16, 1889-1894 (1975).

4. R.L. Singhal and Z. Merali. Biochemical toxicology of cadmium. In "Cadmium in the Environment," (Ed. J.H. Mennear), Modern Pharmacology. (Bousquet and Palmer, Eds.), Marcel Dekker Inc., N.Y. (1976). In press.

(b) Research Papers

1. R.L. Singhal, Z. Merali, S. Kacew and D.J.B. Sutherland. Persistence of cadmium-induced metabolic changes in liver and kidney. Science 183, 1094-1096 (1974).

2. Z. Merali, P.K. Ghosh, P.D. Hrdina and R.L. Singhal. Alterations in striatal acetylcholine, acetylcholine esterase and dopamine after methadone replacement in morphine-dependent rats. Europ. J. Pharmacol. 26, 375-378 (1974).

3. B.K. Tsang, Z. Merali, D.J.B. Sutherland and R.L. Singhal. Testicular and prostatic cyclic AMP metabolism following chronic cadmium treatment and subsequent withdrawal. Environ. Physiol. Biochem. 4, 205-213 (1974).

4. Z. Merali, S. Kacew and R.L. Singhal. Response of hepatic carbohydrate and cyclic AMP metabolism to cadmium treatment in rats. Can. J. Physiol. Pharmacol. 53, 174-184 (1975).

5. Z. Merali and R.L. Singhal. Influence of chronic exposure to cadmium on hepatic and renal cyclic AMP-protein kinase system. Toxicology 4, 207-214 (1975).

6. Z. Merali and R.L. Singhal. Protective effect of selenium on certain hepatotoxic and pancreotoxic manifestations of subacute cadmium administration. J. Pharmacol. Expt. Therap. 195, 58-66 (1975).

7. Z. Merali, S. Kacew and R.L. Singhal. Inhibition of rat liver adenyl cyclase by tricyclohexylhydroxytin (Plictran). Gen. Pharmacol. 6, 299-301 (1975).

8. Z. Merali, B.K. Tsang, R.L. Singhal and P.D. Hrdina. Effect of narcotic dependence and withdrawal on striatal dopamine-sensitive adenylate cyclase and synaptosomal cyclic AMP metabolism. Res. Comm. Chem. Pathol. Pharmacol. 14, 29-37 (1976).

9. Z. Merali and R.L. Singhal. Zinc protection against cadmium-induced biochemical alterations. Brit. J. Pharmacol. 57, 573-579 (1976).

10. A. Stevenson, Z. Merali, S. Kacew and R.L. Singhal. Effects of subacute and chronic lead treatment on glucose homeostasis and renal cyclic AMP metabolism in rats. Toxicology. In press.

11. S. Kacew, Z. Merali and R.L. Singhal. Comparison of the subacute effects of cadmium exposure upon nucleic acid, cyclic adenosine 3',5'-monophosphate and polyamine metabolism in lung and kidney cortex. Toxicol. Appl. Pharmacol. In press.

12. S. Kacew, Z. Merali and R.L. Singhal. Cadmium: sequential changes in nucleic acid synthesis as well as polyamines and cyclic AMP levels of rat pancreas. Gen. Pharmacol. In press.

13. S. Kacew, Z. Merali, A.N. Thakur and R.L. Singhal. Sequential changes in hepatic polyamine, deoxyribonucleic acid and cyclic adenosine 3',5'-monophosphate metabolism following subacute exposure to cadmium in rats. Submitted for publication.

14. S. Kacew, Z. Merali and R.L. Singhal. Cadmium: sequential changes in nucleic acid synthesis as well as polyamines and cyclic AMP levels of rat pancreas. Submitted for publication.

15. R.B. Rastogi, Z. Merali and R.L. Singhal. Cadmium alters behaviour and the biosynthetic capacity for catecholamines and serotonin in neonatal rat brain. Submitted for publication.

(c) Abstracts

1. P.K. Ghosh, Z. Merali, P.D. Hrdina and R.L. Singhal. Ontogenic changes in dopamine, acetylcholine and acetylcholine esterase in brains of normal and thyroidectomized rats. The Pharmacologist 15, 258 (1973).

2. B.K. Tsang, D.J.B. Sutherland, Z. Merali and R.L. Singhal. The influence of chronic cadmium on the cyclic AMP-adenyl cyclase-protein kinase system of rat testes and prostate glands. Fed. Proc. 33, 227 (1974).

3. Z. Merali, S. Kacew and R.L. Singhal. Alterations in rat liver and kidney cortex metabolism after acute and chronic exposure to cadmium. Society of Toxicology Meeting, Washington, D.C. Toxicol. Appl. Pharmacol. 29, 140 (1974).

4. Z. Merali and R.L. Singhal. Hepatic cyclic AMP metabolism in rats treated with cadmium chloride. Proc. Can. Fed. Biol. Soc. 17, 52 (1974).

5. Z. Merali and R.L. Singhal. Effect of selenium on cadmium-induced toxicity in rats. 14th Annual Meeting, Society of Toxicology, March 9-13, 1975. Williamsburg, Va. Toxicol. Appl. Pharmacol. 33, 127 (1975).

ABSTRACT

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Daily intraperitoneal administration of cadmium chloride (0.25 or 1.0 mg/kg) for 21 or 45 days increased the activities of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-diphosphatase (FD-Pase) and glucose 6-phosphatase (G6-Pase) in rat liver. Furthermore, chronic cadmium treatment caused time- and dose-dependent increases in blood glucose and urea concentrations and a decrease in the levels of hepatic glycogen. These alterations were accompanied by a marked elevation in cyclic AMP levels and the activity of hepatic adenylate cyclase. The increase in endogenous cyclic AMP content also was reflected by a decrease in the cyclic AMP binding capacity of hepatic protein kinase and a reduction in the kinase activity ratio. Discontinuation of cadmium administration for 14 or 28 days in rats pre-treated with cadmium chloride (1.0 mg/kg) for 21 or 45 days, respectively, failed to reverse the observed changes in hepatic cyclic AMP or carbohydrate metabolism.

Similarly, daily intraperitoneal injection of cadmium chloride (1.0 mg/kg) for 45 days enhanced kidney cortex PC, PEPCK, FD-Pase and G6-Pase and discontinuation of heavy metal treatment for 28 days failed to restore the observed enzymic alterations. Cadmium treatment led to a significant lowering of cyclic AMP levels of the renal cortex which was accompanied by an increase in the cyclic AMP binding capacity of protein kinase. While the lower dose (0.25 mg/kg) failed to appreciably change the kidney cortex protein kinase ratio, treatment with the higher dose (1.0 mg/kg) of cadmium significantly increased it. Furthermore, administration of cadmium (1.0 mg/kg) for 45 days decreased prostatic and testicular weights. In the prostate gland, treatment with the heavy metal reduced cyclic AMP synthesis but enhanced cyclic AMP binding to prostatic protein kinase as well as the activity of cyclic AMP-dependent protein kinase. Whereas the testicular cyclic AMP levels remained unaffected, the activities of adenylate cyclase and phosphodiesterase were

elevated following exposure to the heavy metal. In contrast, the activities of cyclic AMP-dependent and independent forms of testicular protein kinase were significantly depressed but the cyclic AMP binding activity remained unaffected in cadmium-treated animals. Discontinuation of treatment for 28 days in rats that had previously been given the heavy metal for 45 days resulted in a reversal of several of the cadmium-induced changes in cyclic AMP metabolism of the prostate gland. However, the weight of the prostate glands remained essentially in the same range as that seen in the 45-day "treated group." In the case of testes, cessation of cadmium treatment restored adenylate cyclase and protein kinase (the cyclic AMP-dependent form) activities back to normal. However, endogenous cyclic AMP levels, the cyclic AMP binding capacity of protein kinase, as well as testicular phosphodiesterase and the cyclic AMP-independent form of protein kinase were still significantly reduced in the "withdrawn group."

Treatment with an acute dose of cadmium chloride (60 mg/kg) decreased hepatic glycogen content 1 hr after injection. In addition, acute cadmium exposure increased blood glucose, serum urea and hepatic cyclic AMP synthesis. However, the gluconeogenic enzymes remained relatively unaffected in these animals. In contrast to the acute effects, subacute exposure to cadmium ($CdCl_2$, 1 mg/kg, twice daily for 7 days) was found to induce changes in hepatic carbohydrate metabolism that were qualitatively similar to those resulting from chronic cadmium treatment. Furthermore, the hepatic cyclic AMP level was enhanced, as was the activity of basal, epinephrine- as well as glucagon-stimulated form(s) of adenylate cyclase. Administration of a glucose load in cadmium pretreated animals resulted in decreased glucose tolerance that was associated with a marked reduction in the glucose-stimulated insulin release. Furthermore, the normal sharp increase in serum insulin levels in response to phentolamine was substantially inhibited in cadmium-exposed rats.

Unlike cadmium, administration of selenium (SeO_2, 1 mg/kg, twice daily for 7 days) failed to alter significantly the activities of gluconeogenic enzymes, hepatic cyclic AMP, blood glucose or serum insulin levels, glucose tolerance or the pancreatic secretory activity. However, administration of selenium concurrently with cadmium, completely prevented the cadmium-induced increases in hepatic gluconeogenic enzymes, ameliorated the cadmium-induced hyperglycemia, hypoinsulinemia, glucose intolerance and the suppression of pancreatic secretory activity, but failed to alter significantly the cadmium-induced elevation of hepatic cyclic AMP. Similarly, subacute administration of zinc ($ZnCl_2$, 2 mg/kg, twice daily for 7 days) failed to alter the activities of hepatic gluconeogenic enzymes, cyclic AMP, as well as glucose clearance and insulin release in response to a glucose load. Likewise, testicular cyclic AMP and prostaglandin F levels remained relatively unaltered by zinc. However, when zinc was administered along with cadmium, it effectively prevented the cadmium-induced lesions in hepatic, testicular as well as pancreatic functions.

Oral administration of cadmium chloride (0.1 or 1.0 µg Cd/g, by intubation) to neonatal rats for 45 days also enhanced the hepatic gluconeogenic potential as well as blood glucose and hepatic cyclic AMP levels. The activity of basal, glucagon as well as epinephrine-stimulated form(s) of adenylate cyclase also was enhanced. Although the basal serum insulin levels remained relatively unaltered in rats chronically exposed to cadmium (0.1 µg/g), the levels of pancreatic insulin declined sharply. Furthermore, when the islets isolated from cadmium-exposed rats were incubated in presence of varying glucose concentrations, a marked reduction was seen in glucose-stimulated release of insulin.

Data presented in this dissertation demonstrate that cadmium possesses considerable toxicological potential as this heavy metal induces biochemical or functional lesions in testis, prostate, liver, kidney and pancreas. The observed derangement in carbohydrate metabolism and gonadal function may be

related to alterations in cyclic AMP synthesis. Furthermore, the present study supports the contention that the diabetogenic effects of cadmium may be related to suppressed insulin synthesis and/or release. Oral administration of cadmium to new-born rats also induced lesions that appeared to be qualitatively similar to those produced by parenteral treatment with the metal. The observed cadmium-induced metabolic alterations could be effectively prevented or modified by concurrent treatment with either zinc or selenium. The results have been discussed in relation to the possible mechanisms of cadmium toxicity and to the role of sulphydryl groups in the protection exercised by zinc and selenium.