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THE INFLUENCE OF LEAD EXPOSURE ON GLUCOSE HOMEOSTASIS AND CERTAIN PARAMETERS ASSOCIATED WITH GROWTH IN ADULT AND NEONATAL RATS

A Thesis

Presented to

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CONTENTS

LIST OF ABBREVIATIONS

Acetyl-CoA; acetyl coenzyme A

ADP; adenosine 5'-diphosphate

δ-ALA; δ-aminolevulinic acid

AMP; adenosine 5'-monophosphate

cyclic AMP; cyclic adenosine 3':5'-monophosphate

ATP, ATPase; adenosine 5'-triphosphate, adenosine 5'-triphosphatase

DNA; deoxyribonucleic acid

EDTA; ethylenediaminetetraacetic acid

EGTA; [ethylenebis(oxyethylenenitrilo)]tetraacetic acid

FD-Pase; fructose 1,6-diphosphatase

GSH; reduced glutathione

G6-Pase; glucose 6-phosphatase

IRI; immunoreactive insulin

NADH; reduced nicotinamide-adenine dinucleotide

PC; pyruvate carboxylase

PEPCK; phosphoenolpyruvate carboxykinase

RNA, mRNA, tRNA; ribonucleic acid, "messenger" and "transfer" ribonucleic acid

TEL; tetraethyllead

TML; tetramethyllead

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1. INTRODUCTION

The heavy metal lead is the most ancient environmental pollutant released by man into the biosphere. As a result, occupational lead poisoning has created a major concern amongst metal-processing populations for several centuries. However, in the present circumstances where lead is utilized in the automotive industry and in other widely distributed products, the general populace has become a victim of environmental contamination by this heavy metal (1-4).

Although lead is a non-essential element, there is no effective homeostatic mechanism to deal with an increasing intake of this heavy metal. Consequently, lead accumulates in the body with advancing age (5,6), with large amounts being deposited in bone and to a lesser degree in certain soft tissues. The ubiquitous nature of lead in the environment and its potential hazard to man has stimulated studies designed to elucidate the mechanisms of certain physiological and toxicological reactions to this heavy metal (7-10). In view of the fact that young children may be more susceptible than adults to prolonged low-level lead exposure (11), it has been of considerable interest to determine the effects of this heavy metal on neonatal animals. The present dissertation is concerned primarily with the elucidation of acute, subacute and chronic effects of lead on carbohydrate metabolism and certain biochemical parameters associated with growth in rats.

Since lead was previously found to produce an elevation in the concentration of blood and urinary glucose (12,13), the influence of this heavy metal was examined on the glucose-synthesizing capacity of both liver and kidney cortex. In particular, the effect of lead was investigated on the activities of the four key gluconeogenic enzymes, pyruvate carboxylase, phosphoenolpyruvate

carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. In addition, changes in the concentration of blood glucose and serum urea, a product of protein catabolism, were determined following lead treatment. Since the concentration of blood glucose can be elevated by the process of hepatic glycogenolysis, the effect of lead treatment on hepatic glycogen levels also was determined.

Although the exact mechanism(s) underlying hormonal modulation of hepatic and renal carbohydrate metabolism is still unknown, it has been postulated that 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 thus resulting in an elevation of blood glucose and urea levels (14). This apparent similarity between the effects of cyclic AMP and lead on carbohydrate metabolism prompted studies on the influence of this metal on the renal and hepatic cyclic AMP-adenylate cyclase system. In addition, the effects of subacute and chronic lead treatment on the responsiveness of hormonal-activated adenylate cyclase activity were examined in liver and kidney. Since lead has a long biological half-life, it was of interest to determine whether lead-induced alterations persisted upon cessation of heavy metal administration. Thus, the influence of 4-week cessation of lead treatment was examined on various metabolic alterations in rats previously given the metal for 45 days.

It is of interest that the lead-induced changes in carbohydrate metabolism resemble those of insulin deficiency produced by alloxan or insulin antiserum (15). These observations suggested that metabolic alterations found in lead-exposed animals might be due, at least in part, to lack of insulin. In order to examine this possibility, rats pretreated with lead were challenged

with a glucose load and the subsequent time-course of glucose clearance as well as alterations in serum insulin levels was monitored.

Chronic lead poisoning in man is known to result from inhalation or ingestion, a process which is rather slow but continuous. One of the consequences is the excretion of small amounts of lead through the mother's milk to the neonate. It was therefore of interest to determine whether chronic exposure of neonatal rats, believed to be more susceptible than adults, to oral lead would result in any functional and/or biochemical abnormalities in renal and hepatic tissue. In this regard, new-born rats were administered relatively low amounts of lead either from heavy metal-treated lactating females or by gastric intubation, and the changes in renal gluconeogenic enzymes, hormonal responsiveness of adenylate cyclase as well as endogenous cyclic AMP levels were measured. In addition, since prolonged lead exposure may affect mammalian growth processes, nucleic acids and protein levels were determined in renal, hepatic and pulmonary tissue, as well as the incorporation of thymidine into DNA by these tissues. The observation that a single intracardiac injection of lead produced a wave of mitotic activity and DNA synthesis in mouse kidney (16) prompted us to examine whether lead treatment would also affect nucleic acid levels and synthesis in rat liver, lung and kidney.

Although the precise mechanisms underlying the initiation of cell proliferation remain to be elucidated, various investigators have proposed that alterations in polyamine biosynthesis as well as modulation of the adenylate cyclase-cyclic AMP system may play a role in the stimulation of nucleic acid synthesis (17,18). In order to investigate the mechanisms involved in lead-induced alterations in macromolecular synthesis, we determined the endogenous concentration of putrescine, spermidine and spermine

in rat kidney, liver and lung, as well as adenylate cyclase activity and cyclic AMP levels in these tissues.

Results of the present investigation demonstrate that exposure to lead produces certain functional and biochemical alterations in hepatic, renal, pancreatic as well as pulmonary tissues. The heavy metal-induced changes in renal and hepatic carbohydrate metabolism may be associated with enhanced cyclic AMP synthesis. Since lead treatment suppressed insulin secretory activity, the heavy metal-induced diabetogenic effects may be related to an apparent lack of insulin. In addition, the heavy metal-induced alterations in renal, hepatic and pulmonary DNA synthesis may be related to modulation of the adenylate cyclase-cyclic AMP system in chronically-exposed rats. Data in the present investigation show that young mammals are more susceptible to low levels of lead, and that chronic neonatal studies may provide an appropriate experimental model for the study of the etiology of lead toxicity.

2. LITERATURE REVIEW

I. DISTRIBUTION AND SOURCES OF LEAD IN THE ENVIRONMENT

The existence and mining of lead has been known for approximately 4500 years. Throughout the Egyptian, Greek and Roman Empires lead was utilized in items such as tiles, pipes, cisterns, pewter, cosmetics, drugs, pigments and glazes. Indeed, historical evidence indicates that depletion of lead-silver ores as well as excess dietary levels of lead may have been a contributing factor to the decline of the Roman Empire (1,2,5). In addition to the traditional employment of lead in pigments, pipes, glazes and ammunition, contemporary uses include storage batteries for automobiles (litharge, red lead and other oxides), antiknock components of gasoline (tetraethyllead, tetramethyllead), solders, pesticides (lead arsenate) and stabilizers in the plastics industry. Lead is also employed in sound and radiation shielding, and is alloyed with copper in the production of brass and bronze (3,5,19). Although the largest amount of lead is utilized in the manufacture of lead-acid batteries, emission of this heavy metal into the biosphere is primarily due to the combustion of automotive and aviation fuels containing organic lead additives (approximately 66.6% of the total emissions of lead in Canada in 1970) (19). Lead discharged from automobile exhaust may reach levels of 200,000 tons per year in North America alone. In the production of copper, nickel and iron the amount of lead discharged in Canada in 1970 as waste was 22.2% of 21,417 tons. It is thus evident that the potential for environmental contamination by lead is marked since the metal is utilized and emitted as a by-product in a wide variety of industries.

A. Lead in Soil, Water and Ambient Air

Lead accumulates in the soil surrounding lead-utilizing industries and

heavily travelled roads. However, metallic dispersal through rainfall remains low since its solubility in water is negligible (3). In soil, the concentration of lead may reach approximately 1000 ppm at roadside, and may range from 50 to 100 ppm at a distance of 400 feet from roads as well as in rural districts where automobile traffic is less (2,3,20,21). It has been shown that the lead present in the soil in the form of particulate lead compounds such as lead bromochloride (PbCl.Br) is derived from the exhaust of automobiles through the process of combustion of lead alkyl-containing fuels (3,22). A further source of lead as alkyl vapours (TEL) accounts for only a small fraction of airborne lead, since its atmospheric half-life is short (approximately 1 hr) and less than 0.1 $\mu g/m^3$ of TEL is present in the ambient air (23). The combustion of lead alkyls is responsible for about 90% of the airborne atmospheric lead, which can exist in a wide range of particle sizes (from 0.01 μ to several mm in diameter). In urban areas, the mass median diameter of particulate lead is 0.25 μ, and in general 50-90% of urban atmospheric lead is found in fine particles smaller than 1 μ. Lead particulates emitted in vehicle exhaust are generally smaller than 5 μ in diameter (3,5), and some investigators report that up to 70% of lead-containing particles are smaller than 0.3 μ. It has been established that the fine granules are of particular danger environmentally since both their absorption and half-life is markedly increased as compared to the larger particles (23,24).

Airborne concentrations of lead vary diurnally, seasonally, and geographically. The concentration of metal rises during the fall and winter months (3). In urban areas the average atmospheric lead levels may vary from 1-10 $\mu g/m^3$, although concentrations as high as 40-70 $\mu g/m^3$ have been observed in morning rush-hour traffic or on heavily travelled roads (2,3,6).

During an average 24 hour period lead levels in the largest Canadian cities have been shown to reach values ranging from 1.0 - 4.8 $\mu g/m^3$ (25).

Trace amounts of lead may also be present in water supplies. Soft water sources, where acidic conditions are common, will take up lead into solution more readily than hard water. The average lead content of rivers and lakes is approximately 1-10 µg/l, well within the WHO-recommended limits of 0.1 ppm (3). Although water is not considered to be a hazardous source of environmental lead, metal levels in drinking-water may become elevated in older houses with lead piping (3,6,26).

B. The Presence of Lead in Vegetation

Vegetation is also known to be a source of lead for humans. Various studies demonstrated that the concentration of heavy metal in roadside plants ranged from 100 ppm at roadside to 2 ppm at a distance of 400 feet (2,3). The highest concentrations of lead have been observed in cereals such as corn, soybeans, oats, wheat and rice. Soluble lead compounds were found to be taken up and concentrated in the roots of other types of plants including carrots, potatoes, tomatoes and the roots of several leafy vegetables (3), whereas metal dustfall from vehicle exhaust coated plant leaves and tops (3,27). It is of interest that various plants treated with lead compounds exhibited a marked impairment in photosynthetic reactions, the significance of which remains to be elucidated (28,29).

C. Possible Miscellaneous Environmental Sources of Lead

It is not essential for the population at risk to reside in close proximity to the more common sources of lead emission in order that a hazardous level may be attained within the individual. Indeed, one's personal environment may contain more important sources of lead, including

house paint (interior or exterior), coloured printed material, various foods, or acidic beverages stored in glazed vessels (5,26,30,31). Paint chips contain up to 20% lead, and the soil surrounding lead-painted houses may contain the metal in a concentration range of 200-500 ppm (32). Indeed, the ingestion of lead-containing paint fragments is one of the major causes of lead poisoning in children (11). In addition, lead pigments used in coloured inks and coloured printed material may contain as much as 4000 ppm lead.

Investigations performed on farms in high- and low-level lead production districts indicated that significant amounts of lead were found in milk (6-25 µg/100 ml) from lead-contaminated cattle as compared to animals exposed to the low-level amounts (33). Canned milk concentrates may also contain significant amounts of heavy metal (0.6 - 5.8 µg/100 ml), and these may provide an additional hazard for the infant population, a high-risk group for lead poisoning (34). Other foods containing significant levels of lead include cheese, meat, fish, dried fruit, grains, wine and some canned products. Acidic beverages such as orange juice, coffee, tea or wine may leach up to 1.5 ppm lead from improperly fired glazed vessels in a one-half hour period (35,36). "Moonshine" alcohol, often distilled through lead-containing pipes, also contains appreciable amounts of lead, and consumption of these beverages results in toxic manifestations amongst these individuals (1,3). It is thus evident that the many sources contributing lead to the environment potentiate the hazard of accidental ingestion of this metal in humans.

II. DISTRIBUTION OF LEAD IN MAMMALIAN SYSTEMS

A. Routes of Lead Absorption

The respiratory and gastrointestinal tract provide the major routes of entry for lead into mammalian organisms. Some lead compounds, including

tetraethyllead (TEL), tetramethyllead (TML), lead acetate and lead oleate are also absorbed through the skin (19). The two principal sources of inhaled lead are atmospheric emissions from vehicle exhaust and industrial discharge. The mean daily absorption of this metal through the respiratory tract has been estimated to be between 2-60 µg, dependent on conditions such as the amount of air inhaled and the atmospheric concentration of lead (3,24). Cigarette smoking may also add significantly to the body burden of lead, at a level of about 1 µg of lead per cigarette (24). Various investigators studying experimental lead inhalation have determined that respiratory absorption under controlled conditions ranges from 30-60%, depending on the particle size. It is generally accepted that 40-50% of particles under 1 µ in diameter are deposited in the respiratory tract; however, particles larger than 2 µ are also retained, via deposition on mucous membranes and subsequent transfer to the esophagus where the metal is ingested rather than inhaled (3,24,37-39).

The mean daily intake of lead via ingestion has been estimated to be 200-300 µg (range of 30-500 µg) (3,6,7). A daily ingestion of 1 mg for a period of several months has been found to result in clinically lead-intoxicated children, while a dietary level of 2-3 mg/day is required to produce toxic symptoms in adults. In addition, absorption from the gastro-intestinal tract in adults is about 5-10%, while children absorb 25 to 50% of ingested metal (40,41). In view of the fact that young children display a marked tendency to ingest non-food items such as dirt, paper and paint chips, a condition known as pica, ingestion is thus a critical factor in environmental lead toxicity amongst the young (5,6).

The absorption of various lead compounds depends on certain physical properties including solubility. Inorganic lead acetate, lead chloride, lead

carbonate and lead oxides are readily absorbed via the gut, as are the organic compounds tetraethyllead and tetramethyllead (24,41). Absorption of dietary lead may also be influenced by other factors such as stomach pH, dietary protein, minerals, and vitamins. Lead absorption from the gastrointestinal tract is enhanced in conditions of acidic pH, low dietary calcium or phosphorus, low dietary protein, iron deficiency, vitamin D administration and high dietary fat content (7,42,43). A high mineral intake may reduce lead absorption to a certain degree, and elevated dietary calcium exerts a protective effect as evidenced by the prevention of metal absorption from the gastrointestinal tract, possibly by competition at mucosal binding sites (43,44). In contrast, the addition of 0.4% zinc to 0.1% lead in the diet enhanced metal toxicity as evidenced by increased tissue lead levels and pathological changes (44). The presence of whole milk in the diet enhanced lead absorption in adult animals, which may partially explain the elevated absorption of the metal in young animals whose diet primarily consists of milk (45,46). It is thus evident that the absorption of lead is dependent on a variety of factors which should be considered in any study which deals with environmental lead pollution.

B. Factors Involved in Lead Uptake and Metabolism

Although lead belongs to the IVa group of elements, it exhibits a tendency to parallel the group IIa elements (e.g. calcium, strontium) with respect to certain metabolic parameters (5,6). In duodenum, the presence of lead in the diet (200 µg/day or greater) was found to significantly depress the transport of calcium-45 and strontium-85 (47). However, low levels of dietary calcium are known to enhance the gastrointestinal uptake of several minerals, including magnesium, strontium, zinc and lead, and under these experimental conditions, the concentration of lead was elevated in blood,

bone, liver and kidneys (42,43). Low dietary phosphorus as well as the administration of vitamin D also enhanced the body burden of lead (42). In mammals, the absorbed lead concentrates in regions of bone formation, and this metal has been proposed to interfere with calcification, through competitive inhibition of calcium phosphate formation, the major component of apatite crystal. In addition, lead has been found to occupy the lattice interstices of the apatite crystalline structure, as determined by X-ray diffraction. The high affinity of lead for bone formation sites is important especially with respect to human toxicity, since immobilization of the metal by bone prevents its accumulation in soft tissues (5,48).

C. Lead Retention and Tissue Distribution

Kinetic studies using a radioactive isotope of lead (^{203}Pb) revealed that human subjects retain 50-60% of intravenously injected lead in the blood as compared to 30-40% of an inhaled dose for periods up to 150 hours after exposure (39). Chamberlain et al. (39) estimated that the half-life of inhaled lead was about 15 days. In human volunteers exposure to lead via the oral route resulted in the retention of approximately 10-15% of an administered dose of metal 96 hours later (49). Since chronic ingestion of 3 mg of lead per day for a period of 4 months produced a concentration of 50 µg/100 ml metal in blood, it was estimated that with 8 months of treatment at this dose of lead clinical symptoms of lead intoxication would ensue and the levels of metal would reach 80 µg/100 ml in blood (38). It is of interest that children fed 40-210 µg of lead daily for 3 days absorbed about 53% of the metal and retained an average 18% of the administered dose (40). In rat pups 90% of an intraperitoneal dose of ^{203}Pb was retained after 1 week, while adult animals excreted up to 60% of the injected lead (^{203}Pb or ^{210}Pb) after 7 days (50,51). Although adult rats receiving water retained less than

1% of an oral dose of ^{203}Pb after 6 days, mature animals fed a milk diet retained about 20% of the oral dose of lead. In light of the fact that milk enhances lead absorption it is not surprising that 4 days after an oral dose of ^{203}Pb suckling rat pups (5-7 days old) retained 85% of the metal (52). Thus, it appears that the route of administration, age and dietary factors contribute to lead retention.

In both man and experimental animals, about 90% of the total body burden of lead is stored in bone, and only about 1% remains circulating freely in the blood (2,9). One day after intravenous lead administration, 10% of the metal is present in blood, liver and kidneys, while lung and heart contain about 5% of the element. Studies with radioactive lead showed that a 10-fold decrease in metal occurred by 3 days. In kidney, the disappearance of metal from this tissue was only 2-fold by 3 days, while a 10-fold decrease was observed after 14 days (51). Significant amounts of lead have also been found in brain, teeth and hair of lead-exposed animals (33,44,50). It is of interest that the distribution of lead in the brain was 7-fold greater in suckling rats as compared to adults (50). In humans, lead concentrations in the normal population have been determined by several investigators (53,54), and tissue lead content was found to decrease in the following order: bone > hair > aorta > liver = lymph nodes > kidney cortex > pancreas = prostate or ovary > spleen > lung = brain. Although occupationally-exposed workers exhibited a similar pattern with respect to tissue lead content, noticeably higher lead levels were present in bone, hair, liver and brain (53). With increasing age, the concentration of lead became elevated in renal cortex, lung and bone (53-56). As expected, industrialized populations possessed markedly higher blood lead levels as compared to unacculturated populations (57). Within various organs, lead is tightly

bound to cells, and is probably associated with lipid or lipoprotein components of membranes. In particular, this metal is known to bind strongly to mitochondria in liver, kidney, myocardium and erythrocytes, and may also interfere with membranes of other organelles (58). In addition, lead is stored in the nuclei of renal tubular epithelium and some other cells, in the form of lead-protein intranuclear inclusion bodies (5,7,8). At the molecular level, the metal may interact with sulfhydryl (in particular, dithiol), carboxyl, amino, imidazole and phosphate groups, and thus has a considerable potential for inflicting damage on various enzyme systems (5).

D. Heavy Metal Excretion

Since much of the ingested lead is not absorbed from the gastrointestinal tract, a large proportion of the metal is eliminated unchanged in the feces. For inorganic lead compounds the ratio of fecal to urinary lead may attain a level of 100:1 (5). However, after isotopic lead administration (^{210}Pb or ^{212}Pb) data demonstrated a ratio of 5:1 (39,59). Fecal excretion of intravenously-injected lead was highest during the first 3 days, and reached a plateau by 6 days. Similar observations were made with regard to urinary lead (59). Although the administration of calcium-disodium ethylenediaminetetraacetic acid (EDTA) mobilized bone lead and enhanced urinary excretion, fecal elimination of lead was not altered by this treatment (60). Lead is usually cleared from the blood via glomerular filtration; however, some tubular reabsorption occurred in the presence of excessive amounts of metal in blood (1). Thus, determination of urinary and fecal metal elimination may be useful as a diagnostic tool in the clinical screening of industrial workers and children for lead poisoning (59).

III. CLINICAL PICTURE OF LEAD TOXICITY IN HUMANS

Some of the toxic manifestations associated with chronic inorganic lead

poisoning include pallor, weight loss, constipation and acute abdominal colic. Damage to the central nervous system and kidney characterized by encephalopathy, peripheral neuropathy, and nephropathy (occasionally accompanied by secondary gout) are more severe toxic manifestations. In general, toxicity in adults arises from occupational exposure to lead (the most common symptoms being anemia and colic). While the toxic effects are less severe and more readily reversible in adults, the opposite is true in children. Since early indications of lead poisoning may remain unnoticed in small children, it is conceivable that acute encephalopathy may develop prior to a proper diagnosis of the condition. In light of the fact that brain damage in the form of lead encephalopathy is irreversible in almost all cases (3,5,11), reliable diagnostic tests must be developed.

A. Lead Toxicity in Adults as a Result of Occupational Exposure

The major clinical symptoms associated with occupational inorganic lead poisoning include impaired hematopoeisis, renal dysfunction and peripheral neuropathy (8). In contrast, organic lead produces damage to the central nervous system as evidenced by nervous irritability, emotional instability, increased tendon reflexes, tremor and anorexia without any alterations in porphyrin metabolism (4). If the patient survives the acute phase of organic lead-inflicted central nervous system excitation, complete recovery from the metal-induced effect is possible (4).

Various investigators have shown that lead interferes with the biosynthesis of heme, probably through an inhibition of the enzymes δ-aminolevulinic acid dehydratase and heme synthetase in erythrocytes (9,61-63). In addition, other enzymes involved in heme formation, δ-aminolevulinic acid synthetase and coprogenase were reported to be inhibited in the presence of excess amounts of lead (more than 40 μg/100 ml blood) (5,9). Clinically, the urinary

excretion of intermediary metabolites such as δ-aminolevulinic acid and coproporphyrinogen increased as the concentration of metal rose in the blood. Similarly, δ-ALA and protoporphyrin IX also remained elevated as blood metal levels increased (64,65). It is of interest that δ-aminolevulinic acid dehydratase returned to normal amounts in industrially-exposed men upon termination of exposure to lead (66). Although reduced glutathione (GSH) was decreased after lead exposure, an increase in glutathione reductase activity was observed and Howard (67) suggested that such a mechanism would compensate for the effects of lead binding to sulfhydryl groups. Whereas a reduction in α- and β-chain synthesis was not found in blood obtained from lead workers, a reduction in blood globin synthesis occurred in vitro (68). Other anomalies in blood chemistry attributed to result from elevated metal levels in blood include augmentation in uric acid, bilirubin, serum glutamate-oxaloacetate transaminase and glucose (9,12). The anemia associated with lead poisoning is microcytic and hypochromic, and occasionally there is a significant population of basophilic stippled reticulocytes (9,64).

Acute or chronic lead intoxication often involves nephropathy characterized by proximal tubular dysfunction, associated with mitochondrial degeneration, aminoaciduria and glycosuria. An enhanced excretion of fructose and citrate was also observed in lead-poisoned humans; however, the Fanconi syndrome is not unique to this metal (13,69). In addition, lead-inflicted nephropathy may be associated with "saturnine" gout which results from an interference in the tubular secretion of uric acid and thus plasma levels of uric acid remain elevated (5). In Australia, when cases of childhood lead poisoning were monitored after several years, it was reported that the major cause of death was renal failure which probably occurred during childhood (70). Renal biopsy of industrially lead-exposed workers exhibited diffuse interstitial

fibrosis as well as mitochondrial changes and intranuclear lead inclusion bodies. Although the influence of lead on renal function is not as severe as the hematologic effects of the metal, this non-essential element may be a contributing factor in the etiology of various forms of kidney disease.

Another manifestation of industrial lead toxicity is peripheral neuropathy characterized by weakness in the flexor and extensor muscles of the wrist and arm ("wrist-drop"). This syndrome does not appear to be related to most cases of amyotrophic lateral sclerosis or multiple sclerosis although elevated lead levels have been observed in neuromuscular tissue in patients suffering from amyotrophic lateral sclerosis (71,72). Indeed, the prominent features associated with lead-inflicted neural damage were shown to be a form of motor neuron disease as reflected by reduced maximal motor conduction velocities in the median and ulnar nerves (73). Thus, inorganic lead compounds may exert deleterious effects on hematopoiesis, renal function and the nervous system. It is of interest that these disorders may be effectively treated with various chelating agents (3,5).

B. Environmental Exposure of Children to Lead and Subsequent Symptoms

Although lead poisoning in young children occurs frequently in urban areas, the syndrome is often not diagnosed correctly until severe toxic symptoms become overt. In general, while childhood lead intoxication is asymptomatic, the manifestations seen involve irritability, constipation, ataxia and anorexia. As the disease progresses, vomiting and anemia develop followed by encephalopathy (11). The highest incidence of lead poisoning occurs between the ages of 1-5, predominantly amongst children situated in dilapidated houses or near heavily travelled roads. Whereas lead poisoning in adults is now a rare occurrence, the toxicity of this heavy metal is a major health problem amongst urban children. The main source of lead available to children is ingestion of non-food items such as paint-chips,

coloured paper and road-side dirt, a condition known as pica. It is of interest that acute infection and dietary factors such as iron, protein and caloric intake play no major role in the development of clinical lead poisoning (74,75). It is thus evident that the indiscriminant emission of lead which readily enters the soil subsequently serves as a primary source of poisoning amongst children.

One of the prominent manifestations of lead toxicity commonly seen amongst children is damage to blood-forming elements. Various investigators (8,11,76) noted a reduction in erythrocyte δ-aminolevulinic acid dehydratase activity and elevation in free erythrocyte protoporphyrin accompanied by increased blood lead levels in metal-poisoned children. Chisolm et al. (77) found that by administration of a challenge dose of a chelating agent, a certain amount of lead was mobilized into urine, and it was suggested that the quantity mobilized may be employed as an index of impaired heme synthesis. In addition, acute lead poisoning in children is manifested by the Fanconi syndrome of proximal tubular dysfunction, characterized by enhanced excretion of glucose, amino acids and phosphate (13).

In recent years, much interest concerning lead toxicity has been focussed on the central nervous system. During the most severe stages of lead poisoning encephalopathy develops characterized by ataxia, semi-coma, convulsions, and finally death (11). In patients surviving an attack of acute encephalopathy there is an estimated 30-40% chance of permanent brain damage, associated with cortical atrophy. Less severe signs of neurological damage include learning disabilities, seizures and memory defects. Hyperactive behaviour is a common feature noted in young school children previously exposed to lead (78,79). Evidence indicates that more detailed studies on the influence of lead on the central nervous system are necessary in order to ascertain the effects of this heavy metal on behaviour in children.

IV. TOXICITY OF LEAD IN EXPERIMENTAL ANIMALS

A. General Toxicity

The toxicity of lead compounds in experimental animals is dependent on the chemical form of metallic element used as well as on the route of administration. The intravenous LD_{50} for inorganic lead salts in rats is approximately 70 mg/kg, while the intraperitoneal LD_{50} ranges from 150-1600 mg/kg and is related to the amount of uptake from the peritoneal cavity (3,80). It was found that lead acetate, a soluble lead salt, possessed the lowest LD_{50} (3). In contrast, TEL was found to have higher toxicity when given intravenously to rats at a dose of 15 mg/kg (80). Although data for the acute oral toxicity of most lead compounds in experimental animals still remains to be elucidated, lead arsenate was found to have an oral LD_{50} of 825 mg/kg in rats, and 125 mg/kg in rabbits (3). Exposure of animals to elevated temperatures ($95^{\circ}F = 35^{\circ}C$) reduced the survival time after an acute toxic dose of lead (81). It is of interest that administration of various chelating agents failed to influence the lethal action of a toxic dose of lead (3).

B. Chromosomal and Reproductive Abnormalities

Since lead is known to interfere with processes at the nuclear level and is associated with an increased frequency of miscarriages and abortions amongst female industrial workers (82), several studies were undertaken to examine the effect of lead on chromosomes and certain aspects of fertility. In addition, the observation that lead-poisoned children exhibited a tendency to be shorter and weigh less than normal children (74) further suggested that exposure to this metallic element interfered with the processes of growth and development.

(a) Embryonic Development and Sexual Maturation

Administration of lead to immature male or female rats produced a slight retardation in body weight gain accompanied by a marked inhibition of sexual maturation (83). In female rats treatment with lead produced a decrease in ovarian weight, a delay in vaginal opening and retardation in onset of the estrus cycle, a condition further aggravated upon reduction of dietary protein (83). Immature male mice administered lead displayed a marked reduction in fertility, as evidenced by a reduced rate in pregnancy when metal-treated males were bred with untreated females (84). In addition, an enhancement in the mutagenicity index, a proportion of early fetal deaths, was significantly higher amongst lead-exposed mice. Similarly, Hildebrand et al. (85) showed that treatment with lead reduced fertility in male rats as evidenced by testicular degeneration and oligospermia. It is of interest that intravenous administration of lead to female rats during the 9th through 16th days of gestation induced a high incidence of fetal resorption (86). In this study, external and skeletal anomalies as well as urogenital malformations especially in the kidney were observed predominantly when lead was given on day 9, in doses ranging from 35 to 70 mg/kg. McClain and Becker (86) determined that the amount of lead transported by the placenta was less than 0.10% per hour, which was capable of producing fetal metal levels of about 8 µg per g within 24 hours after maternal lead administration. In the golden hamster, lead also initiated similar fetal malformations characterized by stunting or complete absence of the tail (87). Thus, interference with normal reproduction is one of the symptoms associated with lead-inflicted poisoning in mammals.

(b) Chromosomal Aberrations

In mice fed lead in the diet, leukocyte chromosomes were examined after

preparation of bone marrow cultures. Muro and Goyer (88) found that lead treatment induced a higher frequency of chromosomal aberrations in the form of gaps, breaks and fragments. However, gaps or unstable segments of chromatin were occasionally seen in control animals. The authors suggested that activation of lysosomal enzymes, particularly deoxyribonuclease, may be partially responsible for the increased number of unstable gaps and that their progression into the stable aberrations was evidenced by a breakage in the chromatin (88). Similarly, examination of normal human leukocytes cultured in vitro with lead acetate revealed that this metal was capable of induction of chromosomal aberrations (89). In this study, an increased frequency of breaks was observed in cells incubated with lead for only 24 hours, while some dicentric chromosomes were seen 48 hours later. In contrast, other investigators failed to note any marked effects of lead on chromosomal structure (90). At present, a controversy seems to exist as to whether or not lead has the capacity to inflict damage to cells at the chromosomal level.

C. Central Nervous System

(a) Ultrastructural Alterations

Since the effects of lead on the central nervous system are a prominent feature associated with childhood poisoning, many experiments have been performed to determine the influence of this metal on brain structure and function. Chronic administration of lead to animals from birth resulted in a dose-dependent retardation in body and brain weight gain without any associated alterations in gross pathology (91). Similarly, Pentschew and Garro (92) found a decrease in brain weights which was accompanied by a characteristic brown discolouration of the cerebellum in lead-intoxicated immature rats. In addition, lead appeared to produce a retardation in neuronal maturation, as evidenced by a reduction in myelin content, smaller

neurons, and decreased neurite process proliferation of the neuropil (93). It is of interest that the cerebral cortical and cerebellar regions displayed the highest sensitivity to lead as compared to other brain regions examined (92,93). However, structural alterations in conditions of lead encephalopathy occur predominantly within the cerebral vascular system (94-96). Roy et al. (94) found that treatment of chick embryos with lead acetate produced a thinning of vascular endothelial cells, mitochondrial swelling and alterations in endoplasmic reticular formation, an index of endothelial cell degeneration, and the observed changes were associated with hemorrhage in the brain. In experiments where encephalopathy was produced in infant rats suckling from lead-treated females, alterations in central capillary function were observed, particularly in the cerebellar region (95). This increase in capillary permeability resulted in edema as well as elevated levels of lead, sodium, iron and serum albumin in brains of immature animals, and was followed by hemorrhage in cerebellar and striatal regions leading eventually to death (92,95,96). It is of interest that withdrawal from treatment was followed by complete recovery from the lead-inflicted brain damage (92). Additional evidence to suggest that lead interferes with the function of the blood-brain barrier was provided from studies utilizing the uptake of injected dye (trypan blue) into striatum, cerebellum and spinal cord, in which it was found that the dye entered the brain only after metal treatment (92). Data suggest that damage to the cerebellum is of particular importance, especially since lead primarily causes disturbances in this locus, the region responsible for motor coordination.

As in the case of inorganic metal, organic lead compounds also produce a variety of lesions in the rat central nervous system and appear not to be restricted to any particular neural locus. Administration of TEL produced slight neurone damage, calcification of neurone nuclei, decreased neuroglial

vacuolation and perivascular edema; whereas TML treatment caused extensive neurone damage, nerve tract degeneration, increased neuroglial vacuolation and gliosis (97). In addition cerebellar degeneration, neurofibrillary tangles, a characteristic feature of neurone degeneration, were observed in pyramidal cells of the frontal cortex and hippocampus of rabbits given TEL (98). Irrespective of whether lead exists in the organic or inorganic form, a prominent feature of metal toxicity is damage to the central nervous system of animals.

(b) Metabolic Alterations in the Central Nervous System

The lead-inflicted retardation of rat brain maturation was also demonstrated with the use of certain biochemical parameters. Michaelson (99) noted that lead reduced the cerebellar wet weight in three week-old rat pups and the observed fall in size was accompanied by edema and lower DNA content in this brain region. Since the levels of metal in the cerebral cortex were about one-half the amount seen in cerebellum, it is not surprising that lead-inflicted ultrastructural and biochemical changes occurred less often in the cerebrum (99). Treatment with lead was also found to reduce the conversion of glucose into amino acids, particularly glutamine, in cerebellum, cerebral cortex and forebrain. The observed depression in glycolysis appeared to be independent of permeability across the blood-brain barrier, since equal amounts of radioactive glucose were found in brain regions of control and treated rats (100).

Alterations in various central nervous system mediators have also been postulated to play a role in the toxic effects of lead on the central nervous system. The most prominent change observed in metal-treated animals is an increased turnover of brain norepinephrine (NE) (101). In addition to increased levels of norepinephrine, Silbergeld and Goldberg found that lead enhanced the uptake of ^{14}C-tyrosine into forebrain synaptosomes (101). It

was also demonstrated that this metal increased levels of 3-methoxy-4-hydroxymandelic acid (VMA), the major metabolite of norepinephrine, both in the whole brain and urine of mice (102). Similarly, lead treatment elevated the concentration of norepinephrine in whole brain of rats (103). In contrast, inhibition of the norepinephrine-stimulated form of cerebellar adenylate cyclase was observed *in vitro* using concentrations which approximate those utilized *in vivo* (104). The effects of lead on other mediators are less well defined, although some evidence indicates decreased dopamine levels (105), decreased turnover of acetylcholine (101,106), and a reduction in γ-aminobutyric acid (GABA) synthesis (107).

Organic lead compounds also produce a variety of biochemical alterations in the central nervous system. Triethyllead, at a concentration of 2.5 μM, markedly reduced the incorporation of ^{35}S into sulphatides of rat cerebellum *in vitro* (108). A significant lowering of Cu, Fe and Zn was observed in frontal cortex, cerebellum and hippocampus of organic lead-treated rabbits, suggesting that lead may interfere in a competitive manner with these essential elements for metalloenzymes or other metalloproteins, which are involved in normal central nervous system function (109). At present, the precise mechanisms by which lead affects central nervous system metabolism still remain to be elucidated.

(c) Behavioural Alterations Produced by Lead

Chronic exposure of children to lead has been attributed to result in hyperactivity and other asymptomatic behavioural abnormalities. Exposure of rats or mice to low levels of lead from birth produces a behavioural disorder characterized by elevation in motor activity, aggressiveness, tremors and peripheral ataxia (110). Surprisingly, administration of phenobarbital to lead-treated rats resulted in an increase in hyperactivity, while the reverse occurred in the presence of d- or l-amphetamine (111). In light of this

observation it was found that various amphetamines have been employed in the treatment of "lead-induced hyperactivity" in children (111).

Various investigators have attempted to determine a correlation between metal-inflicted behavioural changes and alterations in the metabolism of neurotransmitters. Some data suggest that enhanced central catecholaminergic function may play a role in lead-induced hyperactivity, although central cholinergic and serotonergic pathways may also play a minor role (101,103,112). However, it is clear that more extensive studies are necessary before any meaningful parallels between neurochemical alterations and behavioural anomalies can be drawn.

Increased lead absorption in children has also been attributed to slow the learning rate, even after withdrawal from metal treatment. Exposure of suckling rat pups to oral or injected lead was found to result in a reduction in the performance in a light/dark-discrimination water T-maze, 5-9 weeks after cessation of treatment. However, no changes in spontaneous locomotor activity were observed in these animals. It is also of interest that blood lead levels in intraperitoneally-injected animals were not significantly different from controls at the time of the T-maze trial, indicating that the actual presence of metal may not be an essential deterrant for learning (113). In addition, decreased T-maze performance was observed in offspring of treated adult rats (114), even without any lead present in the maternal or neonatal diet, suggesting that this metal possesses gametotoxic or teratogenic properties. Although simple learning ability was similar in treated and control animals, discriminatory or habit-reversal tasks were markedly decreased after lead treatment in rats (113,114). In lambs, exposure to low levels of lead through the mothers' milk was found to retard the learning of visual discrimination problems as the concentration of metal reached 24 µg/100 ml in blood, a level known to be considerably lower than the maximum recommended

amount of 40 µg/100 ml (115). The ability of lead to produce abnormal behavioural alterations in animals clearly indicates that further studies are essential in order to re-evaluate the influence of metallic exposure on human behaviour.

D. Effects of Lead on the Hematological System

The greatest proportion (approximately 90%) of lead present in blood is bound to erythrocytes. In conditions where the iron levels are subnormal, the uptake of lead into blood and tissues is enhanced (116). It has been shown that whereas lead does not interfere with iron uptake, high concentrations of iron *in vitro* or *in vivo* can inhibit the uptake of lead by ethyrocytes (117). Farkas (118) postulated that iron and lead compete for a specific low molecular weight binding site, found after fractionation of the hemolysate. It is of interest that addition of iron to the incubation medium failed to affect the binding of lead to hemoglobin and other cellular components, but reduced the amount of lead bound to the low molecular weight protein. Data thus suggest that iron and lead compete for a similar low molecular weight protein in blood.

In rats treated chronically with lead acetate in the diet, an increase in reticulocyte count was accompanied by a decrease in hemoglobin levels, reticulocyte O_2 uptake, δ-aminolevulinic acid dehydratase and ATPase activity (119). In general, hematological changes appeared 8 weeks after treatment (blood lead level approximately 100 µg/ml); however, maximal changes were noted when the concentration of metal reached 150 µg/100 ml, which is about double the concentration found clinically in lead-poisoned humans. In addition, administration of lead was found to reduce the synthesis of globin in red blood cells both *in vivo* and *in vitro* (118). In the presence of Pb^{+2}, Farkas (118) demonstrated a reduction in the incorporation of ^{14}C-

leucine into hemoglobin and non-heme protein, a marked decrease in de novo synthesis of α-chain globin and a slight fall in the amount of β-chain globin formed. It is of interest that this preferential inhibition of α-chain synthesis was also observed in patients suffering from plumbism (68). Furthermore, Farkas (120) found that the lead-inflicted depolymerization of the RNA molecule resulted in inhibition of the translation of isolated globin mRNA to protein. It appeared that globin mRNA was more rapidly and more completely inactivated than polyuridine or tRNA, suggesting that globin mRNA is highly susceptible to lead. However, the observed damage to globin mRNA was reversible since the addition of dithiothreitol abolished the deleterious effects of lead.

In contrast, the use of vitamin E in lead-induced hematological disorders is questionable. It was found that vitamin E-deficient plumbic rats exhibited more pronounced anemia and splenic enlargement than animals given a dietary supplement of α-tocopherol (121). However, elevated dietary vitamin E did not demonstrably affect the severity of lead poisoning in rabbits. Surprisingly, the urinary concentration of δ-aminolevulinic acid and hepatic lead content remained elevated in plumbic rabbits receiving a dietary vitamin E supplement as compared to vitamin-deficient controls (121). Data thus suggest that one of the primary systems affected by lead involves the blood forming elements, and studies are currently underway to attempt to correlate lead toxicity in relation to hematological disorders.

E. Influence of Lead on Liver Function and Metabolism

Although the liver is not considered to be a primary target for lead-induced damage, a variety of enzymic alterations have been observed in this tissue of metal-treated animals. A decrease in hepatic glycogen levels was noted in lead-poisoned rabbits, rats and mice (122-124), accompanied by a reduction in the activity of phosphoenolpyruvate carboxykinase in mice, an

enzyme involved in the formation of glucose from non-carbohydrate precursors (124). In addition, the synthesis of glucose from alanine, lactate and pyruvate was markedly depressed in rat liver, while glucose production from substrates not requiring mitochondrial reactions remained unchanged (125). In contrast, *in vitro* or *in vivo* administration of low concentrations of lead was found to stimulate mouse and rabbit glucose 6-phosphatase, the enzyme responsible for glucose formation from glucose 6-phosphate (122,126). Other hepatic enzymes altered after lead administration include an elevation in acid phosphatase as well as a depression in alkaline phosphatase, various dehydrogenases (lactate, succinate and steroid), and the mixed function oxidase system consisting of cytochrome P_{450} levels, aminopyrine N-demethylase, p-nitroanisole O-demethylase and aniline hydroxylase (122,127). It is of interest that the enzyme activities were reduced in a dose-dependent fashion after lead treatment (127). In contrast, lead increased lipid and phospholipid levels, and produced extensive alterations in the membrane structures of liver Kupffer and parenchymal cells (122,128). Thus, it is evident that although the liver is not a major site of lead accumulation this tissue is susceptible to the effects of this non-essential element.

F. Effects of Lead on Kidney Biochemistry and Function

The most common pathological change noted in chronic plumbism is contracted kidney or interstitial nephritis and is associated with the accumulation of metal in the nuclei of proximal tubular epithelial cells (70). It is noteworthy that the presence of intranuclear inclusion bodies was detected in rat kidneys at concentrations of lead lower than those required to produce hematologic changes suggesting that renal tissue may be more susceptible to the effects of this metal (129). Since the dose-dependent increase in renal lead content was not accompanied by a corresponding enhancement of urinary metal excretion, it was suggested that the storage of lead in intranuclear inclusions

may be partially responsible for this effect (129). Solubility and amino acid composition analysis of these lead-containing bodies revealed that these bodies displayed a strong resemblance to the residual acidic proteins of renal nuclei and that de novo protein synthesis was a critical factor in the development of intranuclear lead inclusions (130,131). Evidence indicates that the intranuclear storage of lead may be a protective mechanism to spare cytoplasmic organelles from damage and preserve metabolic functions.

One of the cell organelles found to be highly susceptible to damage in various tissues, including the kidney, liver, intestine, placenta and heart is the mitochondrion (10). Chronic administration of lead to rats results in mitochondrial swelling in proximal tubular cells (132). In addition, the observed reduction in respiratory control ratio for the substrates pyruvate or malate indicated that this metal exerted a deleterious effect on mitochondrial oxidative phosphorylation. Indeed, Goyer (8) suggested that decreased mitochondrial function may play a role in the lead-inflicted enhancement in the observed renal excretion of amino acids.

A wide variety of metabolic reactions in kidney are also adversely affected by chronic administration of lead. Prolonged heavy metal treatment was found to elevate the activities of the enzymes fetuin:sialic acid transferase, collagen:glucosyltranferase and protease, while a reduction was noted in glucose synthesis and pyruvate metabolism (133,134). In addition, the activities of some lysosomal enzymes tended to increase in metal-treated animals suggesting that cell lysis may play a role in the lead-inflicted kidney damage (133).

Chronic treatment of rats or mice with lead compounds produced adenomatous and carcinomatous tumours in the kidneys of these animals (135-137). Since lead was found to initiate abnormal cell growth and increase kidney weight, various investigators studied the effects of lead on certain aspects of tissue

growth. Choie and Richter (138) reported an increase of mitotic labelling in renal proximal and distal tubular epithelium one day after a single injection of lead in the rat. In the mouse, the elevation in DNA synthesis was preceded by increased RNA and protein synthesis, and augmentation was seen in the RNA:DNA and protein:DNA ratios (16,139). Since the administration of actinomycin D or cycloheximide immediately after lead treatment abolished the alterations in nucleic acid and protein synthesis, Choie and Richter (16,139) suggested that the lead-induced cell proliferation in mouse kidney, as evidenced by increased DNA replication, probably occurs sequentially after RNA transcription and protein synthesis. It is of interest that various types of mammalian cells cultured *in vitro* failed to grow after the addition of low concentrations of lead to the culture medium (140,141). It is evident that lead exerts a rather varying influence on mammalian growth patterns and further studies are essential to understand the manner by which this metal affects tissue development.

G. Cardiovascular Effects Inflicted by Lead

Several reports demonstrated that lead may cause vascular damage characterized by arteriosclerosis, fatty degeneration of blood vessels, and proliferation of perivascular connective tissue (142). In addition, chronic lead poisoning was found to produce arterial hypertension in rabbits (143). Treatment with this metal resulted in abnormal myocardial mitochondrion function as evidenced by histopathological and metabolic changes such as reduced oxygen utilization and decreased oxidative phosphorylation (10,142,144,145). A similar inhibition in oxidative phosphorylation was seen when lead was added to *in vitro* preparations of heart mitochondria (146), and surprisingly, the observed mitochondrial damage was not protected by the addition of chelating agents to the medium. It is of interest that the activities of the cardiac enzymes ferrochelatase and δ-aminolevulinic acid dehydratase, involved in heme

synthesis, were also reduced in chronically lead-treated rats (145). Although lead appears to exert a deleterious effect on myocardial tissue, the nature of the metal-inflicted heart damage requires further investigation.

V. CARBOHYDRATE METABOLISM IN MAMMALIAN TISSUES

The maintenance of glucose homeostasis in mammalian organisms is essential for many cellular activities which require energy derived from the metabolism of carbohydrates. Glucose, the principal carbohydrate found in the body, is metabolized in the presence of oxygen to carbon dioxide and water, releasing 686 kcal/mole of energy in the process of glycolysis. It is of interest that glucose deprivation in the central nervous system leads to convulsions and irregularities in brain function, which may be restored to normal by the administration of glucose.

A. Role of Liver and Kidney in Carbohydrate Metabolism

In mammalian systems, the full enzymatic potential for glucose synthesis from non-carbohydrate precursors as well as glucose degradation via the glycolytic pathway is present in both hepatic and renal cortical tissue. Gluconeogenesis comprises the synthesis of glucose from lactate, pyruvate, glycerol and certain amino acids. When the ingestion of carbohydrates is limited and body glycogen stores are depleted, hepatic and renal tissues provide glucose for the body through the synthetic process of gluconeogenesis. The four key, rate-limiting enzymes of gluconeogenesis are pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. These enzymes catalyze irreversible reactions in the formation of glucose from non-carbohydrate precursors, and are predominantly located in liver and kidney cortex, although minor amounts of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activity have been demonstrated in skeletal muscle (147). Pyruvate carboxylase is the enzyme

involved in the synthesis of oxaloacetate from pyruvate, whereas phosphoenolpyruvate carboxykinase is the enzyme involved in the biotransformation of oxaloacetate into phosphoenolpyruvate. In addition to pyruvate, malate and aspartate can also be converted into phosphoenolpyruvate. The other two important gluconeogenic enzymes are fructose 1,6-diphosphatase and glucose 6-phosphatase. Fructose, 1,6-diphosphate is converted to fructose 6-phosphate by fructose 1,6-diphosphatase, while glucose is the end-product of the reaction catalyzed by glucose 6-phosphatase.

Various factors are known to be involved in the regulation of glucose synthesis in the body. Certain drugs, hormones, dietary factors and the physiological state of the organism are all capable of influencing carbohydrate metabolism in mammals. In addition, gluconeogenesis may be modulated by the availability of substrates essential for this process. 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 supplies of lactate, pyruvate and glycerol are also under the control of catecholamines and insulin (148).

From the circulation, substrates must enter specific cells for transformation into glucose. The uptake of substrates by hepatic cells may 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 (149). Similarly, growth hormone, cortisol and epinephrine have been found to increase hepatic accumulation of various amino acids (150). In contrast, there is evidence that insulin may 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 the entry of pyruvate into liver mitochondria may be a control point at which gluconeogenesis is

influenced by epinephrine, glucagon and cortisol (151).

The synthesis of glucose from non-carbohydrate precursors may also be controlled via modulation of the activities of various enzymes, including the enzymes involved directly in synthesis of glucose or those competing for gluconeogenic substrates. Pyruvate carboxylase is a key enzyme in gluconeogenesis since it is instrumental in determining whether pyruvate is converted to oxaloacetate or oxidized to acetyl-CoA. The mitochondrial level of acetyl-CoA may be a major factor influencing the conversion of pyruvate to glucose since this intermediate is an allosteric activator of pyruvate carboxylase (152). Furthermore, several additional effectors have been reported to influence pyruvate carboxylase activity including Ca^{++}, Mg^{++}, pyruvate, acetoacetyl-CoA, β-hydroxybutyryl-CoA, malonyl-CoA, coenzyme A and aspartate (148). 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 (153). 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 than the kinase for optimal activity. 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 (153).

The enzyme, phosphoenolpyruvate carboxykinase, has been suggested to be a major site at which cyclic AMP acts indirectly or directly to activate gluconeogenesis (154). Regulation of the synthesis of phosphoenolpyruvate carboxykinase is probably an important factor in long-term adaptations of

gluconeogenesis in conditions such as diabetes, starvation, birth and glucocorticoid administration. Phosphoenolpyruvate carboxykinase activity is increased in the liver by glucocorticoids, fasting, glucagon and lactate, or during diabetes induced by alloxan, mannoheptulose or pancreatectomy (155). This stimulation of phosphoenolpyruvate carboxykinase activity is apparently due to increased enzyme synthesis (155). Insulin is effective in suppressing phosphoenolpyruvate carboxykinase, perhaps through decreased cyclic AMP levels (156) or altered supply of hepatic amino acids (157). Stimulation of phosphoenolpyruvate carboxykinase by treatment with glucocorticoids (158), glucagon (159) and isoproterenol has also been reported. Furthermore, glucagon and isoproterenol stimulation of the activity of phosphoenolpyruvate carboxykinase has been shown to be mimicked by cyclic AMP (159).

The reaction sequence between pyruvate and phosphoenolpyruvate represents another major site of control for the process of gluconeogenesis. It is possible that the regulation of this reaction 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 (160). Starvation, diabetes and birth decrease the activity of pyruvate kinase in liver tissue (161).

Fructose 1,6-diphosphatase is another potential site of control of gluconeogenesis and is subject to allosteric control by adenosine 5'-monophosphate (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 (148). 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 (162).

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, adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP). Some of these metabolites may play a role in the physiological regulation of glucose 6-phosphate hydrolysis (163).

Thus, gluconeogenesis is subject to several control processes which include the supply of substrates 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 the levels of ATP, ADP, AMP, cyclic AMP, Mg^{++} and Ca^{++} also seem to play an important role in the regulation of gluconeogenesis.

Schimmel and Knobil (164) found that starvation enhanced serum glucose levels as well as the production of ^{14}C-glucose from labeled alanine, lactate, pyruvate and glycerol in hepatic tissue slices. Starvation also stimulated the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase (165). The elevation of various gluconeogenic enzyme activities was accompanied by a fall in the concentration of valine and alanine (165). 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 (166). 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 (167) found that ^{14}C-lactate, ^{14}C-serine and ^{14}C-aspartate were not incorporated into labeled glucose in 2-day old rats exposed to a 100% nitrogen atmosphere. Similarly, Philippidis and Ballard (168,169) 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 (148). Although the mechanism by which hypoxia suppresses gluconeogenesis is unknown, it is believed to be due to a fall in the concentrations of hepatic pyruvate and oxaloacetate (170,171).

Pregnancy is associated with an altered metabolic state since the fetus is dependent on maternal substrates (172,173). Herrera et al. (174) found an enhanced gluconeogenic capacity in livers of pregnant rats administered ^{14}C-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. Similarly, 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 (148). Alterations in carbohydrate metabolism have also been observed during exercise. Krebs et al. (175) demonstrated an enhanced rate of gluconeogenesis from lactate, pyruvate and fumarate following muscular exercise. Sanchez-Medina et al. (176,177) not only found an increased production of glucose from lactate and pyruvate in exercised rats, but also a 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. (178) demonstrated that in ammonium chloride-induced metabolic acidosis, there was a resultant enhanced renal gluconeogenesis. They observed an increased incorporation of several non-carbohydrate precursors into glucose in rat renal cortical slices. In addition, a marked stimulation in the activity of renal phosphoenolpyruvate carboxykinase was noted during metabolic acidosis induced by ammonium chloride (179). In contrast, metabolic alkalosis induced by sodium bicarbonate loading was found to significantly depress the synthesis of glucose by renal cortical slices (178). As expected, net glucose production in dog kidneys was decreased in metabolic alkalosis (180).

Diabetes in man or experimental animals is characterized by a disturbance in carbohydrate metabolism and is associated with secondary alterations in protein and fat metabolism (181). Weber et al. (182) demonstrated a marked decrease in the activities of various glycolytic enzymes in alloxan diabetic rats. Recently, Singhal et al. (183) 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 (15,155,157,183,184). It is of interest that insulin treatment prevented the diabetes-induced rise in various gluconeogenic enzymes (157). Thus, alterations in gluconeogenesis are important in the metabolic adaptations of man and other mammals to many physiological and pathological conditions. The process of gluconeogenesis is altered under situations including exercise, fasting, diabetes, pregnancy, lactation as well as altered states of acid-base balance. In most physiological situations, the hormones and other factors responsible for the observed changes are still not clearly defined.

B. Role of the Endocrine Pancreas in Carbohydrate Metabolism

The pancreas, via hormones secreted by the islets of Langerhans, plays a prominent role in the regulation of carbohydrate metabolism. Glucagon and insulin are secreted directly into the bloodstream from alpha and beta islet cells, respectively. Pancreatectomy is followed by hyperglycemia, glycosuria and other symptoms of diabetes mellitus, a disease in which the metabolism of carbohydrates, proteins and fats is impaired.

It has been recognized for many years that one of the major metabolic abnormalities in diabetes mellitus is the accelerated breakdown of body protein to yield glucose. Perfused livers from diabetic rats synthesize glucose from

lactate at almost three times the normal rate (185). Human diabetics as well as alloxan diabetic rats also show enhancement of gluconeogenesis from lactate, pyruvate, alanine or glutamate *in vivo* (186,187). It is of interest that insulin administration to diabetic rats reversed the observed changes in blood glucose and hepatic glycogen as well as in various enzymic activities (182,183). Prior administration of either actinomycin D, 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 (182, 183). 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 (15,155,157,183,184). It is interesting that insulin treatment prevented the diabetes-induced rise in the activities of gluconeogenic enzymes (157). Tissue metabolite analysis points to the reaction sequence between pyruvate and phosphoenolpyruvate as a major site for insulin control of gluconeogenesis. Insulin has been found to inhibit protein breakdown in liver, but it is not known whether this is related to a decrease in cyclic AMP levels (148). Thus, a major part of the hypoglycemic action of insulin may be attributed to inhibition of gluconeogenesis from amino acids.

Although metabolic abnormalities in diabetes have largely been attributed to result from a lack of insulin, glucagon has also been recognized as an important factor in the metabolic consequences of diabetes (188). Recent evidence indicates that plasma glucagon levels are normal or high in subjects with diabetes or in animals made insulin-deficient with alloxan or anti-insulin serum, despite the existence of marked hyperglycemia (189). 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 (148). In addition, the action of glucagon on hepatic carbohydrate metabolism is believed to be antagonistic to that of insulin (190). Administration of glucagon was shown to augment hepatic gluconeogenesis from non-carbohydrate precursors in man (191) and in perfused livers (192). Glucagon treatment produced a rise in phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activities whereas the activities of hepatic phosphofructokinase and pyruvate kinase were decreased in the rat (155,190). A similar enzymic response was found in liver when glucagon was administered to humans (190). It is of interest that the concentration of glucose rises during glucagon perfusion and drops when the hormone is removed (192).

From the physiological point of view, the balance between the effects of glucagon and insulin may be the major regulatory influence on the output of glucose by the liver. Menahan and Wieland (193) have shown in perfused rat liver preparations that the activation of endogenous gluconeogenesis by glucagon could be suppressed by insulin. It has been proposed that the glucagon-insulin balance may regulate hepatic glucose output and that this effect may be associated with modulation of hepatic cyclic AMP content (194,195). 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 (156). 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 (195). Thus, interaction of glucagon and insulin may govern the hepatic glucose output and these effects may be associated with changes in hepatic cyclic AMP.

VII. EXPERIMENTAL MODELS OF GROWTH IN MAMMALS

A variety of models have been utilized in the investigation of physiological and biochemical factors implicated to play a role in the regulation of mammalian growth. Numerous studies have demonstrated that hormones secreted by the adenohypophysis, parathyroid and gonads stimulate growth in various tissues. However, the most convenient manner in which to study the physiological and biochemical correlates associated with the regulation of tissue growth involves the use of surgical removal of a portion of certain tissues followed by the investigation of their regenerative processes. Finally, certain drugs capable of augmenting tissue growth have also been employed as experimental tools in the elucidation of the mechanisms involved in cellular proliferation.

A. Hormone-Induced Growth

The most obvious secretagogue which plays a role in growth, that is growth hormone secreted by the adenohypophysis, accelerates body and tissue growth in developing mammals (196,197). In rats and mice, rodents capable of growth throughout their lifespan, administration of growth hormone to adults was found to produce gigantism (196); while in higher-order species acromegaly was observed in response to excess amounts of this peptide hormone (196). Biochemically, growth hormone produced an enhancement in the transport of circulating amino acids into various organs as well as stimulation in the synthesis of RNA and protein within these tissues (196,197). In addition, growth hormone increased the concentration of free fatty acids and glucose in the blood as well as augmented the capacity of liver to convert non-carbohydrate precursors into glucose (196,197). Whereas deprivation of growth hormone in young developing mammals inflicted by hypophysectomy or congenital factors leads to pituitary dwarfism, replacement therapy with growth hormone was found to successfully reverse this effect (196,197). It

is of interest that growth hormone produced an elevation in RNA polymerase activity not only in intact rat liver (198), but cell proliferation and increased DNA synthesis were also observed in thymic lymphocytes cultured in the presence of this hormone (199). In addition to growth hormone, other hormones secreted by the adenohypophysis, such as the gonadotrophins, are capable of initiating hyperplasia and hypertrophy of cells of the accessory sex glands including the testes, prostate, ovaries and uterus. It is of interest that in castrated male and female animals, replacement with testosterone and estradiol, respectively was found to increase accessory sex gland size as well as enhance the biosynthesis of DNA and RNA in these organs (200-202). Similarly, administration of estradiol-17β to immature chicks or testosterone to immature rodents produced an elevation in nucleic acid synthesis and increased accessory sex gland size (203,204).

Parathyroid hormone, along with vitamin D, is essential for the regulation of serum calcium levels and bone formation. Treatment with this hormone was found to enhance the intestinal absorption and proximal tubular reabsorption of calcium as well as reduce the retention of phosphate. In conditions where a deficiency of parathyroid hormone resulting from parathyroidectomy exists, this abnormal hormonal imbalance leads to dental and skeletal malformations and subcutaneous calcification. It is noteworthy that the ability of the thymic lymphoblast to undergo DNA synthesis and cell proliferation is dependent on the presence of parathyroid hormone (205), such that thymic atrophy has been attributed to result from a lack of parathyroid hormone.

Although the precise biochemical basis of cellular proliferation in mammalian systems still remains to be elucidated, numerous studies indicate that alterations in polyamine metabolism play an important role in growth processes, in particular macromolecule biosynthesis (17,206). Pegg and Williams-Ashman (200) demonstrated that the testosterone-induced increase in prostatic

weights of orchidectomized rats was associated with a marked enhancement in the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase, two of the enzymes involved in polyamine formation. Similarly, administration of estradiol to ovariectomized animals produced a significant increase in uterine weights which was accompanied by a concomitant elevation in the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase as well as the endogenous levels of putrescine, spermidine and spermine (207). In addition, treatment of developing chicks with estradiol produced a parallel increase in the accumulation of spermidine, spermine and RNA in hepatic tissue (203). Various investigators (208-210) have proposed that growth hormone may play a role in mammalian tissue proliferation through modulation in polyamine metabolism. Administration of growth hormone to normal or hypophysectomized rats produced a marked elevation in the activity of ornithine decarboxylase activity as well as in the endogenous putrescine and spermidine levels in liver and kidney (209,210). Since polyamines have been demonstrated to exhibit a stabilizing and stimulatory effect on nucleic acid synthesis in mammals (17, 206), it is of interest that the growth hormone-induced elevation in hepatic ornithine decarboxylase activity was accompanied by a rise in the activity of RNA polymerase in fed, fasted and adrenalectomized animals (208).

Recently, a model has been proposed which suggests that the process of cellular growth in mammals involves stimulation of both polyamine formation and the adenylate cyclase-cyclic AMP system. In castrated male or female rats, replacement therapy with testosterone or estradiol, respectively produced an increase in accessory sex gland weight, cyclic AMP levels and the activities of polyamine biosynthetic enzymes (200-202,204). The growth hormone-induced proliferation of rat thymic lymphocytes was also accompanied by an elevation in cyclic AMP levels in these cells (199). Whitfield et al. (199) have demonstrated that parathyroid hormone produced an enhancement in DNA synthesis

and mitotic activity in thymic lymphocytes which was preceded by an increase in the concentration of cyclic AMP. However, parathyroid hormone-induced stimulation of cellular proliferation has not as yet been reported to be associated with enhanced polyamine biosynthesis, and it is conceivable that this hormone exerts its effects through an action on intra- and extra-cellular calcium homeostasis (211).

B. Surgically-Induced Compensatory Growth in Certain Tissues

In recent years, various investigators have employed surgically-induced compensatory growth as an experimental model in an effort to elucidate the metabolic steps involved in the initiation of cell proliferation. Surgical ablation of portions of certain tissues, including liver, kidney, muscle and lung, has been demonstrated to produce an interval of compensatory growth subsequent to partial removal of these organs (212-216). It has been established that partial hepatectomy results in a dramatic enhancement in DNA synthesis and is associated with elevation in the activities of the enzymes DNA polymerase, thymidine kinase and ribonucleoside diphosphate reductase during the phase of regenerative growth (212,217). After unilateral nephrectomy, compensatory hyperplasia of the contralateral kidney was accompanied by a rise in the concentration of DNA and RNA as well as increased renal oxygen uptake (213,218). In rat skeletal muscle, incapacitation of the gastrocnemius muscle was followed by cell proliferation in the soleus (215) and plantaris muscles (214), characterized by an increase in wet weight as well as enhanced DNA and RNA synthesis. It is of interest that unilateral extirpation of rat lung was followed by an elevation in mitotic activity in alveolar cells of the contralateral lung (216).

Various authors have suggested that the observed enhancement in nucleic acid and protein biosynthesis in rapidly growing tissues is related to increased polyamine and cyclic nucleotide synthesis. Partial hepatectomy was found to

initiate cellular growth and enhance the polyamine-synthesizing potential of this tissue (219). In addition, partial hepatectomy resulted in a biphasic rise in tissue cyclic AMP levels and the observed cyclic nucleotide enhancement preceded the elevation in DNA formation (220). It is of interest that hypertrophy of the kidney in response to unilateral nephrectomy was accompanied by an elevation in the activity of ornithine decarboxylase (218), and the observed increase was further enhanced by treatment with hydrocortisone or growth hormone during the period of compensatory growth. The role of polyamines and cyclic AMP in pulmonary and muscular growth following surgical injury still remain to be resolved.

C. Chemical Induction of Mammalian Tissue Growth

A variety of chemical compounds are known to produce stimulation of cell proliferation in certain tissues. Short et al. (221) demonstrated that administration of a solution containing triiodothyronine, amino acids, glucagon and heparin produced a marked increase in nuclear DNA formation in hepatocytes of unoperated rats. In addition, treatment with thioacetamide or azacytidine also enhanced hepatic DNA synthesis and this was accompanied by cellular proliferation (222,223). Guidotti et al. (224) found that treatment with isoproterenol produced augmentation in the incorporation of thymidine into salivary gland DNA. Exposure to noxious gases such as nitrogen dioxide, ozone and high concentrations of oxygen resulted in proliferation of type II epithelial cells that was accompanied by an enhancement in the capacity of lung to incorporate thymidine into DNA (225-227). Recently, the paraquat-inflicted increase in type II pulmonary cells was found by Kacew and Witschi (228) to be associated with stimulation in the activity of thymidine kinase as well as the incorporation of thymidine into DNA. In addition, Witschi et al. (229,230) demonstrated that the butylated hydroxytoluene-stimulated increase in lung cells was accompanied by enhanced thymidine kinase activity and DNA formation. In a

recent study, Palmer et al. (231) demonstrated that in rats exposed to cadmium aerosol, a significant rise was noted in lung weight, cellular proliferation and incorporation of thymidine into DNA. Similarly, Kacew et al. (232) found that exposure to cadmium via the intraperitoneal route augmented the capacity of pulmonary tissue to incorporate thymidine into DNA. It is of interest that cellular proliferation in renal tissue can also be initiated by a variety of agents. Comber and Taylor (233) noted a significant elevation in kidney DNA synthesis of folic acid-treated rats. In recent reports, Choie and Richter (16,139) demonstrated that in mice given a single intracardiac dose of lead, a marked elevation resulted in the biosynthesis of renal DNA as well as in the formation of RNA and protein. A similar augmentation in the incorporation of thymidine into mouse kidney DNA was noted by Cihak and Seifertova (223) in mice administered lead acetate by the intracardiac route, however, the observed increase in nucleic acid biosynthesis was independent of any apparent change in the activity of thymidine kinase, an important enzyme involved in DNA synthesis. It is of interest that Kacew et al. (232) demonstrated that the cadmium-inflicted alterations in renal function were associated with a depression in the incorporation of thymidine into DNA. Data thus indicate that various chemicals can be employed as experimental tools in the investigation of growth in renal tissue.

Recently, various investigators have proposed that alterations in polyamine metabolism are closely associated with nucleic acid biosynthesis, in particular RNA, in rapidly growing tissues (17,206). Increases in tissue polyamine content associated with elevated RNA synthesis have been observed in livers of thioacetamide-treated rats as well as in various tissues of developing neonatal rats (222,234). It is of interest that α-methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, has been demonstrated to produce a marked depletion in polyamine content and a subsequent reduction in thymidine incorporation

into DNA in hepatoma tissue culture (235). A similar positive correlation between enhanced incorporation of thymidine into pulmonary DNA and a rise in the levels of putrescine, spermidine and spermine was recently found in lungs of cadmium-exposed rats (236). Similarly, hypertrophy of the kidney in response to folic acid treatment was accompanied by an elevation in the activity of ornithine decarboxylase and augmentation in DNA synthesis (206,233). In contrast, the reduction in the incorporation of thymidine into renal DNA was associated with a significant rise in the endogenous concentrations of putrescine, spermidine and spermine in kidneys obtained from animals given cadmium (232). It is thus evident that polyamine metabolism may be associated with the biosynthesis of DNA as well as RNA in intact mammalian tissues. Indeed, the observed enhancement in nucleic acid and protein synthesis initiated by polyamines *in vitro*, has led several investigators (17,206) to suggest that the *in vivo* growth and development of certain tissues is dependent upon the presence of polyamines.

Although considerable experimental data indicate that polyamine metabolism is closely associated with the synthesis of nucleic acids, there is a growing body of evidence to suggest that modulation in the adenylate cyclase-cyclic AMP system may be a prerequisite for triggering mammalian DNA synthesis. Short *et al.* (236) found that administration of cyclic AMP to rats produced a marked increase in the incorporation of thymidine into liver DNA as well as in the activity of ornithine decarboxylase. It may be noted that in parotid glands, the isoproterenol-stimulated elevation in the adenylate cyclase-cyclic AMP system was also followed by a subsequent increase in the incorporation of thymidine into DNA (224,237). Similarly, Kacew *et al.* (232) demonstrated that the cadmium-inflicted augmentation in the incorporation of thymidine into pulmonary DNA was preceded and accompanied by a rise in the endogenous levels of the triad of polyamines as well as cyclic AMP. A similar positive correlation between the rise in cyclic AMP and the augmentation in DNA synthesis was

observed in kidneys of folic acid-treated rats (233). The observed depression in the incorporation of thymidine into renal DNA was also associated with a fall in the endogenous levels of cyclic AMP in rodents administered cadmium (232). Indeed, administration of aminophylline, a cyclic nucleotide phosphodiesterase inhibitor, produced an elevation in endogenous cyclic AMP levels in the rat adrenal medulla, followed by a marked stimulation in ornithine decarboxylase activity (238), suggesting that activation of the adenylate cyclase-cyclic AMP system may also play a role in the regulation of polyamine biosynthesis. However, the phenomenon of alterations in the adenylate cyclase-cyclic AMP system as a necessary trigger for DNA synthesis is not universal. Indeed, in transformation viruses and cultured fibroblast 3T3 cells, enhancement in DNA formation was associated with depressed cyclic AMP levels (239). Recently, Witschi et al. (230) demonstrated that the dramatic increase in thymidine kinase, DNA polymerase and the incorporation of thymidine into DNA in mouse lung induced by butylated hydroxytoluene was not associated with any alterations in the adenylate cyclase-cyclic AMP system.

It is thus evident that the process of mammalian tissue growth can be studied with the use of a variety of hormonal, surgical and chemical techniques. Whereas polyamines, cyclic AMP and calcium ions have been proposed to play a role in the initiation of cellular proliferation in mammalian organisms, there still remains considerable uncertainty as to the possible biochemical mechanisms involved in the regulation of mammalian growth.

3. MATERIALS AND METHODS

I. ANIMALS

All animals were obtained from Canadian Breeding Farms and Laboratories (CBFL), St. Constant, Quebec, and maintained on Master Laboratory Chow and tap water ad libitum throughout the treatment periods. In subacute studies, male rats of the Sprague-Dawley strain weighing about 125 g were used, while the initial weight of animals in chronic studies was approximately 100 g. Unless otherwise stated, all rats were starved overnight (16 hours) prior to sacrifice.

II. STUDIES ON NEONATAL RATS

Lactating female Sprague-Dawley rats with one-day old pups were obtained from CBFL for use in chronic oral studies. In the first series of experiments, lactating females were administered 2% Pb (as lead acetate) in the drinking water, according to a modification of the method of Pentschew and Garro (92). Pups were then weaned at the age of 21 days and randomized into groups of 10 animals. Subsequently each set of rats received either 20, 40 or 80 ppm Pb (as lead acetate) in the drinking water until the age of 56 days, while controls received a solution containing 80 ppm sodium acetate. Water intake and body weights were recorded throughout the study.

Another study to examine the effects of lead on growing rat pups was performed according to a modified method of Golter and Michaelson (103). Rat pups were treated with a dose of 50 µg lead (as lead acetate) via gastric intubation daily for the first 21 days after birth. These animals were subsequently weaned on Master Laboratory Chow and water containing 80 ppm Pb (as lead acetate) for a duration of 5 weeks. Pups were randomized into groups of 10. Controls received corresponding amounts of the vehicle (sodium acetate). In this study at least 8 animals per group were also killed 2 and 4 weeks after

the start of the treatment. Corresponding controls (8 rats per group) were sacrificed at 2, 4 and 8 weeks.

III. PREPARATION OF SAMPLES FOR ASSAYING GLUCOSE, GLYCOGEN, UREA, INSULIN AND GLUCONEOGENIC ENZYMES

Animals were killed by decapitation and blood was collected for the determination of glucose and urea. The kidneys were quickly excised and their cortices dissected and weighed on a torsion balance. A small portion of liver was also rapidly removed, weighed and used for the assay of glycogen. The remainder of the liver was removed, weighed and 5% homogenates of both hepatic and renal cortex tissue were prepared in 0.15 M KCl, pH 7.4. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a Teflon plastic pestle spinning at about 700 rpm for exactly 90 seconds. After removing an aliquot of the homogenate for the determination of glucose 6-phosphatase and protein, the homogenate was centrifuged for 30 minutes at 100,000 x g at 0°C in a refrigerated International preparative model B-60 ultracentrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant fluids were decanted and stored in polypropylene test tubes immersed in crushed ice for the assay of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and protein.

A. Blood Glucose, Serum Urea and Liver Glycogen Determinations

Blood glucose was measured according to the method of Somogyi (240) and expressed as milligrams per 100 ml. The concentration of urea was determined in serum according to the urease method of Bernt and Bergmeyer (241), and given as milligrams per 100 ml. The anthrone method of Seifter et al. (242) was used for the assay of hepatic glycogen and data are expressed as g per 100 g tissue. Protein was determined in both homogenate and supernatant

fluids of kidney and liver according to the method of Lowry et al. (243), and given as mg per g tissue.

B. Preparation of Tissue Samples for the Assay of Gluconeogenic Enzymes

The activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase were measured in the 100,000 x g supernatant fluids. In contrast, the whole homogenate was used for the assay of glucose 6-phosphatase activity in liver and kidney cortex. The activity of pyruvate carboxylase was determined at 340 nm in a constant recording Unicam spectrophotometer model SP 800 (Unicam Instruments Ltd., Cambridge, England) thermostated at 37°C. Preliminary experiments were performed to establish linearity with regard to time and amount of tissue and in each case, enzyme activities were assayed under strictly linear kinetic conditions. Pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase activities were calculated as micromoles of substrate metabolized per hour per g of tissue and expressed as specific activity per milligram protein in the supernatant fluid. In contrast, the activity of glucose 6-phosphatase was calculated as micromoles of glucose 6-phosphate metabolized per hour per g tissue and expressed as specific activity per milligram protein in the homogenate.

(a) Pyruvate Carboxylase

Pyruvate carboxylase activity was measured according to the method of Scrutton et al. (244). The assay was based on the rate of disappearance of NADH in a system coupled to malic dehydrogenase. The following components, at the designated final concentrations, were added in the reaction mixture (final volume, 1.0 ml) in the sequence given: 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 in optical density were recorded for at least 5 minutes against a blank which contained no pyruvate.

(b) Phosphoenolpyruvate Carboxykinase

Enzyme activity was determined according to the procedure of Phillips and Berry (245) based on the conversion of oxaloacetate to phosphoenol-pyruvate which is subsequently allowed to react with alkaline hypoiodide to produce a resultant inorganic phosphate. The reaction mixture (final volume 2.0 ml) contained the following components added in the given order: Tris buffer (pH 7.5), 88 mM; $MnCl_2$, 40 mM; KF, 200 mM; inosine triphosphate, 45 mM; oxaloacetate (pH 7.0), 32 mM. The blanks were devoid of the substrate, oxaloacetate. The reaction was initiated by the addition of the supernatant fluid corresponding to 5 mg wet weight of tissue and stopped by 10% trichloroacetic acid. The supernatant was decanted, reacted with sodium hypoiodide for 10 minutes, and then centrifuged at 1,000 x g in a clinical centrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant was decanted to remove the precipitate(iodoform) and inorganic phosphate was measured colorimetrically according to the method of Fiske and Subbarow (246) using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Co., N.Y.).

(c) Fructose 1,6-Diphosphatase

Fructose 1,6-diphosphatase activity was determined by measuring the release of inorganic phosphate from fructose 1,6-diphosphate and complexing the remaining substrate with borate to allow the reaction to proceed to completion according to the method of Weber and Singhal (162). The following components, at the designated final concentrations, were added to the reaction mixture (final volume 1.0 ml) in the given sequence: 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 contained no fructose 1,6-diphosphate. The reaction was started by adding supernatant fluid corresponding to 5 mg wet weight of the tissue and terminated by 10% trichloroacetic acid. The supernatants were decanted and inorganic phosphate was measured according to the method of Fiske and Subbarow (246) using a Klett-Summerson Photoelectric Colorimeter.

(d) Glucose 6-Phosphatase

Glucose 6-phosphatase was measured according to the method of Singhal (247). The assay was based on the rate of formation of glucose and inorganic phosphate 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 were devoid of any glucose 6-phosphate. The reaction was initiated by adding the homogenate corresponding to 5 mg wet weight of the tissue and stopped by 10% trichloroacetic acid. The supernatants were decanted and inorganic phosphate was determined by the method of Fiske and Subbarow (246) using a Klett-Summerson Photoelectric Colorimeter.

C. Insulin Determination

Insulin was determined as immunoreactive insulin (IRI) by the method of Hales and Randle (248) using the commercial kit available from Amersham/Searle Co. (Don Mills, Ontario). 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, Bagsvaerd, Denmark).

IV. PREPARATION OF TISSUE SAMPLES AND PROTEIN KINASE FOR ASSAYING ENDOGENOUS LEVELS OF CYCLIC AMP

Approximately 100 mg of renal cortex, hepatic and pulmonary tissue were quickly frozen in liquid nitrogen and homogenized in 3 ml of 5% trichloroacetic acid. Homogenization was effected with a chilled Potter-Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.), fitted with a plastic Teflon pestle spinning at 700 rpm for exactly 90 seconds. The homogenate was then spun for 15 minutes at 1,000 x g in a refrigerated PR-6000 centrifuge (International Equipment Co., Needham Heights, Mass.) ahd 0.2 ml of 1 N HCl was added to the supernatant. The trichloroacetic acid was removed by extracting with 4 volumes of anhydrous ether and the remaining supernatant was lyophilized overnight using a freeze-drying lyophilizer (Virtis Co., Gardiner, N.Y.). The freeze-dried residue was dissolved in 0.5 ml of distilled water and the concentration of cyclic AMP was determined according to the protein-binding method of Tovey et al. (249).

A. Preparation of Cyclic AMP-Dependent Protein Kinase

The procedure described by Kuo and Greengard (250) 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°C. 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 with acetic acid (1 M). The precipitate was then removed by centrifugation at 27,000 x g for 30 min 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 µg 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. Cyclic AMP Determination

Cyclic AMP levels were measured according to the method of Tovey et al. (249) based on a modification of the protein-binding method of Gilman (251). Cyclic AMP was allowed to compete with a known amount of tritiated cyclic AMP for binding sites on a cyclic AMP-dependent protein kinase. The cyclic nucleotide-protein complex was separated from free nucleotide by adsorption of the unbound nucleotide onto a charcoal adsorbent. The activity of a 200 µl

aliquot of the nucleotide-protein complex was then measured employing phase-combining system cocktail (PCS, Amersham/Searle), a scintillation cocktail for aqueous solutions. The following components, at the designated final concentrations were added to the reaction mixture (final volume, 100 µl) in the given sequence: tritiated cyclic AMP (50 µl, 0.9 picomoles); homogenate fluid corresponding to 10 mg wet weight of the tissue (50 µl). The reaction was initiated by the addition of 100 µl of binding protein and terminated by 100 µl of charcoal suspension. The ratio of counts per min (cpm) bound in the absence of unlabeled cyclic AMP to that 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 per incubation tube). The concentration of cyclic AMP was determined from this standard curve and expressed as picomoles of cyclic AMP per milligram wet weight of tissue.

V. MEASUREMENT OF ADENYLATE CYCLASE ACTIVITY

A. Tissue Preparation

10% homogenates of kidney cortex, liver and lung were prepared in a homogenizing buffer containing Tris-maleate 2 mM and EGTA 2 mM (pH 7.4). 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 rpm for exactly 90 seconds. An appropriate aliquot of the homogenate was then used for the assay of adenylate cyclase and phosphodiesterase activity.

B. Assay for Adenylate Cyclase Activity

Adenylate cyclase activity was measured according to a modified procedure of Kebabian et al. (252). The assay was based on the biotransformation of adenosine triphosphate into cyclic AMP, and the nucleotide formed was measured by the protein-binding method of Tovey et al. (249) as previously described.

An inhibitor of phosphodiesterase, isobutylmethyl xanthine, was added to prevent the breakdown of the cyclic nucleotide. The reaction mixture (final volume, 500 µl) contained the following components added in the given order: Tris-maleate (pH 7.4), 200 mM; $MgSO_4$, 100 mM; ATP, 1 mM; EGTA, 50 mM; isobutyl-methyl xanthine, 10 mM; and 10% homogenate fluid. Sodium fluoride, 10 mM; epinephrine, 0.05 mM; or glucagon, 0.01 mM were added to measure the fluoride- or hormone-stimulated forms of adenylate cyclase. The reaction was initiated by the addition of ATP (15 mM), stopped by boiling for 2.5 minutes and samples were then centrifuged for 10 minutes at 1,000 x g in an IEC PR-6000 refrigerated centrifuge. An appropriate aliquot of the supernatant was used for assaying adenylate cyclase activity. Enzyme activity was calculated as picomoles of cyclic AMP formed per 10 minutes per mg wet weight of tissue and expressed as specific activity per mg protein.

IV. PREPARATION OF TISSUES FOR MEASURING ENDOGENOUS NUCLEIC ACID LEVELS AND SYNTHESIS

A. Measurement of Endogenous RNA levels and ^{14}C-Orotic Acid Incorporation Into RNA

Fresh tissues weighing approximately 1 gram were homogenized in 10 ml distilled water using a Polytron (Brinkmann Instruments Canada Ltd., Toronto, Ontario) and the homogenates then added to 2.5 ml of 0.6 N perchloric acid. The homogenate was then centrifuged at 2000 rpm in an IEC PR-6000 refrigerated centrifuge for 15 min at 0°C. The resulting pellet was washed twice with 0.2 N perchloric acid. After centrifugation, the pellet was dissolved in 0.3 N potassium hydroxide, incubated for 90 min at 37°C, and the reaction stopped by the addition of 1.2 N perchloric acid. The supernatant obtained after centrifugation was retained for RNA determination. Subsequently the pellet was again washed with 0.2 N perchloric acid and the resultant supernatant

obtained after centrifugation was added to the initial supernatant fluid to make up a final volume of 15 ml. The pellet which still contained DNA was thus retained. RNA was assayed according to the method of Volkin and Cohn (253). A ferric ammonium sulphate-orcinol reagent was added to aliquots of the extracted samples. After boiling for 20 min in a 100°C water bath, the OD_{660} was read on a Klett-Summerson Photoelectric Colorimeter. The tissue concentration of RNA was calculated from a set of standards assayed under similar conditions, and expressed as milligrams RNA per gram tissue.

In order to determine the incorporation of ^{14}C-orotic acid into RNA, animals were administered 5 µCi of orotic acid (specific activity 49.95 mCi per mmole) in a volume of 0.2 ml *via* the tail vein, 15 minutes prior to sacrifice, according to the method of Witschi (254). A 3 ml aliquot of the extracted RNA sample was added to 10 ml of Scintiverse scintillation cocktail (Fisher Scientific Co., Ottawa, Ontario) and counted in a Beckman liquid scintillation counter (Beckman Instruments Inc., Toronto, Ontario). The incorporation of orotic acid into RNA was expressed as dpm per milligram RNA.

B. Assay of Tissue DNA Concentration and ^{14}C-Thymidine Incorporation Into DNA

After extraction of RNA from various tissues, the pellets were resuspended in 0.5 N perchloric acid, and incubated for 20 min at 80°C. The above procedure was repeated, the supernatants obtained after both sets of incubations were retained, and made up to a final volume of 10 ml. DNA was assayed in these samples according to the method of Burton (255). Four ml of diphenylamine reagent consisting of diphenylamine (1.5 g), glacial acetic acid (100 ml) acetaldehyde (0.5 ml), and concentrated sulfuric acid (1.5 ml) was added to aliquots of the supernatant. Samples were incubated 16 hours in a 30°C water bath, and the OD_{590} was then read on a Klett-Summerson Photoelectric Colorimeter.

The tissue concentration of DNA was calculated from a set of standards assayed under similar conditions, and expressed as milligrams DNA per gram tissue.

In the assay for the incorporation of ^{14}C-thymidine into DNA, animals were injected via the tail vein with 1 µCi of radioactive-labelled thymidine (specific activity 58.9 mCi/mmole) dissolved in 0.2 ml distilled water and all animals killed 90 min later, according to the method of Witschi and Saheb (229). An aliquot of 3 ml of the extracted DNA sample was counted in 10 ml of Scintiverse scintillation cocktail. The incorporation of thymidine into DNA was expressed as dpm per milligram of DNA.

VII. PREPARATION OF TISSUE SAMPLES FOR ASSAYING ENDOGENOUS LEVELS OF POLYAMINES

The concentration of polyamines in various tissues was measured by the method of Dion and Herbst (256). A portion of tissue was homogenized in 1 N perchloric acid (300 mg/ml) and a 200 µl aliquot of the supernatant was neutralized with sodium carbonate. After incubation with dansyl chloride for 16 hr in the dark, the dansylated samples were extracted into benzene. Subsequently these samples were spotted on thin layer chromatographic plates and run in a cyclohexane ethyl acetate (3:2) solvent system for two 90 min periods. For fixation the plates were sprayed with a triethanolamine/isopropanol (1:4) solution and then vacuum dried in a Thelco vacuum drier (GCA/Precision Scientific Co., Chicago) for 16 hr. The fluorescent spots were then visualized using an ultraviolet lamp. The spots were cut from the plate, placed in benzene and read in a Turner spectrofluorometer (Fisher Scientific Co., Ottawa, Ontario) with a primary filter at 365 nm and a secondary filter at 512 nm. The endogenous levels of putrescine, spermidine and spermine are given as picomoles per g tissue.

VIII. PREPARATION OF TISSUE SAMPLES FOR MEASURING LEAD CONTENT

The concentration of lead in liver, kidney and lung was measured according to the method of Gross and Parkinson (257). Approximately 300 mg of each tissue was added to 0.6 ml of tetramethyl ammonium hydroxide (25% in methanol) in a polyethylene scintillation vial, and heated for 2 hours in a 70°C water bath. The resulting viscous solutions were diluted to 3.0 ml with 1N nitric acid. An aliquot of 25 µl was introduced into a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer "Pb Electrodeless Discharge", Norwalk, Connecticut) equipped with an automatic integrator and background corrector. The absorption of the samples was recorded at a wavelength of 283.3 nm under the following instrumental conditions:

	Temperature (°C)	Time (sec)
Dry	105	30
Ash	550	30
Atomize	2300	10

A set of standards (30-1000 ppb Pb) were prepared in 1N nitric acid and the concentration of lead in tissue samples was determined from the standard curve obtained.

IX. CHEMICALS

All reagents were of the purest grade available and were dissolved in glass distilled water unless stated otherwise. Cyclic 3',5'-AMP, ATP, pyruvate, NADH; malic dehydrogenase, bovine serum albumin, inosine triphosphate, oxaloacetate, fructose 1,6-diphosphate, glucose 6-phosphate, EGTA, tris-maleate, spermine, spermidine, putrescine, phosphoenolpyruvate and pyruvate kinase were purchased from the Sigma Chemical Co. (St. Louis, Mo.). Dansyl chloride (Sigma) was dissolved in acetone. (8-^{3}H) adenosine 3',5'-cyclic phosphate and the insulin immunoassay kit were obtained from Amersham/Searle Corp.

(Don Mills, Ontario), while rat insulin was obtained from the NOVO Research Institute (Bagsvaerd, Denmark). Tetramethyl ammonium hydroxide (25% TMAH in methanol) was purchased from Matheson, Coleman and Bell (Canadian Laboratory Supplies Ltd., Ottawa, Ontario). The radioactive pyrimidine derivatives (6-^{14}C)-orotic acid hydrate (specific activity 49.95 mCi/mmole) and (2-^{14}C)-thymidine (specific activity 58.9 mCi/mmole) were obtained from New England Nuclear (Lachine, Quebec). Lead chloride was dissolved in distilled water and injected intraperitoneally in a final volume of 0.2 ml. Control animals always received an equal volume of physiological saline. Lead acetate was dissolved in distilled water for oral administration and controls received an equal concentration of sodium acetate.

X. STATISTICAL ANALYSIS

The results were subjected to statistical evaluation using Student's "t-test" and significant differences between the means (calculated as p values) are shown. No statistical significance is indicated when the p value was >0.05.

4. RESULTS

I. INFLUENCE OF SUBACUTE LEAD TREATMENT ON VARIOUS METABOLIC PARAMETERS IN KIDNEY AND BLOOD

A. Effects of Lead on Kidney and Body Weight, Liver Glycogen as well as Urea, Glucose and Insulin in Blood

Male Sprague-Dawley rats, initially weighing approximately 125 g, were injected intraperitoneally with lead chloride (5 mg/kg/day x 2) for 7 days. Data presented in Table 1 demonstrate that although treatment with lead for 7 days significantly lowered the body weight, kidney size was not markedly altered. Administration of this heavy metal tended to produce a decrease in liver glycogen (26%) and serum immunoreactive insulin (14%) as well as elevation in serum urea (129%); however, these metabolic parameters were insignificantly different from control values. In addition, the concentration of blood glucose appeared not to be dramatically altered in plumbic rats.

B. Influence on Renal Gluconeogenic Enzymes

Exposure to lead for 7 days produced an increase in the activities of renal pyruvate carboxylase to 122%, fructose 1,6-diphosphatase to 189% and glucose 6-phosphatase to 130% (Table 2). Whereas the activity of kidney phosphoenolpyruvate carboxykinase displayed a tendency towards a rise in plumbic rats (125%) enzymic activity remained insignificantly different from control values.

C. Responsiveness of Renal Cyclic AMP and Adenylate Cyclase to Heavy Metal Treatment

Modulation in the cyclic AMP-adenylate cyclase system has been suggested to play a role in the control of renal gluconeogenesis (258). Since lead increased the activities of various gluconeogenic enzymes in kidney

TABLE 1

EFFECT OF SUBACUTE ADMINISTRATION OF LEAD CHLORIDE ON BODY WEIGHT AND SOME TISSUE AND BLOOD PARAMETERS

Means ± S.E.M. represent at least 5 animals in each group. 5 mg/kg lead chloride was administered intraperitoneally twice daily to male rats for 7 days, and these animals were sacrificed 24 hr following the last injection. Blood glucose and serum urea are expressed as milligrams per 100 millilitres, while liver glycogen is expressed as grams per 100 grams tissue. Serum immunoreactive insulin is given as microunits per millilitre. Data are also given as percentages (in parentheses) with control values taken as 100%.

Parameters	Treatment	
	Control	Lead Chloride
Initial Body Weight (g)	123±3 (100)	124±2 (101)
Final Body Weight (g)	160±1 (100)	132±3 (82)*
Kidney Weight (g)	1.43±0.06 (100)	1.39±0.03 (97)
Liver Glycogen (g/100 g)	1.00±0.36 (100)	0.74±0.42 (74)
Serum Urea (mg/100 ml)	18.6±2.9 (100)	24.1±3.1 (129)
Blood Glucose (mg/100 ml)	72±4 (100)	79±3 (109)
Serum Insulin (μU IRI/ml)	9.9±0.9 (100)	8.5±0.5 (86)

*Statistically significant difference when compared with the values of control rats ($p<0.05$).

TABLE 2

GLUCONEOGENESIS IN RAT KIDNEY CORTEX AFTER SUBACUTE ADMINISTRATION OF LEAD

Means ± S.E.M. represent at least 5 animals in each group. 5 mg/kg lead chloride was administered intraperitoneally twice daily to rats for 7 days, and sacrifice followed 24 hr after the last injection. Enzyme activities are expressed as micromoles of substrate metabolized per hour per mg protein. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

	Enzymes			
Treatment	PC	PEPCK	FD-Pase	G6-Pase
Control	238±17 (100)	21.5±1.4 (100)	5.2±0.5 (100)	7.5±1.2 (100)
Lead Chloride	289±9 (122)*	27.0±3.2 (125)	9.7±0.8 (189)*	9.7±0.9 (130)*

*Statistically significant difference when compared with the values of control rats ($p<0.05$).

TABLE 3

EFFECTS OF SUBACUTE LEAD TREATMENT ON CYCLIC AMP LEVELS AND METABOLISM IN KIDNEY CORTEX

Means ± S.E.M. represent at least 5 animals in each group. 5 mg/kg lead chloride was administered intraperitoneally twice daily to rats for 7 days and animals were sacrificed 24 hr following the last injection. Cyclic AMP levels are expressed as picomoles per milligram tissue. The activity of adenylate cyclase was calculated as picomoles cyclic AMP formed per 10 minutes per milligram protein. Data are also presented in percentages (in parentheses) taking the values of control rats as 100%. The activity of adenylate cyclase in the presence of NaF or epinephrine is also given as a percentage [in parentheses] with the respective basal value taken as 100%.

		Adenylate Cyclase		
Treatment	Cyclic AMP	Basal	+NaF	+Epinephrine
Control	4.92±0.36 (100)	3.48±0.47 (100)	14.98±2.06 (100) [430]†	5.18±0.77 (100) [149]†
Lead Chloride	3.94±0.17 (80)*	3.50±0.38 (101)	15.64±1.93 (104) [447]†	4.96±0.41 (96) [142]†

* Statistically significant difference when compared with the values of control animals ($p<0.05$).

† Statistically significant difference when compared with the respective basal adenylate cyclase activity ($p<0.05$).

cortex, it was of interest to examine whether heavy metal treatment also exerted an effect on the adenylate cyclase-cyclic AMP system. Results in Table 3 illustrate that lead unexpectedly decreased the endogenous concentration of renal cyclic AMP to 3.94 ± 0.17 from a control value of 4.92 ± 0.36 picomoles per milligram tissue. In contrast, lead failed to markedly alter the basal activity of kidney adenylate cyclase. Table 3 also shows that fluoride and epinephrine produced enhancement in the activity of basal adenylate cyclase. However, the fluoride- and epinephrine-stimulated forms of adenylate cyclase remained unchanged in kidneys obtained from plumbic rats.

D. Influence on Blood Sugar and Serum Immunoreactive Insulin After a Glucose Load

In order to examine the ability of subacute lead-administered rats to tolerate high levels of blood glucose, studies were undertaken to examine the time-course of the elevation of blood glucose after a single intraperitoneal injection of dextrose (2 g/kg). Although lead failed to alter the basal concentration of blood glucose, a marked rise in blood sugar was observed at various times in animals given an injection of dextrose (Fig. 1). The observed increase was maintained at 15 minutes (147%), 30 minutes (135%) and 60 minutes (151%) after glucose administration, suggesting that the capacity of lead-exposed rats to compensate for high levels of blood sugar may be disturbed. It is of interest that in response to a rise in blood glucose levels, increased amounts of insulin are usually released into the circulation (259), suggesting that the serum immunoreactive insulin (IRI) levels may serve as an important index of glucose tolerance in mammals. Whereas lead did not alter the basal levels of serum IRI, the concentration of this hormone fell to 58% of the control values 15 minutes after glucose administration (Fig. 1). However, the initial depression in serum IRI was followed by a subsequent elevation (135% of control values) at 30 minutes and a return to control levels after 1 hour.

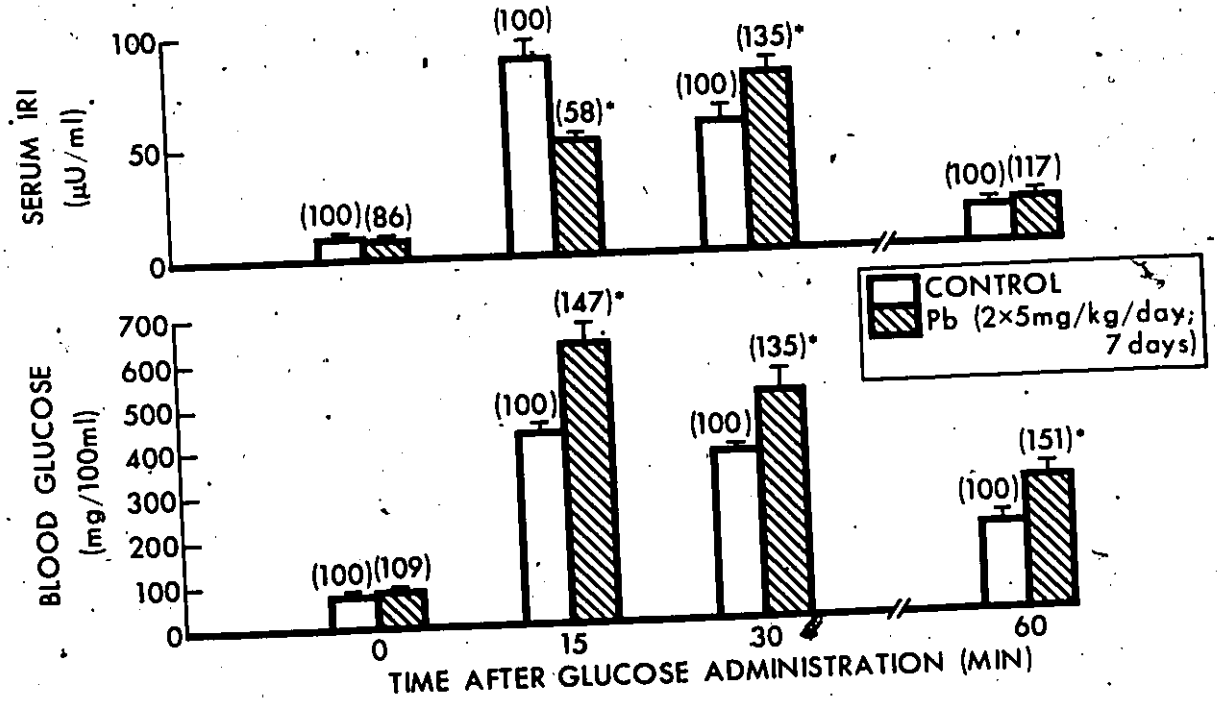

FIG. 1

Effects of subacute lead treatment on tolerance to an injected glucose lead. Each bar represents the mean ± S.E.M. of at least 5 animals in each group. Rats were injected intraperitoneally with lead chloride (5 mg/kg) twice daily for a one week period. Following a 16 hour fast, animals were administered glucose (2 g/kg, i.p.) and killed at 0, 15, 30 and 60 minutes following the glucose load. Blood glucose levels are expressed as mg per 100 ml, and serum immunoreactive insulin (IRI), as μU per ml. Data are also given in percentages (in parentheses) with the values for control animals taken as 100%. *Statistically significant difference when compared with the control values ($p<0.05$).

Although a similar pattern of responsiveness in serum IRI following a glucose load was noted in control rats, the observed changes in normal animals preceded those in metal-treated animals, indicating that lead may delay the release of this hormone from the pancreas.

E. Effect on Insulinogenic Index in Rats Subjected to a Glucose Load

Since lead impaired the ability of rats to tolerate a glucose load, it was of interest to examine the influence of this metal on the insulinogenic index, a measure of pancreatic secretory activity. Table 4 shows that the insulinogenic index was markedly decreased in heavy metal-treated rats 15 minutes after glucose administration (52%). At none of the other time periods studied was there a significant difference noted in the insulinogenic indices of lead-treated animals as compared to controls. It is of interest that 60 minutes after the injection of glucose, the insulinogenic index was decreased in both control (54%) and treated animals (49%).

II. METABOLIC ALTERATIONS INDUCED BY CHRONIC INJECTION OF LEAD IN RATS

A. Effects on Body and Tissue Weights

Male rats, initially weighing about 100 g, were injected daily by the intraperitoneal route with lead chloride (0.2 or 1.0 mg/kg) for 45 days. The weights of kidney, testes, heart and whole body remained unaltered in rats treated with either dose of lead (Table 5). However, liver weight was reduced by approximately 35% in both treatment groups. In contrast, adrenal and thymus weights were significantly increased in rats receiving either amount of heavy metal.

B. Changes in Liver Glycogen, Blood Glucose and Urea in Plumbic and Subsequently Withdrawn Animals

Data presented in Table 6 illustrate the effects of lead injection and subsequent withdrawal on liver glycogen, serum urea and blood glucose. Whereas

TABLE 4

INSULINOGENIC INDEX FOLLOWING SUBACUTE LEAD ADMINISTRATION IN RATS GIVEN AN INTRAPERITONEAL GLUCOSE LOAD

Means ± S.E.M. represent at least 5 animals in each group. 5 mg/kg lead chloride was administered intraperitoneally twice daily to rats for 7 days, and animals were sacrificed 24 hours after the last injection. Insulinogenic index represents a ratio of serum immunoreactive insulin (μU IRI/ml) to blood glucose (mg/ml), at various times following an injected glucose load (2 g/kg i.p.). Data are also expressed as percentages of respective controls ( ) or as percentages of the initial control value [ ], with the appropriate control value taken as 100%.

Treatment	Time following glucose injection (min.) 0	15	30	60
Control	13.2±1.8 (100) [100]	17.4±1.6 (100) [132]	15.3±1.7 (100) [116]	6.1±0.7 (100) [46]†
Lead chloride	12.1±1.3 (92) [92]	8.3±1.0 (48)* [63]†	17.3±0.9 (113) [131]	6.7±1.5 (109) [51]†

*Statistically significant difference from control values at a given time ($p<0.05$).

†Statistically significant difference from control values at zero time ($p<0.05$).

TABLE 5

EFFECTS OF CHRONIC INJECTION OF LEAD ON BODY AND TISSUE WEIGHTS

Means ± S.E.M. represent at least 5 animals in each group. Rats were treated daily with lead chloride (0.2 or 1.0 mg/kg) injected intraperitoneally for 45 days and killed 24 hours after the last injection. Weights are given in grams, except for adrenal and thymus weights, given in milligrams. Data are also given in percentages (in parentheses) with the values of control animals taken as 100%.

Parameters	Treatment		
	Control	Lead Chloride 0.2 mg/kg	Lead Chloride 1.0 mg/kg
Body Weight (g)	386±15 (100)	363±17 (94)	351±19 * (91)
Liver (g)	13.6±1.0 (100)	8.7±0.2 (64)*	9.1±0.6 (67)*
Kidney (g)	2.9±0.2 (100)	2.7±0.1 (92)	2.8±0.3 (95)
Adrenal (mg)	46±1.4 (100)	53±1.3 (115)*	63±1.0 (139)*
Thymus (mg)	455±59 (100)	673±62 (147)*	660±20 (140)*
Testes (g)	3.2±0.1 (100)	3.3±0.1 (102)	3.3±0.2 (102)
Heart (g)	1.1±0.0 (100)	1.1±0 (100)	1.1±0 (100)

*Statistically significant difference when compared with the values of control rats ($p<0.05$).

TABLE 6

LIVER GLYCOGEN, BLOOD GLUCOSE AND SERUM UREA FOLLOWING CHRONIC INJECTION OF LEAD AND SUBSEQUENT WITHDRAWAL

Means ± S.E.M. represent at least 5 animals in each group. Rats were treated daily with lead chloride (0.2 or 1.0 mg/kg) injected intraperitoneally for 45 days and killed 24 hours after the last injection. Some rats treated with the 1.0 mg/kg, dose were withdrawn from the injections for an additional 28 days before sacrifice. Blood glucose and serum urea are given in milligrams per 100 millilitres, while liver glycogen is given as grams per 100 grams. Data are also given in percentages (in parentheses) with the values of control animals taken as 100%.

Treatment	Liver Glycogen (g/100 g)	Serum Urea (mg/100 ml)	Blood Glucose (mg/100 ml)
Control	2.2±0 (100)	26±1 (100)	81±2 (100)
*Lead Chloride 0.2 mg/kg, 45 days	1.3±0.1 (59)*	37±1 (142)*	124±2 (153)*
1.0 mg/kg, 45 days	0.4±0.1 (18)*	47±1 (181)*	158±3 (195)*
1.0 mg/kg, 45 days; 28 days withdrawal	1.2±0.2 (53)*	39±1 (151)*	144±2 (178)*

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

treatment with either 0.2 or 1.0 mg/kg lead markedly reduced liver glycogen content, a significant rise was noted in the concentration of serum urea and blood glucose. The observed alterations were related to the dose used and greater quantitative changes were seen in rats receiving the higher amount of lead. Some of the rats previously treated with 1.0 mg/kg lead were maintained for an additional period of 28 days to determine the effects of withdrawal in previously exposed animals. Termination of the treatment regimen for 28 days in rats previously given the metal for 45 days resulted in partial restoration of these metabolic alterations; however, the decrease in liver glycogen as well as elevation in serum urea and blood glucose still remained significantly different from controls.

C. Influence on Serum Immunoreactive Insulin and Insulinogenic Index

Since chronic lead treatment produced alterations in glucose homeostasis, it was of interest to examine the effects of this metal on serum immunoreactive insulin. Results presented in Table 7 demonstrate that a dose of 0.2 mg/kg lead failed to alter serum IRI. In contrast, the higher amount of metal (1.0 mg/kg) significantly decreased the concentration of IRI in serum by 18%. Withdrawal of lead for 28 days in rats previously given the metal for 2 months produced a return in the level of serum IRI to control amounts. Insulinogenic index, a measure of pancreatic secretory activity, was also depressed by either dose of metal. Termination of the treatment regimen restored partially the insulinogenic index, although the values still remained significantly lower than those of controls.

D. Response of Hepatic Gluconeogenic Enzymes to Chronically-Injected Lead

Administration of either 0.2 or 1.0 mg/kg lead produced a rise in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase (Fig. 2). Withdrawal from

TABLE 7

SERUM IMMUNOREACTIVE INSULIN AND INSULINOGENIC INDEX FOLLOWING CHRONIC INTRAPERITONEAL INJECTION OF LEAD AND SUBSEQUENT WITHDRAWAL

Means ± S.E.M. represent at least 5 animals in each group. Rats received either 0.2 or 1.0 mg/kg lead chloride for 45 days and were sacrificed 24 hours following the last injection, except for a portion of the 1.0 mg/kg group which underwent 28 days withdrawal from the injections before sacrifice. Immunoreactive insulin is expressed as microunits per millilitre serum. Insulinogenic index represents a ratio of serum IRI to blood glucose. Data are also expressed as percentages (in parentheses) with the values of control animals taken as 100%.

Treatment	Immunoreactive Insulin (μU IRI/ml serum)	Insulinogenic Index
Control	58.8±3.8 (100)	72.6±6.4 (100)
Lead 0.2 mg/kg	53.2±2.2 (90)	42.9±2.4 (59)*
1.0 mg/kg	48.4±1.1 (82)*	30.6±1.3 (42)*
1.0 mg/kg, 28 day withdrawal	58.6±2.4 (100)	40.7±2.2 (56)*

*Statistically significant difference when compared with the values of control animals ($p<0.05$).

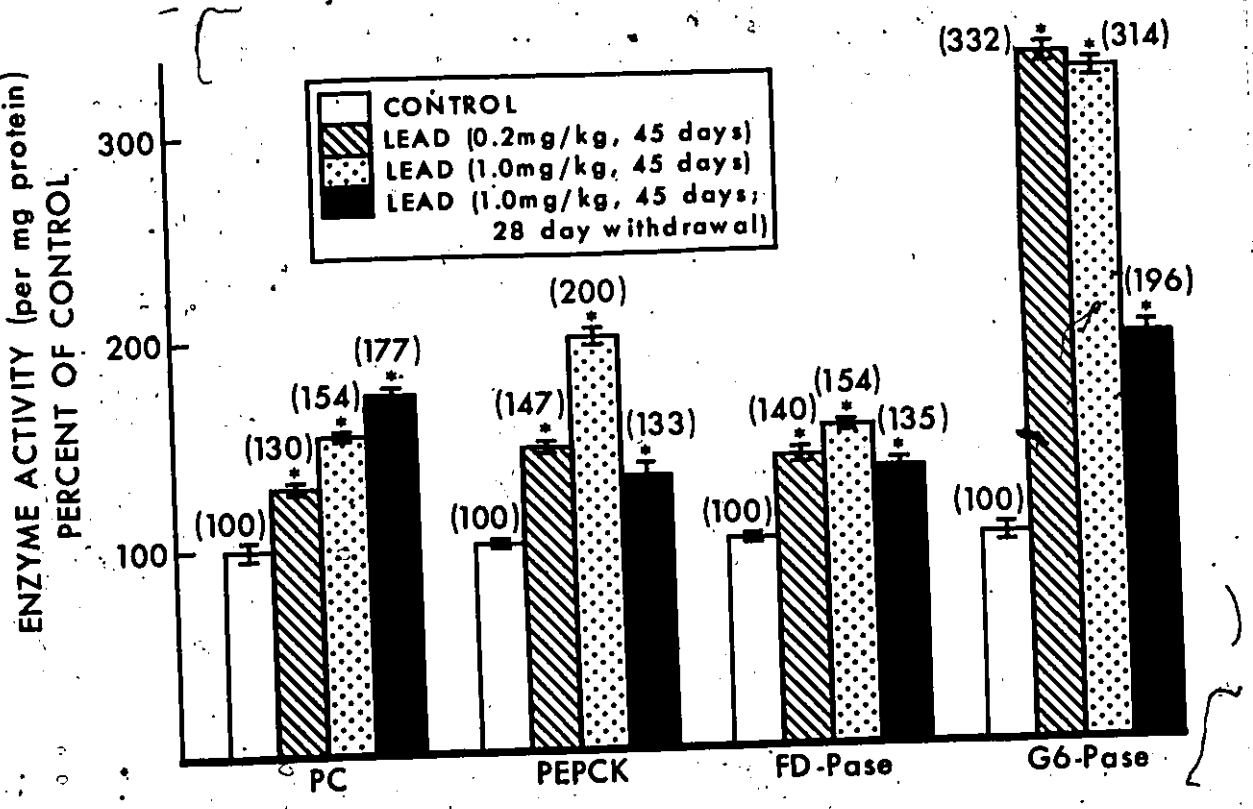

FIG. 2

Influence of chronic lead and subsequent withdrawal on hepatic gluconeogenic enzymes. Each bar represents the mean value ± S.E.M. of 5 animals in each group. Animals were given lead daily by the intraperitoneal route (0.2 or 1.0 mg/kg $PbCl_2$) for 45 days and killed 24 hours after the last injection. Five rats pretreated with 1.0 mg/kg lead were maintained for an additional period of 28 days without any treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per mg protein. Data are given in percentages, taking the values of control animals as 100%. *Statistically significant difference when compared with the values of control rats ($p<0.05$).

treatment for 28 days in previously injected rats partially restored the activities of PEPCK, FD-Pase and G6-Pase to control values, although the observed alterations still remained significantly higher than controls. In contrast, the metal-induced enhancement in the activity of pyruvate carboxylase in withdrawn rats remained elevated to the same extent as that seen in 45 day-injected animals.

Influence on Renal Gluconeogenic Enzymes

Since kidney is also an important site of glucose production (148), it was of interest to determine whether lead treatment also affected the activities of renal gluconeogenic enzymes. Results presented in Fig. 3 show that injection of either amount of lead for 45 days elevated the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in rat kidney cortex. As in the case of liver, withdrawal from treatment for 28 days in rats previously given lead for 45 days restored the activities of PEPCK, FD-Pase and G6-Pase to the levels seen in controls. In contrast, the activity of pyruvate carboxylase still remained significantly elevated (181%), although enzymic activity was quantitatively lower than that seen in 45-day treated animals (307%).

F. Lead-Induced Alterations in the Hepatic Cyclic AMP-Adenylate Cyclase System

Chronic administration of lead (1.0 mg/kg) produced an elevation in cyclic AMP levels and a rise in the activity of adenylate cyclase in rat liver (Table 8). However, the lower amount (0.2 mg/kg) of heavy metal failed to alter hepatic cyclic AMP content and the activity of adenylate cyclase. Table 8 also shows that withdrawal from lead treatment for 28 days in animals previously given the metal for 45 days, restored both cyclic AMP levels and the activity of adenylate cyclase to control levels.

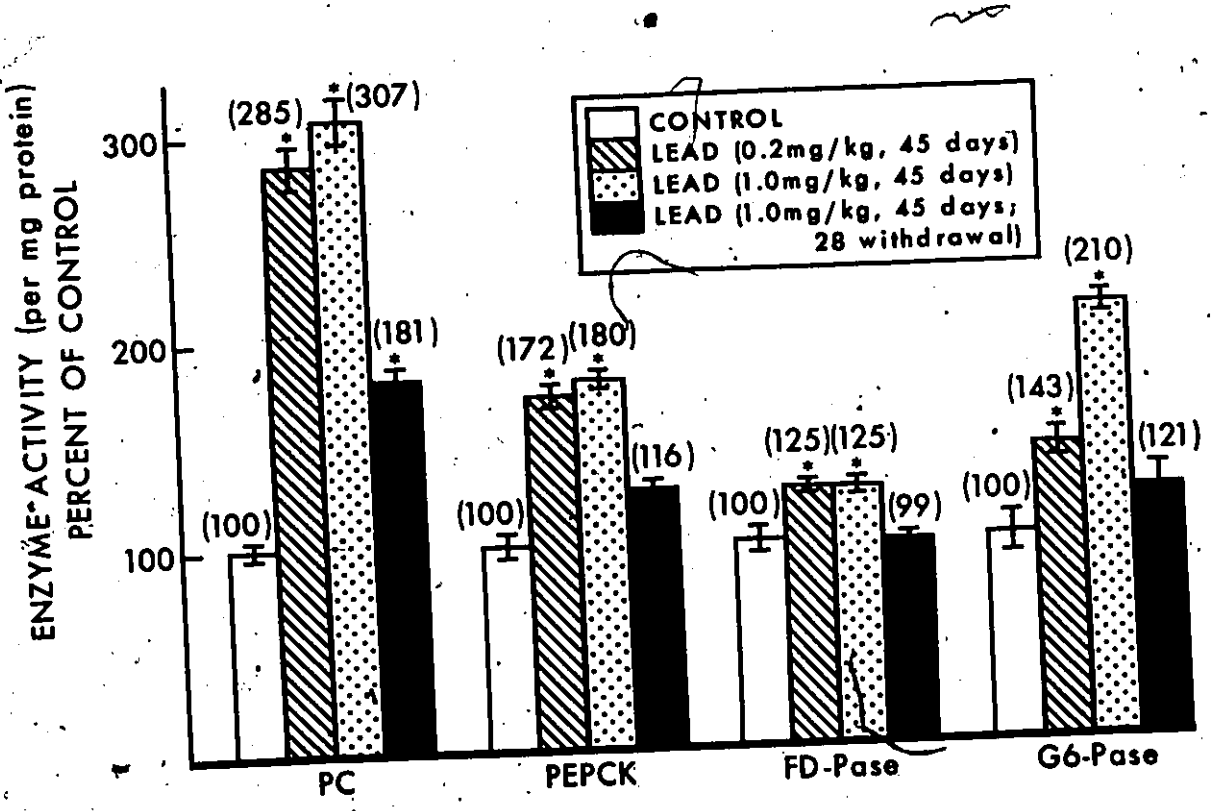

FIG. 3

Effect of chronic lead and subsequent withdrawal on renal gluconeogenic enzymes. Each bar represents the mean value ± S.E.M. of 5 animals in each group. Animals were given lead daily by the intraperitoneal route (0.2 or 1.0 mg/kg $PbCl_2$) for 45 days and killed 24 hours after the last injection. Five rats pretreated with 1.0 mg/kg lead were maintained for an additional period of 28 days without any treatment. Enzyme activities were calculated as micromoles of substrate metabolized per hour per mg protein. Data are given in percentages, taking the values of control animals as 100%. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

TABLE 8

EFFECTS OF CHRONICALLY ADMINISTERED LEAD ON HEPATIC CYCLIC AMP-ADENYLATE CYCLASE

Means ± S.E.M. represent at least 5 animals in each group. Rats were treated daily with lead chloride (0.2 or 1.0 mg/kg) injected intraperitoneally for 45 days and killed 24 hours after the last injection. Some rats treated with the 1.0 mg/kg dose were withdrawn from the injections for an additional 28 days before sacrifice. Cyclic AMP levels are expressed as picomoles per milligram tissue, whereas the activity of adenylate cyclase is given as picomoles cyclic AMP formed per 10 minutes per milligram protein. Data are also given in percentages (in parentheses) with the values of control animals taken as 100%.

Treatment	Cyclic AMP	Adenylate Cyclase
Control	0.93±0.05 (100)	1.74±0.27 (100)
Lead 0.2 mg/kg	1.01±0.06 (109)	2.19±0.15 (126)
1.0 mg/kg	1.26±0.14 (135)*	3.04±0.31 (174)*
1.0 mg/kg, 28 day withdrawal	1.02±0.08 (110)	2.20±0.22 (126)

*Statistically significant difference when compared with the values of control animals ($p<0.05$).

G. Effects of Certain Compounds on Liver Adenylate Cyclase Activity

Various agents are known to stimulate adenylate cyclase activity *in vitro* (260). Lead treatment and subsequent withdrawal appeared not to affect the 5- to 8-fold increase in the activity of adenylate cyclase produced by fluoride ions (Table 9). Similarly, chronic heavy metal administration failed to alter the activity of the epinephrine-stimulated form of the enzyme; however, upon termination of heavy metal administration for 28 days, a fall in the activity of this form of adenylate cyclase was noted. It is of interest that epinephrine produced no additional stimulation in adenylate cyclase activity as compared to basal enzymic activity in the livers of animals administered 1.0 mg/kg lead chloride. Chronic exposure to lead produced a marked enhancement in the glucagon-stimulated form of adenylate cyclase. The rise in activity of the glucagon-stimulated form of adenylate cyclase was maintained even after lead was removed for 28 days in rats previously administered the metal.

H. Lead-Induced Changes in Renal Cyclic AMP-Adenylate Cyclase System

Lead at a dose of 0.2 mg/kg failed to alter renal cyclic AMP levels or the activity of basal adenylate cyclase (Table 10). However, increasing the dose of metal to 1.0 mg/kg produced a fall in the levels of cyclic AMP and reduced the activity of adenylate cyclase by 61%. Indeed, the lead-induced decrease in the activity of adenylate cyclase was maintained even 28 days after withdrawal from treatment (52%). In contrast, subsequent termination of injection in rats previously given the metal for 28 days restored the endogenous concentration of cyclic AMP to control amounts. As in the case of liver, chronic lead treatment had no significant effects on the activity of the epinephrine-stimulated form of adenylate cyclase. Although treatment with 0.2 mg/kg lead produced a reduction in the fluoride-stimulated form of

TABLE 9

INFLUENCE OF VARIOUS STIMULATORS ON LEAD-INFLICTED ALTERATIONS IN HEPATIC ADENYLATE CYCLASE

Means ± S.E.M. represent at least 5 animals in each group. Rats were treated daily with lead chloride (0.2 or 1.0 mg/kg) injected intraperitoneally for 45 days and killed 24 hours after the last injection. Some rats treated with the 1.0 mg/kg dose were withdrawn from the injections for an additional 28 days before sacrifice. The activity of adenylate cyclase is given as picomoles cyclic AMP formed per 10 minutes per milligram protein. Data are also given in percentages (in parentheses) with the values of control animals taken as 100%. The activity of adenylate cyclase in the presence of NaF, epinephrine or glucagon is also given as a percentage [in parentheses] with the respective basal value taken as 100%.

Treatment	Adenylate Cyclase			
	Basal	+NaF	+Epinephrine	+Glucagon
Control	1.74±0.27 (100)	13.44±1.80 (100) [772]†	3.37±0.35 (100) [194]†	8.43±0.41 (100) [484]†
Lead 0.2 mg/kg	2.19±0.15 (126)	14.75±1.47 (110)* [674]†	3.76±0.25 (111) [172]†	18.59±1.89 (220)* [849]†
1.0 mg/kg	3.04±0.31 (174)*	14.50±0.87 (108) [477]†	3.67±0.59 (109) [121]†	13.06±1.75 (155)* [430]†
1.0 mg/kg, 28 day withdrawal	2.20±0.22 (126)	14.89±0.70 (111) [677]†	2.43±0.25 (72)* [110]†	15.21±1.13 (180)* [691]†

*Statistically significant difference as compared to the values of control animals ($p<0.05$).

†Statistically significant difference as compared to the basal values in respective treatment groups ($p<0.05$).

TABLE 10

CHANGES IN RENAL CORTICAL CYCLIC AMP AND ADENYLATE CYCLASE AFTER CHRONIC LEAD ADMINISTRATION

Means ± S.E.M. represent at least 5 animals in each group. Rats were treated daily with lead chloride (0.2 or 1.0 mg/kg) injected intraperitoneally for 45 days and killed 24 hours after the last injection. Some rats treated with the 1.0 mg/kg dose were withdrawn from the injections for an additional 28 days before sacrifice. The activity of adenylate cyclase is expressed as picomoles cyclic AMP formed per 10 minutes per milligram protein. Data are also given in percentages (in parentheses) with the values of control animals taken as 100%. The activity of adenylate cyclase in the presence of NaF or epinephrine is also given as a percentage [in parentheses] with the respective basal values taken as 100%.

Treatment	Cyclic AMP	Adenylate Cyclase		
		Basal	+NaF	+Epinephrine
Control	4.41±0.11 (100)	1.39±0.04 (100)	9.63±0.91 (100) [696]†	3.04±0.15 (100) [219]†
Lead 0.2 mg/kg	4.49±0.32 (102)	1.41±0.24 (102)	4.86±0.32 (50)* [345]†	3.12±0.38 (102) [221]†
1.0 mg/kg	4.06±0.15 (92)	0.54±0.07 (39)*	9.75±1.76 (101) [1806]†	2.45±0.29 (80) [454]†
1.0 mg/kg, 28 day withdrawal	4.47±0.13 (101)	0.66±0.13 (48)*	18.24±1.85 (189)* [2764]†	3.39±0.30 (100) [514]†

*Statistically significant difference as compared to the values of control animals ($p<0.05$).

†Statistically significant difference as compared to the basal values in respective treatment groups ($p<0.05$).

adenylate cyclase (50%), enzymic activity remained unchanged in kidney cortices obtained from 1.0 mg/kg treated rats. Withdrawal from injection for 28 days markedly enhanced the fluoride-stimulated form of renal adenylate cyclase (189%). It is of interest that, unlike the responsiveness of the basal activity of adenylate cyclase in lead-treated animals, a marked enhancement in the fluoride-stimulated form was noted in animals given 1.0 mg/kg metal as well as in rats withdrawn from treatment for 28 days. A similar elevation in the epinephrine-stimulated form of adenylate cyclase was noted as compared to the respective basal activity of the enzyme in rats given 1.0 mg/kg lead chloride and also after withdrawal from metal injection.

III. EFFECT OF CHRONIC ORAL LEAD ADMINISTRATION ON VARIOUS METABOLIC PARAMETERS IN KIDNEY AND BLOOD

A. Influence on Body and Kidney Weight, Liver Glycogen and Serum Urea

Since chronic oral ingestion of lead by children has been attributed to be a major environmental hazard (11), it was of interest to examine the effects of this mode of metal administration for a period of 8 weeks from birth. Dams received 2% lead (as lead acetate) in the drinking water. This dietary dose of lead has been reported to result in lead concentrations ranging from 25-40 ppm in the milk (261). When the pups reached 21 days of age, they were weaned and separated randomly into groups receiving 20, 40 or 80 ppm lead (as lead acetate) in the drinking water for a further 35 days. Data given in Table 11 demonstrate that lead failed to alter body and kidney weight. In contrast, liver glycogen was markedly reduced by all 3 amounts of heavy metal and the lowest value (19% of control) was obtained with the 40 ppm concentration. Whereas serum urea was decreased in animals given 20 ppm lead (22%), an increase was seen in the 80 ppm group (133%) and no change was noted in rats receiving 40 ppm.

TABLE 11

EFFECTS OF CHRONIC ORAL LEAD ON BODY WEIGHT, KIDNEY WEIGHT, LIVER GLYCOGEN AND SERUM UREA

Mean ± S.E.M. represent at least 5 animals in each group. Lead was administered to rats for 8 weeks from birth. Dams received 2% lead (as lead acetate) in the drinking water, and 21 days after parturition, pups were separated randomly into groups receiving 20, 40 or 80 ppm lead (as lead acetate) in the drinking water for the remaining 35 days. Body and kidney weights are given in grams. Liver glycogen is expressed as grams per 100 grams tissue, and serum urea is expressed as milligrams per 100 milliliters. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Treatment	Body Weight at 56 days (g)	Kidney Weight (g)	Liver Glycogen (g/100 g)	Serum Urea (mg/100 ml)
Control	214±18 (100)	1.85±0.14 (100)	3.6±0.6 (100)	19.2±1.5 (100)
Lead 20 ppm	211±15 (99)	1.92±0.13 (104)	1.2±0.4 (33)*	15.0±1.1 (78)*
40 ppm	223±9 (104)	1.77±0.08 (96)	0.7±0.3 (19)*	19.6±1.7 (102)
80 ppm	206±11 (96)	1.84±0.11 (99)	0.8±0.2 (23)*	25.6±1.5 (133)*

*Statistically significant difference when compared with values of control animals ($p<0.05$).

B. Lead-Induced Alterations in Blood Glucose, Serum Immunoreactive Insulin and Insulinogenic Index

The concentration of blood glucose was elevated in rats given either 20, 40 or 80 ppm lead for 8 weeks in a dose-dependent manner and greater quantitative changes were seen as the dose was increased (Table 12). The observed hyperglycemia was associated with a fall in the concentration of serum IRI in animals receiving the highest amount of metal. In addition, exposure to 40 or 80 ppm lead significantly decreased the insulinogenic index, a measure of pancreatic function.

C. Influence on Gluconeogenic Enzymes in Rat Kidney Cortex

Data presented in Fig. 4 illustrate that lead at a dose of 20 or 40 ppm significantly elevated the quartet of renal gluconeogenic enzymes. It is of interest that treatment with 80 ppm metal produced a rise in phosphoenol-pyruvate carboxykinase to 273%, pyruvate carboxylase to 227%, glucose 6-phosphatase to 180% and fructose 1,6-diphosphatase to 260% of control values, respectively.

D. Lead-Induced Alterations in Renal Cyclic AMP Levels and Adenylate Cyclase Activity

Administration of all 3 doses of lead increased the concentration of cyclic AMP in kidney cortex (Table 13). It is worthwhile to note that the metal-induced rise in cyclic nucleotide levels was related to the amount of lead ingested and greater quantitative changes were observed when the administered dose was increased. Under the experimental regimen employed, maximal stimulation to 295% of the control values was seen with the 80 ppm concentration. Whereas treatment with either 20 or 40 ppm lead failed to alter renal adenylate cyclase, a significant elevation in enzymic activity was noted with the 80 ppm dose.

TABLE 12

EFFECTS OF CHRONIC ORAL LEAD ON BLOOD GLUCOSE, SERUM IMMUNOREACTIVE INSULIN, AND INSULINOGENIC INDEX

Means ± S.E.M. represent at least 5 animals in each group. Lead was administered to rats for 8 weeks from birth. Dams received 2% lead (as lead acetate) in the drinking water, and 21 days after parturition, pups were separated randomly into groups receiving 20, 40 or 80 ppm lead (as lead acetate) in the drinking water for the remaining 35 days. Blood glucose is expressed as milligrams per 100 millilitres, while serum immunoreactive insulin is given as microunits per millilitre. Insulinogenic index represents a ratio of serum IRI to blood glucose. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Treatment	Blood Glucose (mg/100 ml)	Serum Immunoreactive Insulin (μU IRI/ml)	Insulinogenic Index
Control	82.0±4.1 (100)	57.3±1.9 (100)	65.8±3.4 (100)
Lead			
20 ppm	109.1±6.7 (133)*	58.7±3.2 (102)	65.3±3.1 (99)
40 ppm	147.1±9.2 (179)*	53.8±3.6 (94)	39.5±3.3 (60)*
80 ppm	179.3±4.8 (219)*	48.9±3.6 (85)*	27.5±2.6 (42)*

*Statistically significant difference as compared to the values of control animals ($p<0.05$).

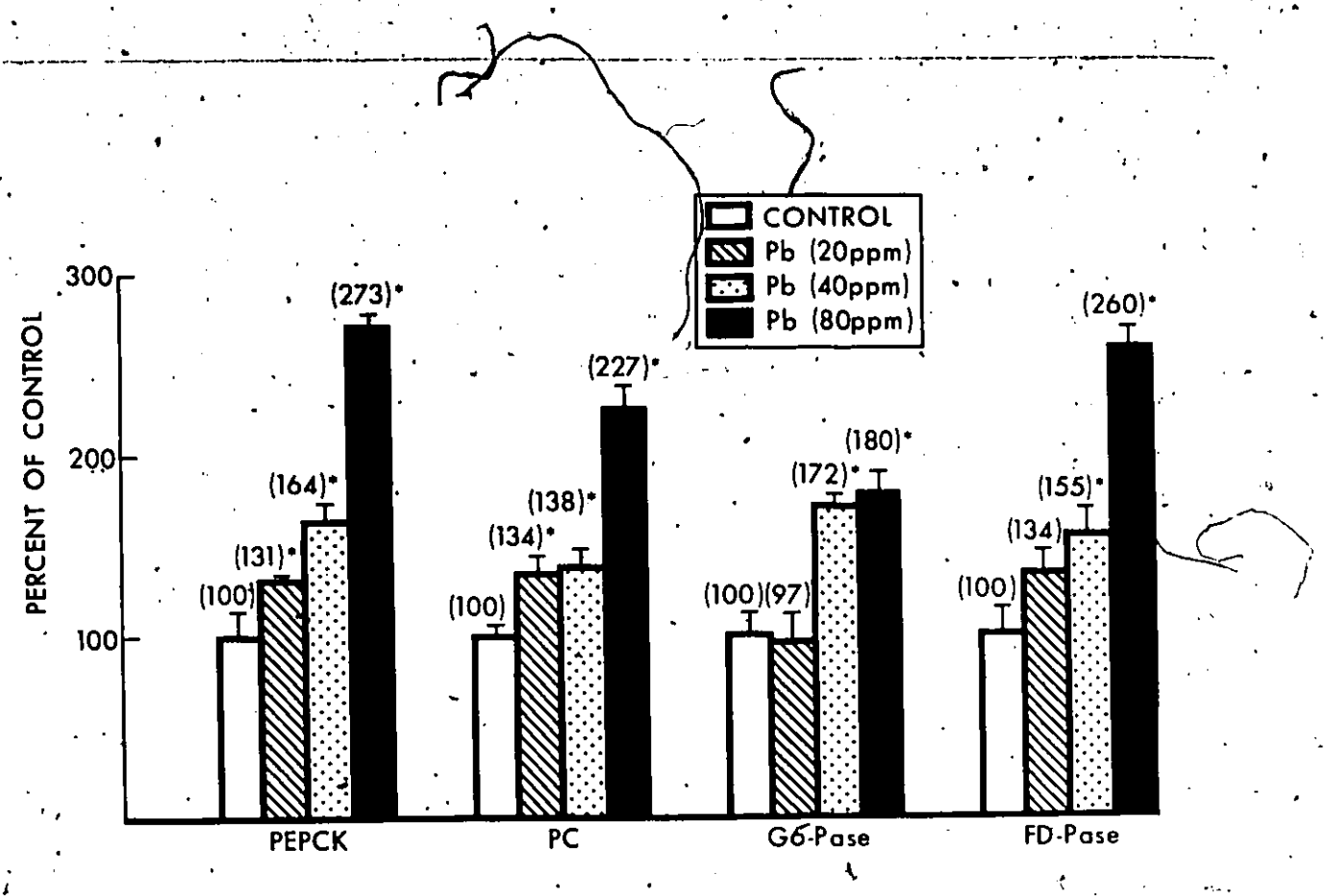

FIG. 4

Effect of chronic lead ingestion on the quartet of key gluconeogenic enzymes in kidney cortex. Each bar represents the mean value ± S.E.M. of at least 5 animals in each group. Mothers received 2% lead (as lead acetate) in their water supply for the first 21 days and, subsequently, groups of infants had access to either 20, 40 or 80 ppm heavy metal in the drinking water for an additional period of 35 days. Enzyme activities were calculated as micromoles of substrate metabolized per hour per mg protein. Data are expressed in percentages, taking the values of control animals as 100%. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

TABLE 13

EFFECTS OF CHRONIC ORAL LEAD ON ADENYLATE CYCLASE ACTIVITY IN RAT KIDNEY CORTEX

Means ± S.E.M. represent at least 5 animals in each group. Lead was administered to rats for 8 weeks from birth. Dams received 2% lead (as lead acetate) in the drinking water, and 21 days after parturition, pups were separated randomly into groups receiving 20, 40 or 80 ppm lead (as lead acetate) in the drinking water for the remaining 35 days. The endogenous concentration of cyclic AMP is expressed as picomoles per milligram tissue (wet weight). Enzyme activity is expressed as picomoles cyclic AMP formed per 10 min per milligram protein. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%. The activity of adenylate cyclase in the presence of NaF or epinephrine is also given as a percentage [in parentheses] with the respective basal value taken as 100%.

Treatment	Cyclic AMP	Adenylate Cyclase		
		Basal	+NaF	+Epinephrine
Control	1.15±0.03 (100)	2.53±0.12 (100)	12.89±0.61 (100) [509]†	6.69±0.24 (100) [264]†
Lead				
20 ppm	1.29±0.05 (112)*	2.73±0.15 (110)	13.17±0.79 (102) [482]†	6.80±0.39 (102) [249]†
40 ppm	1.55±0.06 (135)*	2.94±0.31 (116)	14.65±0.90 (114) [498]†	7.68±0.52 (115) [261]†
80 ppm	2.25±0.15 (195)*	3.30±0.22 (130)*	16.17±1.99 (125) [490]†	8.19±1.20 (122) [248]†

*Statistically significant difference when compared with the values of control animals ($p<0.05$).

†Statistically significant difference when compared with the respective basal adenylate cyclase activity ($p<0.05$).

It is of interest that the activities of the fluoride- and epinephrine-stimulated forms of renal adenylate cyclase remained unchanged in metal-treated rats.

IV. EFFECT OF CHRONIC ORAL LEAD ADMINISTRATION ON VARIOUS PARAMETERS ASSOCIATED WITH GROWTH IN LIVER, KIDNEY AND LUNG

A. Influence on Body Weight and Tissue Lead Levels

In order to examine the influence of chronic lead ingestion on various parameters associated with growth, rat pups were given daily oral doses of 50 µg lead for 21 days from birth. Thereafter, for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Body weight was monitored throughout the experimental period, and no significant differences were noted between control and treated animals (Fig. 5). The tissue lead levels at various time intervals during the treatment period are illustrated in Table 14. Treatment of rat pups from birth with lead revealed that the kidney retained the metal during the entire exposure period with the highest amount being noted after 2 weeks. Although the liver contained higher levels of lead 2 weeks after intubation was started, continuation of this treatment regimen resulted in decreased retention as compared to 2 week-old animals. However, the concentration of metal retained by this tissue at 4 and 8 weeks was still significantly greater than controls. In contrast, at none of the time intervals studied did pulmonary lead values exceed control values.

B. Effect on ^{14}C-Thymidine Incorporation Into Tissue DNA

The sequential alterations in the capacity of kidney, liver and lung of lead-exposed rats to incorporate labelled thymidine into DNA is illustrated in Fig. 6. Whereas lead treatment reduced the incorporation of thymidine into hepatic DNA after 2 weeks, a return to control levels was seen at 4 weeks which

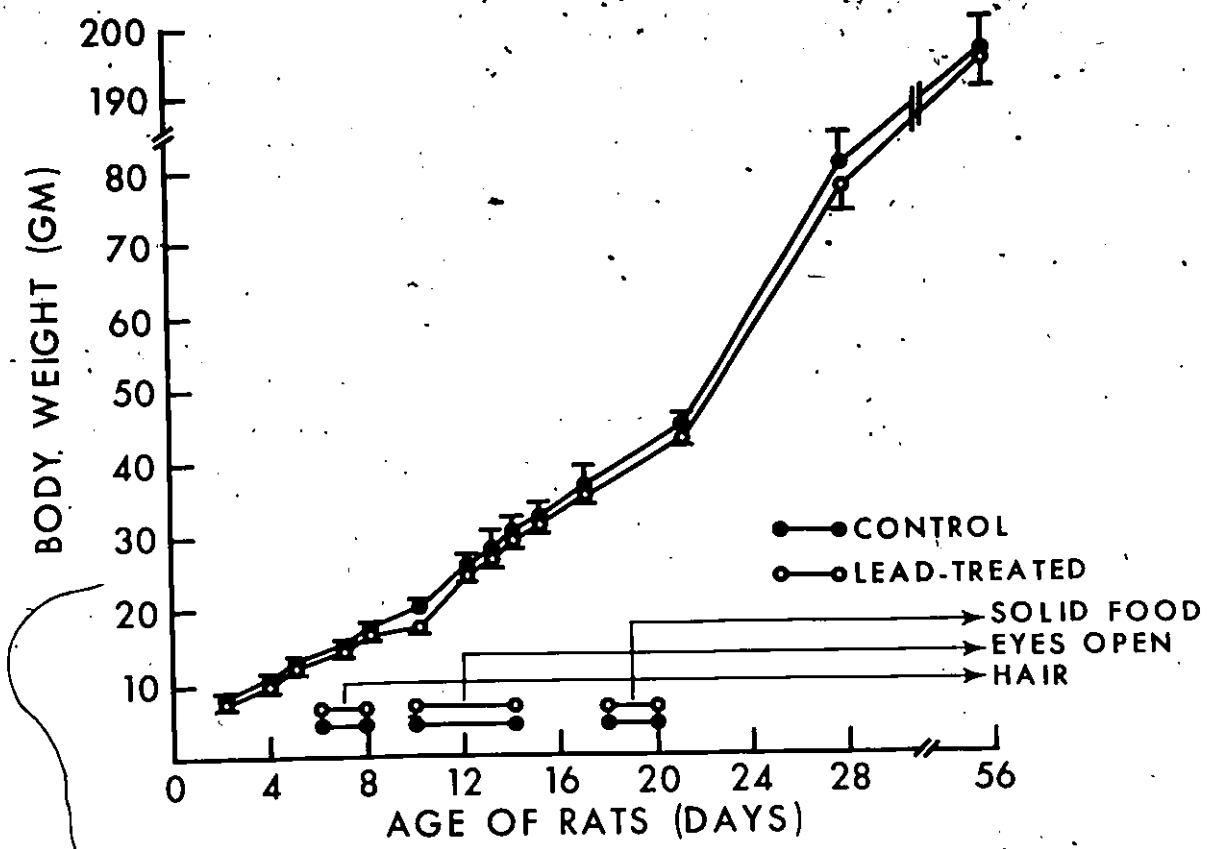

FIG. 5

Comparison between the growth and development of control and lead-treated rats. Each point represents the mean ± S.E.M. of at least 6 animals in each group. Animals were given daily oral doses of 50 µg of lead for 21 days from birth. Thereafter, for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. *Statistically significant difference as compared with the values of control animals ($p<0.05$).

LEAD CONTENT OF LIVER, KIDNEY AND LUNG AFTER CHRONIC TREATMENT

Means ± S.E.M. represent at least 6 animals in each group. The treatment regimen involved p.o. administration of 50 µg lead for 21 days from birth followed by administration of 80 ppm lead in drinking water for the next 35 days. Animals were killed at 2, 4 or 8 weeks during this treatment regimen. Retention of lead in tissues is expressed as the percent of the total dose in µg/g for each time period. The total available dose was estimated assuming 50-80% absorption from the gastrointestinal tract in immature rats, and 90% deposition of absorbed lead into bone (i.e. 10% of the absorbed dose was available for retention in all soft tissues).

Tissue Examined	Treatment Period (Weeks)	Lead Content (µg/g)		Percent total dose in tissue		Percent total available dose in tissue	
		Control	Treated	Control	Treated	Control	Treated
Liver	2	0.102±0.003 (100)	0.402±0.038 (394)[b]	0	0.64	0	11-14
	4	0.101±0.003 (100)	0.169±0.034 (165)[b]	0	0.18	0	2- 3
	8	0.104±0.003 (100)	0.169±0.007 (165)[b]	0	0.14	0	2- 3
Kidney	2	0.080±0.010 (100)	0.463±0.031 (579)[b]	0	0.74	0	13-16
	4	0.082±0.010 (100)	0.334±0.039 (418)[b]	0	0.35	0	4- 6
	8	0.083±0.010 (100)	0.362±0.036 (453)[b]	0	0.31	0	4- 6
Lung	2	0.088±0.015 (100)	0.080±0.005 (91)	0	0	0	0
	4	0.080±0.015 (100)	0.091±0.007 (104)	0	0	0	0
	8	0.088±0.015 (100)	0.090±0.010 (103)	0	0	0	0

*Statistically significant difference as compared with control values ($p<0.05$).

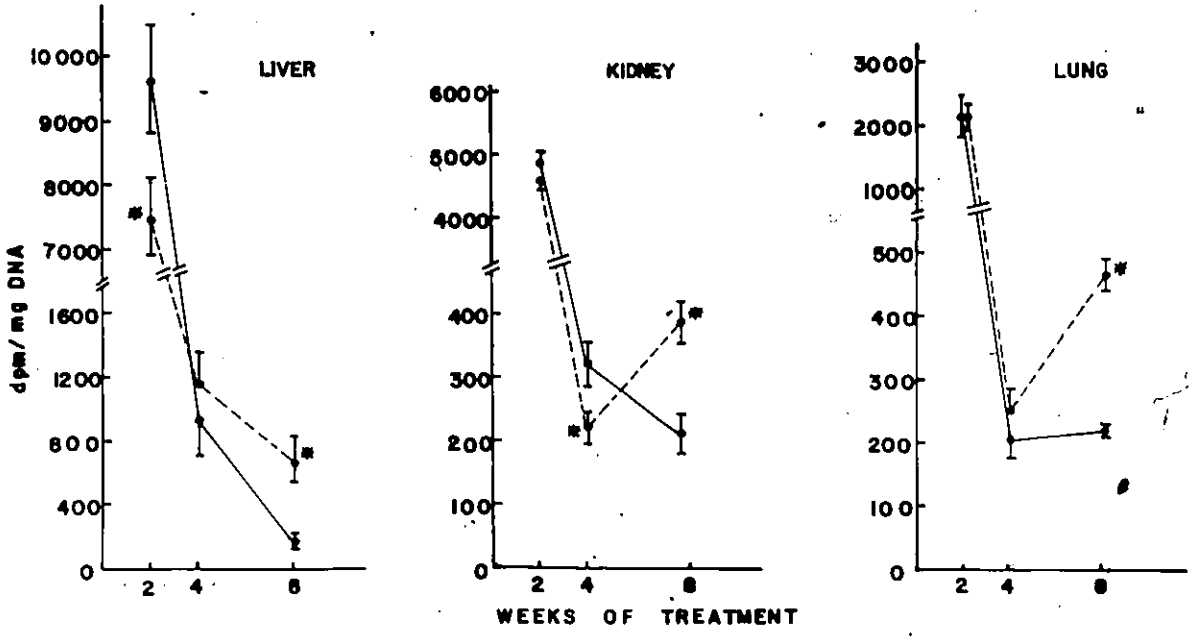

FIG. 6

Time-course of lead-inflicted changes in the incorporation of ^{14}C-thymidine into hepatic, renal and pulmonary DNA in neonatal rats. Each point represents the mean ± S.E.M. of at least 6 animals in each group. Animals were given daily oral doses of 50 µg of lead for 21 days from birth. Thereafter, for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. The incorporation of ^{14}C-thymidine into DNA is expressed as dpm per mg DNA. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

was followed by a subsequent 3.5-fold elevation at 8 weeks. Although metal administration failed to alter thymidine incorporation into renal DNA after 2 weeks, a reduction in incorporation was noted after 4 weeks. As in the case of liver, a significant elevation in the incorporation of thymidine into kidney DNA to 160% of control values was observed in 8 week-treated rats. Similarly, the incorporation of thymidine into pulmonary DNA was elevated after 8 weeks of lead treatment; however, the incorporation of thymidine into DNA remained unaltered at the other time periods examined.

C. Alterations in Hepatic, Renal and Pulmonary DNA Content

Data in Table 15 show the influence of prolonged lead administration on tissue DNA content. In liver, administration of lead for 2 or 8 weeks produced a significant reduction in the concentration of DNA. A similar fall in DNA content was noted in kidneys at all time periods studied, the maximal decrease (73% of control values) occurring after 2 weeks. Although pulmonary DNA levels remained insignificantly different from control in 1- or 2-month old metal-treated animals, a slight yet significant fall (14%) was noted after 2 weeks.

D. Changes in RNA Content of Liver, Kidney and Lung

Data in Table 16 show that administration of lead for 2 or 4 weeks produced a decrease in hepatic RNA content, followed by a subsequent return to control amounts by 2 months. The concentration of RNA was also reduced in lungs of 2 week-treated rats with no apparent marked alterations noted after 1 or 2 months. Whereas renal RNA levels remained unchanged after 2 or 4 weeks, a fall was observed in 8-week treated rats.

E. Influence on RNA/DNA Ratio in Various Tissues

The effects of chronic lead treatment on the hepatic, renal and pulmonary RNA/DNA ratio are presented in Table 17. Whereas the hepatic ratio of RNA/DNA was reduced after 2 and 4 weeks of metal treatment, a return to control values

TABLE 15

INFLUENCE OF CHRONIC LEAD TREATMENT ON DNA CONTENT OF VARIOUS TISSUES

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily doses of 50 µg lead per day for 21 days from birth. After weaning, rats were given 80 ppm lead in the drinking water for the remaining 35 days. DNA content of the tissues studied is expressed as milligrams per gram tissue. Data are also expressed as percentages (in parentheses) with the values of control animals taken as 100%.

Age of rats	DNA (mg/g tissue) Liver	Kidney	Lung
2 weeks			
Control	3.21±0.04 (100)	6.19±0.16 (100)	4.28±0.25 (100)
Treated	3.01±0.06 (94)*	4.55±0.10 (73)*	3.69±0.16 (86)*
4 weeks			
Control	2.92±0.07 (100)	4.34±0.06 (100)	4.46±0.11 (100)
Treated	2.69±0.08 (92)	3.92±0.07 (90)*	4.18±0.11 (94)
8 weeks			
Control	2.51±0.08 (100)	2.86±0.10 (100)	6.26±0.32 (100)
Treated	2.08±0.07 (83)*	2.49±0.12 (87)*	5.78±0.14 (93)

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

TABLE 16.

CHANGES IN RNA CONTENT OF LIVER, KIDNEY AND LUNG AFTER CHRONIC LEAD ADMINISTRATION

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily doses of 50 µg lead (as lead acetate) per day for 21 days from birth. After weaning, rats were given lead at a concentration of 80 ppm in the drinking water for the remaining 35 days. RNA content of the tissues studied is expressed as milligrams per gram tissue. Data are also expressed as percentages (in parentheses) with the values of control animals taken as 100%.

Age of rats	RNA (mg/g tissue) Liver	Kidney	Lung
2 weeks			
Control	7.34±0.15 (100)	5.90±0.17 (100)	3.66±0.13 (100)
Treated	6.05±0.34 (82)*	5.73±0.14 (97)	2.92±0.15 (80)*
4 weeks			
Control	8.87±0.52 (100)	5.86±0.08 (100)	2.92±0.16 (100)
Treated	6.79±0.27 (77)*	5.40±0.25 (92)	2.93±0.18 (101)
8 weeks			
Control	7.55±0.24 (100)	4.60±0.18 (100)	2.51±0.09 (100)
Treated	7.01±0.13 (94)	4.04±0.17 (88)*	2.43±0.07 (97)

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

TABLE 17

EFFECTS OF CHRONIC LEAD TREATMENT ON THE RNA/DNA RATIO IN LIVER, KIDNEY AND LUNG

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily doses of 50 µg lead per day for 21 days from birth. After weaning, rats were given 80 ppm lead in the drinking water for the remaining 35 days. The RNA/DNA ratio was calculated from the values obtained for tissue nucleic acid content. Data are also expressed as percentages (in parentheses) with the values of control animals taken as 100%.

Age of rats	RNA/DNA ratio		
	Liver	Kidney	Lung
2 weeks			
Control	2.29±0.02 (100)	0.99±0.03 (100)	0.77±0.00 (100)
Treated	2.05±0.09 (89)*	1.26±0.03 (127)*	0.79±0.03 (103)
4 weeks			
Control	3.02±0.13 (100)	1.35±0.02 (100)	0.69±0.02 (100)
Treated	2.54±0.10 (84)*	1.31±0.01 (97)	0.73±0.02 (106)
8 weeks			
Control	3.13±0.20 (100)	1.61±0.03 (100)	0.40±0.02 (100)
Treated	3.26±0.14 (104)	1.63±0.06 (101)	0.42±0.01 (105)

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

was seen by 2 months. In kidney, 2 weeks of lead ingestion produced a significant increase in kidney RNA/DNA ratio without any apparent changes at the other time points. No alterations in the ratio of RNA/DNA were observed in lungs of metal-treated animals at any of the times examined.

F. Effects on Tissue Protein Levels

Results in Table 18 illustrate the influence of lead on tissue protein content. In liver, administration of lead for 2 weeks produced a significant reduction in the concentration of protein, followed by a return to control levels at 1 and 2 months after treatment. In contrast, lead produced a rise in the concentration of renal protein after 2 weeks without any associated alterations in 4 or 8 week-administered animals. As in the case of kidney, the concentration of protein in lung was significantly elevated after 2 and 8 weeks of lead administration, the maximal increase (154% of control values) seen at 2 weeks.

G. Changes in Tissue Polyamine Content

In order to examine the role of polyamine metabolism in the lead-induced alterations in nucleic acid synthesis, the effects of this heavy metal were examined on the concentration of putrescine, spermidine and spermine in liver, kidney and lung. Although lead administration failed to alter the hepatic concentrations of putrescine or spermidine at any of the time periods examined (Table 19), spermine levels were elevated as early as 2 weeks, decreased by 30% at 4 weeks and subsequently returned to control levels at 8 weeks. In kidney, the concentration of putrescine was significantly reduced by approximately 50% after 4 and 8 weeks (Table 20). Whereas renal spermidine fell at 4 weeks, a significant increase to 269% of control values was noted in 2 month-treated rats. However, the levels of spermine remained unchanged throughout the course of the experiment. Data in Table 21 show that one month after treatment the

TABLE 18

PROTEIN CONTENT OF HEPATIC, RENAL AND PULMONARY TISSUE AFTER CHRONIC LEAD INGESTION

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily doses of 50 µg lead per day for 21 days from birth. After weaning, rats were given 80 ppm lead in the drinking water for the remaining 35 days. Protein content of the tissues examined is expressed as milligrams per gram tissue. Data are also expressed as percentages (in parentheses) with the values of control animals taken as 100%.

Age of rats	Protein (mg/g tissue) Liver	Kidney	Lung
2 weeks			
Control	158.7±3.6 (100)	135.2±5.8 (100)	78.9±7.7 (100)
Treated	140.6±4.7 (89)*	161.5±9.9 (119)*	121.7±8.9 (154)*
4 weeks			
Control	182.5±10.1 (100)	252.8±10.8 (100)	182.5±16.1 (100)
Treated	167.5±9.8 (92)	233.4±16.8 (92)	168.5±12.8 (92)
8 weeks			
Control	166.9±14.4 (100)	266.0±23.9 (100)	170.0±18.1 (100)
Treated	202.5±19.1 (121)	221.3±7.5 (83)	224.1±13.7 (132)*

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

TABLE 19

INFLUENCE OF CHRONIC LEAD INGESTION ON HEPATIC POLYAMINES

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily oral doses of 50 µg lead for 21 days from birth. Thereafter for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. Polyamine content is given as picomoles per milligram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Age of rats	Polyamines (pmoles/mg tissue)		
	Putrescine	Spermidine	Spermine
2 weeks			
Control	7.27±0.35 (100)	10.73±1.39 (100)	72.7±4.8 (100)
Treated	6.30±0.47 (87)	7.73±0.89 (72)	97.2±9.4 (134)*
4 weeks			
Control	2.09±0.35 (100)	1.15±0.13 (100)	63.7±9.3 (100)
Treated	1.91±0.11 (91)	0.92±0.14 (80)	44.7±1.9 (70)*
8 weeks			
Control	1.05±0.13 (100)	0.60±0.07 (100)	34.5±3.9 (100)
Treated	1.18±0.06 (113)	0.72±0.08 (120)	29.0±4.3 (84)

*Statistically significant difference as compared with control values ($p<0.05$).

TABLE 20

KIDNEY POLYAMINE CONTENT AFTER CHRONIC LEAD ADMINISTRATION

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily oral doses of 50 µg lead for 21 days from birth. Thereafter for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. Polyamine content is given as picomoles per milligram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

	Polyamines (pmoles/mg tissue)		
Age of rats	Putrescine	Spermidine	Spermine
2 weeks			
Control	7.38±0.75 (100)	10.45±1.07 (100)	70.2±6.0 (100)
Treated	6.14±0.34 (83)	11.14±1.19 (107)	62.4±3.5 (89)
4 weeks			
Control	6.57±0.07 (100)	1.82±0.28 (100)	73.3±16.1 (100)
Treated	4.01±0.23 (61)*	1.04±0.10 (57)*	84.8±2.3 (116)
8 weeks			
Control	5.44±0.54 (100)	0.78±0.13 (100)	60.3±7.7 (100)
Treated	2.86±0.42 (53)*	2.1 ±0.49 (269)*	53.0±6.8 (88)

*Statistically significant difference as compared with control values ($p<0.05$).

TABLE 21

EFFECT OF CHRONIC LEAD TREATMENT ON PULMONARY POLYAMINES

Means ± S.E.M. represent at least 6 animals in each group. Animals were given daily oral doses of 50 µg lead for 21 days from birth. Thereafter for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. Polyamine content is given as picomoles per milligram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

	Polyamines (pmoles/mg tissue)		
Age of rats	Putrescine	Spermidine	Spermine
2 weeks			
Control	5.16±0.50 (100)	3.36±0.33 (100)	33.0±5.0 (100)
Treated	4.80±0.34 (93)	3.14±0.23 (93)	40.5±3.6 (123)
4 weeks			
Control	2.05±0.15 (100)	1.90±0.18 (100)	63.9±9.2 (100)
Treated	1.08±0.08 (53)*	0.84±0.07 (44)*	28.5±2.2 (45)*
8 weeks			
Control	1.17±0.06 (100)	1.18±0.14 (100)	41.5±2.8 (100)
Treated	1.10±0.11 (95)	1.07±0.17 (91)	48.6±2.8 (117)

*Statistically significant difference as compared with control values ($p<0.05$).

concentrations of pulmonary putrescine, spermidine and spermine were significantly reduced to approximately 50% of control values; however, no apparent changes were detected at any of the other time intervals examined.

H. Influence on Endogenous Cyclic AMP Levels

Since modulation in the adenylate cyclase-cyclic AMP system may be involved in the initiation of DNA synthesis in certain mammalian tissues (18), it was of interest to examine the role of cyclic nucleotide metabolism in the lead-inflicted augmentation of thymidine incorporation into hepatic, renal and pulmonary DNA. In the triad of tissues examined, the endogenous levels of cyclic AMP increased approximately 2-fold after 4 weeks of lead treatment and the observed elevation in cyclic nucleotide content was maintained only in kidney cortex after 8 weeks (Fig. 7). It is of interest that the increase in cyclic AMP levels noted in all 3 tissues examined preceded the observed enhancement in the incorporation of thymidine into DNA. In addition, the reduction of thymidine incorporation into renal DNA seen at 4 weeks was accompanied by a fall in cyclic nucleotide content in animals given lead for 2 weeks. Data thus suggest that augmentation in the incorporation of thymidine into tissue DNA is associated with alterations in cyclic AMP metabolism.

I. Effect on Adenylate Cyclase Activity

The influence of lead treatment on the activity of adenylate cyclase is illustrated in Fig. 8. In liver, the activity of this enzyme remained unchanged after 2 weeks, rose significantly to 154% of control values after 4 weeks and was followed by a subsequent return to control amounts by 8 weeks. Two weeks after initiation of treatment, renal adenylate cyclase activity was depressed, returned to control values after 4 weeks, and subsequently increased to 169% of control after 8 weeks. Whereas the activity of pulmonary adenylate cyclase remained at control levels after 4 weeks, a significant fall in enzymic

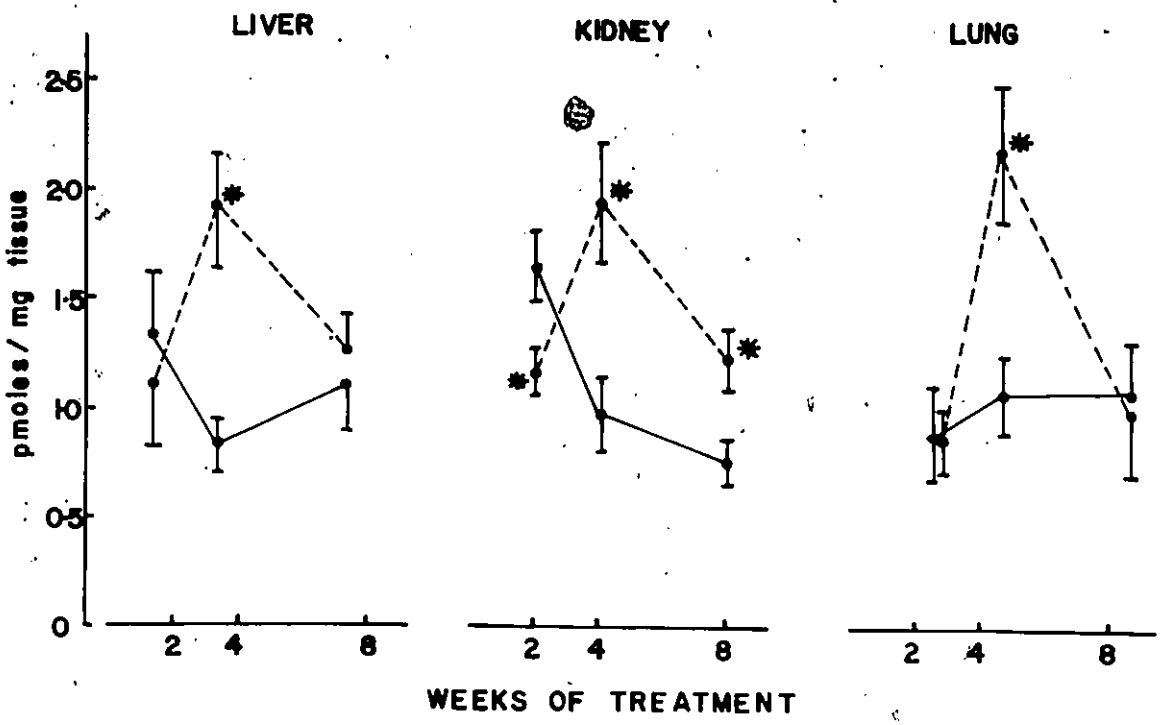

FIG. 7

Sequential changes in hepatic, renal and pulmonary cyclic AMP levels after prolonged exposure to lead. Each point represents the mean ± S.E.M. of at least 6 animals in each group. Animals were given daily oral doses of 50 µg of lead for 21 days from birth. Thereafter, for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. The endogenous concentration of cyclic AMP is expressed as pmoles per mg tissue. *Statistically significant difference when compared with the values of control animals ($p<0.05$)

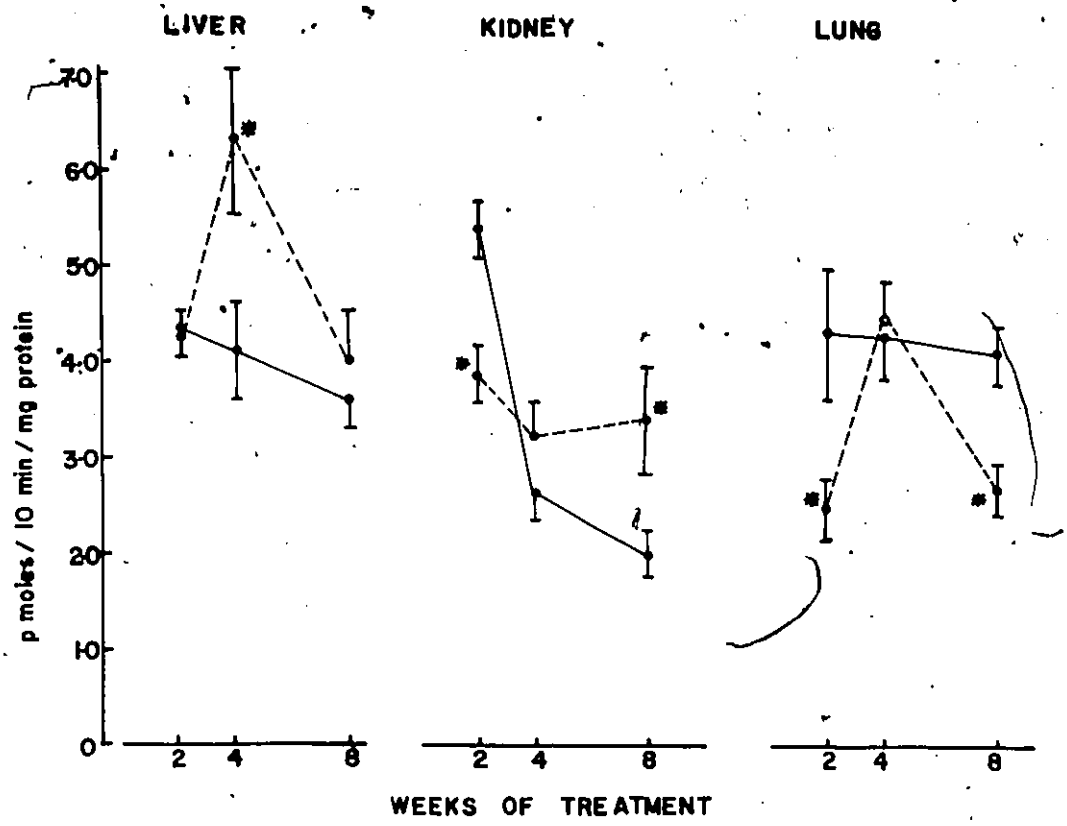

FIG. 8.

Influence of chronic lead administration on hepatic, renal and pulmonary adenylate cyclase activity. Each point represents the mean ± S.E.M. of at least 6 animals in each group. Animals were given daily oral doses of 50 µg of lead for 21 days from birth. Thereafter, for the next 35 days, groups of rats were given 80 ppm lead in the drinking water. Animals were killed 24 hours after the cessation of treatment. The activity of adenylate cyclase was calculated as picomoles cyclic AMP formed per 10 minutes per mg tissue and is expressed as specific activity per mg protein. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

activity was seen after 2 or 8 weeks. It is possible that a reduction in the activity of lung cyclic nucleotide phosphodiesterase may account for the elevated concentration of cyclic AMP observed in this tissue at 4 weeks. It is of interest that the changes in renal and hepatic adenylate cyclase activity also preceded and accompanied the observed alterations in the incorporation of thymidine into DNA.

V. INFLUENCE OF ACUTE LEAD TREATMENT ON VARIOUS PARAMETERS ASSOCIATED WITH GROWTH IN LIVER, KIDNEY AND LUNG

A. Influence on the Incorporation of ^{14}C-Thymidine Into Tissue DNA

Several investigators have reported that a single administration of lead via the intracardiac route produced cell proliferation in mouse kidney (16, 223). It was thus of interest to examine the effects of acute lead treatment on various parameters of growth in rat kidney and also in liver and lung, in order to ascertain whether treatment with this heavy metal produced similar effects in a variety of organs. Male Sprague-Dawley rats were given a single intraperitoneal injection of lead chloride (10 mg/kg) and sacrificed after either 1, 3, 5 or 7 days. Corresponding controls for each set of treated animals received an equal volume (0.2 ml) of physiological saline. Fig. 9 illustrates the time-course of the lead-induced alterations in the capacity of liver, kidney and lung to incorporate labelled thymidine into DNA. Although a slight but insignificant rise was noted in the incorporation of thymidine into hepatic DNA after 24 hours, no marked alterations were observed until the seventh day, where DNA synthesis fell to 39% of control values. In contrast, whereas heavy metal treatment for one day failed to markedly alter the incorporation of thymidine into renal DNA, a significant 2-fold rise was noted after 72 hours followed by a subsequent return to control values by the fifth day. In the lung, lead administration decreased the incorporation

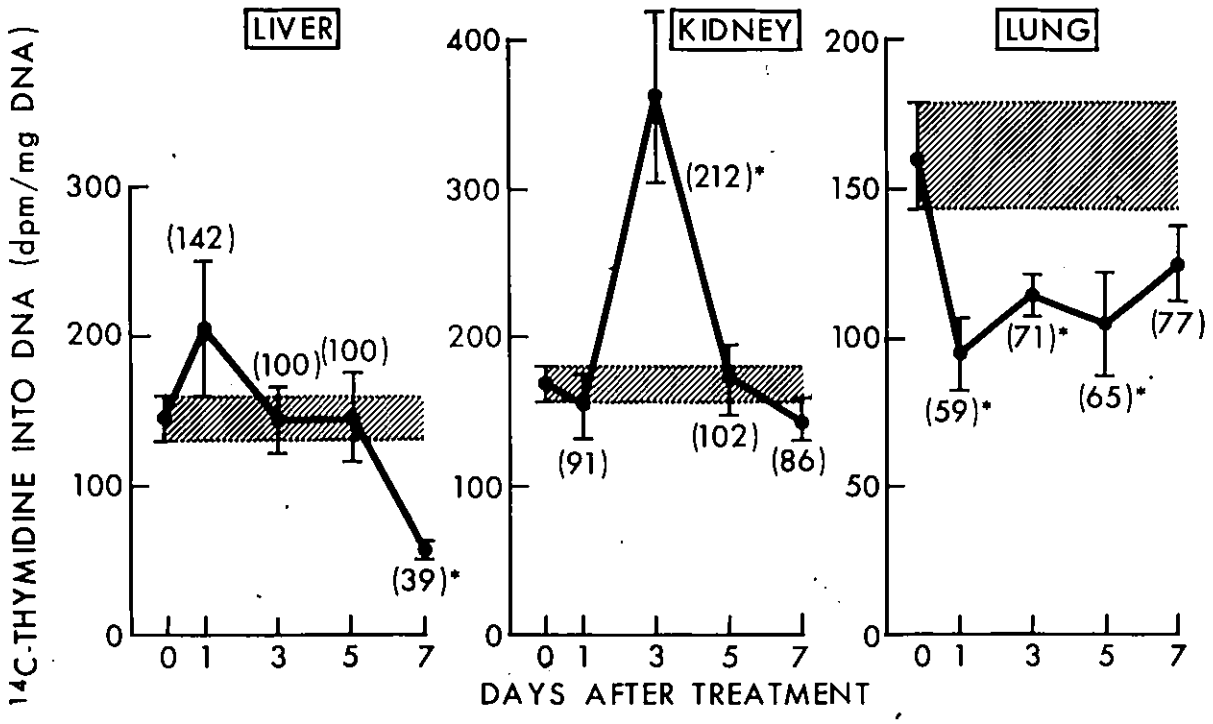

FIG. 9

Effects of lead on the incorporation of ^{14}C-thymidine into DNA in several tissues. Each point represents the mean ± S.E.M. of at least 5 animals in each group. Rats were administered a single intraperitoneal dose of lead (10 mg/kg $PbCl_2$) and killed 1, 3, 5 or 7 days thereafter. The mean ± S.E.M. of 24 vehicle-treated animals is shown at point zero and is represented by the area between the two dotted lines. The incorporation of ^{14}C-thymidine into hepatic, renal and pulmonary DNA is expressed as dpm per mg DNA. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.
*Statistically significant difference when compared with the values of control animals ($p<0.05$).

of thymidine into DNA after 1 day and remained lower than control values throughout the treatment period.

B. Effect on Incorporation of ^{14}C-Orotic Acid Into Tissue RNA

The sequential changes in the incorporation of ^{14}C-orotic acid into hepatic, renal and pulmonary RNA after a single administration of lead are demonstrated in Fig. 10. Unlike the differences noted with respect to DNA synthesis in these organs, heavy metal treatment was found to produce an initial, significant elevation in the incorporation of orotic acid into hepatic, renal and pulmonary RNA after 24 hours. In liver and kidney, the observed increase in RNA synthesis was followed by a subsequent marked reduction in the incorporation of orotic acid into RNA after 5 days, while pulmonary RNA synthesis was not significantly reduced until one week after treatment.

C. Alterations in Tissue DNA Content

Data in Table 22 show the influence of lead on tissue DNA content. The lead-inflicted rise in hepatic DNA content observed after 3 days was maintained throughout the exposure period. Similarly, heavy metal administration increased the concentration of DNA in kidney cortex by approximately 25% both after 5 and 7 days. A significant elevation in pulmonary DNA content was only observed after one week of lead exposure.

D. Influence on RNA Content of Liver, Kidney and Lung

The concentration of hepatic RNA was elevated 3, 5 and 7 days after metal treatment (Table 23), in a manner similar to the observed alterations in DNA content. Similarly, the lead-induced elevation in renal DNA content seen after 7 days was accompanied by a rise in the concentration of RNA in this tissue. In contrast, the initial rise in pulmonary RNA content was followed by a significant reduction in RNA levels in this tissue after 5 and 7 days of metal treatment.

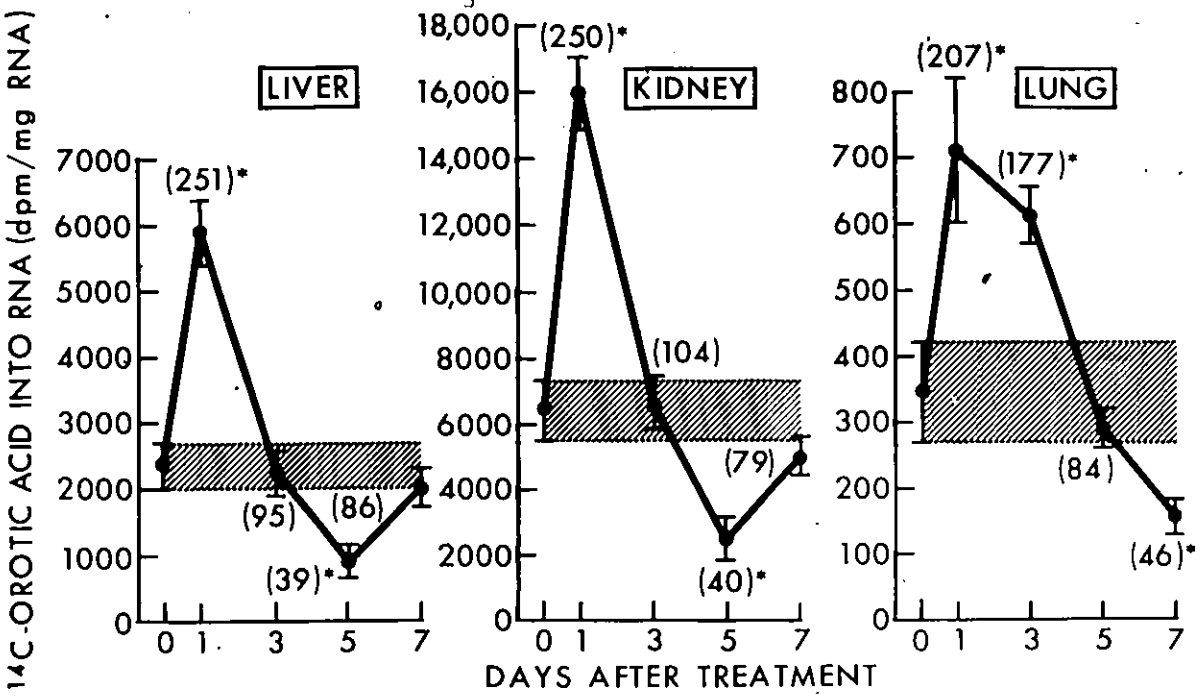

FIG. 10

Influence of lead on incorporation of ^{14}C-orotic acid into hepatic, renal and pulmonary RNA. Each point represents the mean ± S.E.M. of at least 5 animals in each group. Rats were administered a single intraperitoneal dose of lead (10 mg/kg $PbCl_2$) and killed 1, 3, 5 or 7 days thereafter. The mean ± S.E.M. of 24 vehicle-treated animals is shown at point zero and is represented by the area between the two dotted lines. The incorporation of ^{14}C-orotic acid into hepatic, renal and pulmonary RNA is expressed as dpm per mg RNA. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

Table 22

HEPATIC, RENAL AND PULMONARY DNA CONTENT AFTER A SINGLE DOSE OF LEAD

Means ± S.E.M. represent at least 5 animals in each group. Animals were injected intraperitoneally with a single dose of lead chloride (10 mg/kg), and were sacrificed 1, 3, 5 or 7 days following lead administration. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. DNA content of the tissues studied is expressed as milligrams per gram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	DNA (mg/g tissue) Liver	Kidney	Lung
0	3.20±0.26 (100)	3.92±0.27 (100)	5.73±0.22 (100)
1	3.11±0.23 (97)	3.26±0.17 (83)	5.63±0.20 (98)
3	4.08±0.12 (128)*	3.47±0.06 (89)	5.43±0.13 (95)
5	4.48±0.37 (140)*	4.93±0.09 (126)*	5.97±0.24 (104)
7	4.37±0.28 (137)*	4.80±0.09 (122)*	6.85±0.26 (120)*

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

TABLE 23

RNA CONTENT OF LIVER, KIDNEY AND LUNG AFTER ACUTE LEAD ADMINISTRATION

Means ± S.E.M. represent at least 5 animals in each group. Animals were injected intraperitoneally with a single dose of lead chloride (10 mg/kg), and were sacrificed 1, 3, 5 or 7 days following lead administration. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. RNA content of the tissues studied is expressed as milligrams per gram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	RNA (mg/g tissue)		
	Liver	Kidney	Lung
0	6.78±0.23 (100)	3.71±0.14 (100)	3.32±0.15 (100)
1	6.73±0.15 (99)	3.84±0.15 (103)	3.76±0.12 (113)*
3	8.45±0.28 (125)*	3.58±0.10 (96)	3.09±0.06 (93)
5	7.56±0.18 (112)*	3.85±0.28 (104)	1.96±0.09 (59)*
7	8.29±0.40 (122)*	4.41±0.20 (119)*	2.71±0.10 (81)*

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

E. Changes in RNA/DNA Ratio of Certain Tissues Examined

The numerical ratio of RNA to DNA is an index of the capacity of DNA to synthesize RNA. The time-course of the lead-induced alterations in the ratio of RNA to DNA is illustrated in Table 24. A significant reduction in the RNA to DNA ratio was observed in kidney and lung throughout the course of the experiment. Although the ratio of RNA to DNA in liver remained unchanged after 1, 3 or 5 days, a significant decrease resulted after 1 week.

F. Influence on Tissue Protein Content

Whereas lead administration failed to significantly alter the concentration of protein in hepatic and pulmonary tissue at any of the time periods examined, (Table 25), heavy metal treatment increased the level of renal protein after 3 days. The observed elevation in kidney protein content was maintained for 7 days in our experimental conditions.

G. Alterations in Hepatic, Renal and Pulmonary Polyamine Levels

In order to examine the role of polyamine metabolism in the lead-induced alterations in nucleic acid synthesis, the effects of this heavy metal were examined on the concentration of putrescine, spermidine and spermine in liver, kidney and lung. The concentration of putrescine in hepatic tissue was markedly reduced throughout the treatment period (Table 26). Although hepatic spermine and spermidine levels also tended to decrease, a significant reduction was only noted on the 5th and 7th days after lead administration, respectively. In kidney, no significant alterations were noted in the concentration of putrescine and spermidine at any of the time periods examined (Table 27). However, whereas renal spermine levels tended to decrease after one day, a significant elevation to 140% of control values was observed after 5 days, followed by a subsequent restoration to control levels after one week.

TABLE 24

CHANGES IN THE RNA/DNA RATIO IN LIVER, KIDNEY AND LUNG AFTER ACUTE LEAD ADMINISTRATION

Means ± S.E.M. represent at least 5 animals in each group. Animals were injected intraperitoneally with a single dose of lead chloride (10 mg/kg), and were sacrificed 1, 3, 5 or 7 days following lead administration. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	RNA/DNA Ratio		
	Liver	Kidney	Lung
0	2.22±0.16 (100)	1.20±0.08 (100)	0.67±0.02 (100)
1	2.34±0.20 (105)	0.95±0.04 (80)*	0.58±0.01 (87)*
3	2.08±0.09 (94)	1.03±0.04 (86)	0.57±0.01 (86)*
5	1.82±0.16 (82)	0.78±0.04 (65)*	0.33±0.02 (50)*
7	1.69±0.09 (76)*	0.85±0.05 (71)*	0.40±0.01 (59)*

*Statistically significant difference as compared with control values ($p<0.05$).

TABLE 25

INFLUENCE OF LEAD ON PROTEIN CONTENT IN HEPATIC, RENAL AND PULMONARY TISSUE

Means ± S.E.M. represent at least 5 animals in each group. Animals were administered a single dose of lead chloride (10 mg/kg, i.p.) and killed after 1, 3, 5 or 7 days. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. Protein content of the tissues examined is expressed as milligrams per gram tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	Protein (mg/g tissue) Liver	Kidney	Lung
0	163.1±4.6 (100)	121.2±7.1 (100)	106.5±10.2 (100)
1	163.1±2.8 (100)	110.9±6.8 (92)	101.3±7.0 (95)
3	181.3±8.3 (111)	142.2±4.4 (117)*	95.3±4.8 (89)
5	161.7±6.3 (99)	150.4±8.5 (124)*	106.4±5.2 (100)
7	185.4±9.4 (114)	148.8±7.1 (123)*	96.5±4.9 (91)

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

TABLE 26

TIME-COURSE OF THE LEAD-INDUCED CHANGES IN HEPATIC POLYAMINES

Means ± S.E.M. represent at least 5 animals in each group. Rats were injected intraperitoneally with a single dose of lead chloride (10 mg/kg) and were killed 1, 3, 5 or 7 days after lead administration. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. Polyamine content is expressed as picomoles per milligram liver. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	Polyamines (pmoles/mg tissue) Putrescine	Spermidine	Spermine
0	128.1±13.6 (100)	46.2±4.4 (100)	218.4±14.8 (100)
1	76.5±6.9 (60)*	46.7±2.2 (101)	190.7±10.4 (87)
3	75.7±7.4 (59)*	43.8±2.1 (95)	181.2±12.7 (83)
5	87.7±11.5 (68)*	35.0±3.3 (76)	173.4±5.0 (79)*
7	85.1±8.3 (66)*	29.4±1.5 (64)*	189.7±6.6 (87)

*Statistically significant differences as compared with the values of control animals ($p<0.05$).

TABLE 27

INFLUENCE OF ACUTE LEAD TREATMENT ON RENAL POLYAMINES

Means ± S.E.M. represent at least 5 animals in each group. Rats were injected intraperitoneally with a single dose of lead chloride (10 mg/kg) and were killed 1, 3, 5 or 7 days after lead treatment. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. Polyamine content is expressed as picomoles per milligram kidney cortex. Data are also expressed as percentages (in parentheses) with the control values taken as 100%.

Days after Treatment	Polyamines (pmoles/mg tissue) Putrescine	Spermidine	Spermine
0	10.8±1.5 (100)	18.5±1.6 (100)	137.0±19.2 (100)
1	9.1±0.7 (84)	15.5±1.1 (84)	92.0±11.8 (67)
3	9.8±0.4 (90)	17.9±2.3 (96)	160.0±34.9 (117)
5	10.0±1.5 (92)	16.1±2.4 (87)	191.7±1.5 (140)*
7	9.3±0.9 (86)	17.1±1.2 (92)	119.1±26.3 (87)

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

The time-course of the lead-induced alterations in pulmonary polyamine content is illustrated in Table 28. Whereas the concentration of putrescine in lungs of metal-treated animals fell to 50% of control values after 1 or 3 days, no significant alterations in pulmonary spermidine or spermine levels were noted at either time period. However, 5 days and 1 week after lead administration, the pulmonary concentration of putrescine, spermidine and spermine fell markedly, in a manner similar to the changes noted in liver of lead-administered animals.

H. Changes in Cyclic AMP Levels

Since modulation in the adenylate cyclase-cyclic AMP system is believed to be involved in the initiation of DNA synthesis (18), the influence of lead was examined on the endogenous levels of cyclic AMP in liver, kidney and lung (Fig. 11). The hepatic concentration of cyclic AMP was reduced to 60% of control after 3 days, and no significant alterations were noted at any of the other time periods examined. In kidney, the lead-induced reduction in the concentration of cyclic AMP was maintained from the 3rd through 7th day of the treatment period. In contrast, pulmonary cyclic AMP content rose to 152% of control values after one day, which was followed by a subsequent restoration to control values throughout the remainder of the treatment period. It is of interest that the reduction in hepatic cyclic AMP levels preceded the observed decrease in thymidine incorporation into DNA in livers of lead-administered animals.

TABLE 28

PULMONARY POLYAMINE CONTENT AFTER ACUTE LEAD ADMINISTRATION.

Means ± S.E.M. represent at least 5 rats in each group. Animals were intraperitoneally injected with a single dose of lead chloride (10 mg/kg) and were killed 1, 3, 5 or 7 days after lead treatment. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. Polyamine content is expressed as picomoles per milligram lung. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%.

Days after Treatment	Polyamines (pmoles/mg tissue) Putrescine	Spermidine	Spermine
0	19.5±0.9 (100)	6.4±0.7 (100)	266.0±47.6 (100)
1	9.9±1.2 (51)*	4.7±1.1 (74)	181.8±45.3 (68)
3	10.0±0.3 (51)*	5.0±0.8 (77)	216.5±47.9 (81)
5	6.7±0.2 (34)*	3.0±0.2 (46)*	113.1±19.0 (43)*
7	6.3±0.3 (32)*	2.6±0.2 (40)*	103.5±11.9 (39)*

*Statistically significant difference as compared with the values of control animals ($p<0.05$).

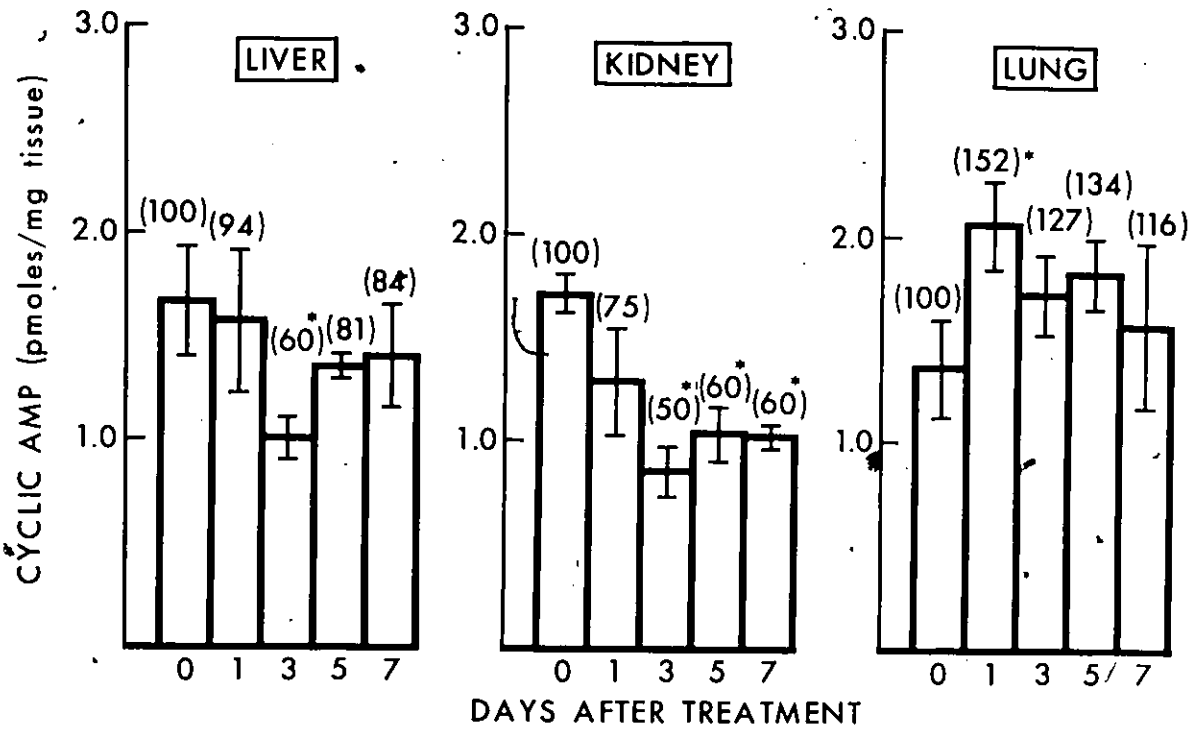

FIG. 11

Influence of lead treatment on cyclic AMP levels in certain tissues. Each bar represents the mean ± S.E.M. of at least 5 animals in each group. Rats were administered a single intraperitoneal dose of lead (10 mg/kg $PbCl_2$) and killed 1, 3, 5 or 7 days thereafter. The mean ± S.E.M. of 24 vehicle-treated animals is given at day zero. The endogenous concentration of cyclic AMP is expressed as picomoles per mg tissue. Data are also given as percentages (in parentheses) with the values of control animals taken as 100%. *Statistically significant difference when compared with the values of control animals ($p<0.05$).

5. DISCUSSION

I. INFLUENCE OF VARYING LEAD TREATMENT REGIMENS ON GLUCOSE HOMEOSTASIS

The continuous emission of lead into the environment from industrial sources and automobile exhaust, the accumulation and persistence of this heavy metal in the atmosphere as well as the high affinity of lead to remain bound to mammalian tissues emphasizes the need for a more complete understanding of the metabolic consequences of heavy metal toxicity. Although the role played by certain metallic elements in mammalian functions is well known, little attention has been focussed on the environmental health hazard resulting from the use of non-essential heavy metals in industry and agriculture. The present study shows that daily administration of lead over a 45-day period produced augmentation in the activities of pyruvate carboxylase, phosphoenol-pyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in liver and kidney, the two major tissues which possess the complete enzymatic potential for glucose synthesis from non-carbohydrate precursors (148). In addition to elevating the concentration of blood glucose and urea, chronic lead treatment significantly reduced the levels of hepatic glycogen. Abstinence from treatment for 28 days in rats previously given lead for 45 days reduced the heavy metal-inflicted biochemical alterations. However, certain changes observed in the "withdrawal" group were still significantly different from controls, indicating the persistent nature of some of the lead-induced alterations in liver and kidney carbohydrate metabolism even when the exposure to heavy metal was terminated for 4 weeks. It is interesting that chronic lead treatment increased the urinary excretion of glucose and protein (30-60 mg% above control) as measured by using the Dextrostix and Combistix tapes, respectively, and the observed glycosuria and proteinuria also persisted 4 weeks after stopping the treatment. Recently, Goyer (262) found that whereas chronic lead treatment also increased the urinary excretion of protein, glucose

and phosphate, the excretion of uric acid was decreased and the observed functional abnormalities were associated with kidney damage. It is of interest that two other heavy metals, mercury and cadmium, which exert similar toxic actions on the kidney (10), also produced marked proteinuria and glycosuria (263,264). In recent reports, Singhal et al. (265) and Merali et al. (266) have demonstrated that the organomercurial- and cadmium-induced rise in urinary excretion of glucose and protein may also have been related to stimulation in the gluconeogenic capacity in liver and kidney cortex. In contrast to the findings with lead, withdrawal from organomercurial or cadmium treatment in rats which had been injected with these heavy metals for 45 days was found to produce persistent changes in the quartet of renal and hepatic gluconeogenic enzymes, liver glycogen, serum urea and glucose (265,266). Since the metabolic alterations produced by lead persisted only partially after 4 weeks withdrawal, it is possible that the immobilization of lead in bone stores results in a lower effective toxicity of a large body burden of the heavy metal.

A more relevant experimental model of heavy metal intoxication was employed in which animals were given either 20, 40 or 80 ppm lead for a period of 56 days. Chronic oral administration of lead at a concentration of 40 ppm in the drinking water produced significant reduction of liver glycogen, elevation of blood glucose as well as augmentation of the activities of renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. These lead-induced alterations in glucose synthesis were dose-related and significant enhancement in kidney gluconeogenesis could be seen with a concentration as low as 20 ppm given for 56 days. Changes in glucose homeostasis as evidenced by glycosuria also have been reported by Chisolm (13) and Goyer (262) following exposure to lead. Berteloot and Hugon (126) found that incubation of rat liver homogenates with less than 0.5 mM lead nitrate enhanced the activity of glucose 6-phosphatase,

although increasing the concentration of the heavy metal depressed enzymic activity. In acute studies, Filkins (123) noted a decreased capacity of liver tissue slices to biotransform alanine or pyruvate to glucose in animals given a 5 mg/kg dose of lead acetate. It may be noteworthy that in contrast to the present study where lead was administered in a level as low as 20 ppm for 56 days, Hirsch (134) using a dietary concentration of heavy metal as high as 2% (an amount known to exceed the level to which humans may be environmentally exposed), observed a decrease in glucose production from pyruvate in rat kidney cortex slices.

One of the prominent toxic manifestations related to plumbism is renal damage associated with an increased urinary excretion of glucose (13). In the present study, the observed augmentation in kidney glucose synthesis may thus contribute to enhanced excretion of glucose in the urine. The reduction in hepatic glycogen stores following lead treatment suggests that the process of glycogenolysis may also provide a source for the increased levels of glucose present in blood and urine. The maintenance of blood glucose levels is dependent, at least partially, on the hormones secreted by the pancreas. Chronic lead exposure resulted in a dose-related increase in blood glucose and a decrease in insulinogenic index. Further, in rats subacutely exposed to lead and subsequently challenged with a glucose load, the insulin secretory response was both delayed and suppressed, and the ability to tolerate a glucose load was significantly impaired. Data thus suggest that alterations in glucose homeostasis may indeed be related to the action of lead on the pancreas. A similar dysfunction in pancreatic activity characterized by inhibition of the glucose-stimulated release of insulin has been reported in animals given other heavy metals such as nickel and cadmium (267-269). The precise mechanisms by which lead affects pancreatic function still remains to be elucidated; however, it is conceivable that this heavy metal may interfere with certain divalent

ions such as zinc and calcium, known to be essential for normal pancreatic activity. Indeed, Merali and Singhal (270) demonstrated that the concurrent administration of zinc produced a partial protection against the pancreatotoxic actions of cadmium.

Our results also demonstrate that chronic administration of lead produces an elevation in the concentration of serum urea. Since urea represents the chief metabolic product of protein and amino acid catabolism (271), the present data seem to be consistent with the suggestion that lead alters protein metabolism. Indeed, ultrastructural studies revealed that administration of lead produces characteristic lead-protein intranuclear inclusion bodies in kidney as well as reduced protein synthesis in rabbit reticulocytes (118,130). The observed elevation of serum urea in rats chronically-exposed to lead may thus be related to enhanced glucose synthesis from proteins and amino acids. It is of interest that other heavy metals such as mercury and cadmium also produced an elevation in serum urea and gluconeogenesis in hepatic and renal tissue (265,266). These metal-inflicted biochemical alterations persisted even after withdrawal from treatment for 4 weeks (265,266).

At present, the mechanism(s) by which the kidney responds to various nephrotoxic agents such as lead remains obscure. Kirschbaum et al. (133) noted that lead poisoning increased the activities of renal glycoprotein: glycosyltransferase, protease, acid phosphatase, N-acetyl glucosaminidase, α-galactosidase and β-galactosidase suggesting that acid hydrolases might participate in cellular damage to the kidney. Cellular oxidation processes and mitochondrial structures also were found to be altered in livers of lead-treated rats and rabbits (10,115). Since energy production appears to be altered by this heavy metal (10), it is possible that lead enhances the glucose-synthesizing enzymatic potential of both renal and hepatic tissue in order to meet the increased demand for energy. In addition, the glycosuria

observed after chronic lead treatment might be related to a decreased reabsorption of glucose as a result of kidney failure (70).

II. ROLE OF ADENYLATE CYCLASE-CYCLIC AMP SYSTEM IN LEAD-INDUCED METABOLIC CHANGES

In mammals, modulation of the adenylate cyclase-cyclic AMP system is believed to be responsible for a variety of hormonal- and drug-induced alterations in carbohydrate and lipid metabolism (14). Administration of cyclic AMP was found by Sutherland and Robison (14) to produce a rise in blood glucose and the cyclic nucleotide-induced hyperglycemia was accompanied by a concomitant fall in liver glycogen. In addition, treatment with cyclic AMP increased hepatic and renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase activities (272) and enhanced the formation of glucose from non-carbohydrate sources in both tissues (14). In the present study, chronic ingestion of lead *via* drinking water elevated the endogenous levels of cyclic AMP, augmented the activity of adenylate cyclase and enhanced the gluconeogenic capacity of the renal cortex, indicating that the observed alterations in renal carbohydrate metabolism may be related to modulation in the adenylate cyclase-cyclic AMP system. Whereas the ingestion of lower amounts of heavy metal tended to produce only a slight rise in adenylate cyclase activity, significant increases in endogenous cyclic AMP levels were noted in kidney cortices of rats receiving either 20 or 40 ppm lead for 56 days. A similar elevation in the endogenous concentration of hepatic cyclic AMP and adenylate cyclase activity was noted in rats chronically-injected with 1.0 mg/kg lead for 45 days. In contrast, prolonged treatment with lead *via* the intraperitoneal route produced a significant decrease in cyclic AMP levels of kidney cortex. In addition, subacute exposure to this metal failed to markedly alter the activity of adenylate cyclase and only slightly reduced the concentration of renal cyclic AMP. It is difficult at present to ascertain the reason for the observed decrease in renal cyclic AMP levels after intraperitoneal lead

administration, since both lead and cyclic AMP were previously found to produce a marked enhancement in renal gluconeogenesis (14). However, the possibility exists that the observed fall in kidney cortex cyclic AMP may be due to enhanced breakdown by cyclic nucleotide phosphodiesterase and/or increased urinary excretion of cyclic AMP due to tubular damage. Indeed, chronic exposure to lead in man and experimental animals has been found to produce renal tubular dysfunction (Fanconi syndrome) which includes glycosuria, aminoaciduria and fructosuria (13). It is of interest that treatment with cadmium, which produced disturbances in kidney function characterized by glycosuria and proteinuria, also resulted in a significant depression in the concentration of renal cyclic AMP (273).

III. LEAD-INFLICTED ALTERATIONS IN PANCREATIC FUNCTION

In mammals, the maintenance of glucose homeostasis is dependent to some extent on the hormones secreted by the pancreas. Our data demonstrate that chronic intraperitoneal administration of lead resulted in enhancement of renal and hepatic gluconeogenic enzymes, cyclic AMP levels and the concentration of blood glucose. Since insulin deficiency inflicted either by insulin antiserum or alloxan treatment also increased blood glucose, cyclic AMP as well as the rate of hepatic gluconeogenesis (15,185), it was of interest to examine whether the metabolic alterations caused by exposure to lead might be due, at least in part, to a lack of insulin. Employing a 7-day multiple dosing schedule of lead administration, it was found that this metal enhanced the gluconeogenic potential of kidney similar to that seen in chronically-treated animals. Although subacute lead administration failed to produce any apparent hyperglycemia, this treatment did result in stimulation of the activities of renal pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase. Whereas blood glucose and serum immunoreactive insulin levels were not markedly altered by subacute lead administration, injection of

a glucose load in lead-treated animals revealed a marked glucose intolerance which was associated with a reduction in the glucose-stimulated insulin release. A marked reduction in the insulinogenic index was noted in lead-exposed rats, suggesting that lead produced a decrease in pancreatic secretory activity. It is of interest that Ghafghazi and Mennear (268) also found that subacute administration of cadmium significantly decreased the resting IRI concentrations in mice. However, since no glucose intolerance was observed, it is conceivable that enhanced glucose excretion due to cadmium-induced renal damage may have masked the glucose intolerance. Indeed, Merali and Singhal (273) demonstrated that subacute exposure to cadmium produced a marked glucose intolerance characterized by a reduction in the release of insulin as well as in the clearance of glucose load from the blood. It is of interest that administration of selenium or zinc concurrently with cadmium afforded a partial protection against the pancreatotoxic effects of cadmium as evidenced by normalization of the tolerance to an injected glucose load (270,274). Although the mechanism(s) by which lead exerts an adverse effect on pancreatic function are as yet unknown, it is possible that this heavy metal may interfere with selenium, zinc or calcium, divalent ions known to play a role in the maintenance of pancreatic activity (275,276).

IV. INFLUENCE OF LEAD TREATMENT ON CERTAIN BIOCHEMICAL PARAMETERS ASSOCIATED WITH TISSUE GROWTH

In recent years, it has been shown (6,11) that a correlation exists between the incidence of lead poisoning and the prevalence of pica (ingestion of non-food items) in young children. Since children exhibit a tendency to consume paper, paint chips and dirt, possible environmental sources of lead (3), investigators have focussed their attention on the effects of exposure to low levels of this heavy metal on certain aspects involved in the process

of growth and development in young mammals. Thus, in any consideration of the interpolation and comparison of data to human lead poisoning, it is essential to determine the concentrations of this heavy metal in various tissues. Data in the present study demonstrate that exposure to lead results in heavy metal retention by certain tissues. Although lead tended to accumulate to a greater extent in livers of young (2 week-old) rats, studies in 4 or 8 week-treated animals revealed a decline in the amount of metal retained by this tissue. In contrast, high quantities of lead were retained by kidney throughout the experimental period and by 8 weeks twice as much metal was present in renal tissue as in the liver. It is of interest that Azar et al. (277) found that treatment of rats with 60 ppm lead for 1 year produced a 3-fold greater accumulation of the metal in kidney in comparison to hepatic tissue. In rats treated for one year with 60 ppm dietary lead, significant amounts of metal were retained in the kidney (0.85 ppm), liver (0.31 ppm), brain (0.28 ppm) and bone (17.5 ppm), while the metal content of pulmonary tissue was not reported. These results together with our findings suggest that unlike liver, kidney possesses a greater capacity to accumulate and store administered lead in the rat. In addition, the present investigation shows that even though the lung is the only organ which receives the entire output of the right heart and is thus highly susceptible to ingested chemicals, no detectable amounts of lead were present in pulmonary tissue. It may be noteworthy that examination of children with no known exposure to lead revealed the presence of trace concentrations of metal in lung (0.1 ppm) and the levels observed were similar to those obtained in this study (53). Why only trace amounts of lead were detected in lung as compared to other tissues is unknown; however, it is conceivable that the mechanism(s) which clears this metal from the pulmonary tissue may be more efficient than that found in kidney and liver. Since the levels of lead noted in the present investigation closely resemble those

previously seen in unexposed children, the possibility remains that the data obtained with the use of this experimental model may indeed reflect metabolic and functional alterations which occur within humans suffering from lead intoxication.

The knowledge that children are more prone to ingest lead-containing materials (11) and display a greater susceptibility to the toxic effects of the metal as evidenced by hematopoietic disorders and hyperexcitability (76,79) prompted studies on metabolic consequences of lead exposure in young developing animals. In an early study, Sauerhoff and Michaelson (105) found that dietary administration of lead to lactating mothers resulted in a reduction of rat pup body weight and brain dopamine content accompanied by enhanced motor activity. However, on repetition of the identical experimental protocol, lead failed to affect brain dopamine levels but increased the concentration of norepinephrine in this tissue suggesting that diet, rather than the non-essential element, played an important role in observed responses (103). In attempts to eliminate the problem associated with dietary habits of rats, Golter and Michaelson (103) reported that administration of lead directly to rat pups produced a marked increase in motor activity with no significant alterations in body weight gain between control and treated animals. Similarly, no difference in body weight gain between corresponding controls and groups of rats receiving lead for either 2, 4 or 8 weeks was found in our investigation. Although there was a lack of any apparent effect of lead on the ability of animals to gain weight, exposure to this heavy metal induced an approximately 2- to 4-fold enhancement in the capacity of liver, kidney and lung to incorporate thymidine into DNA 2 months after the initiation of treatment. It is of interest that other heavy metals such as cobalt, nickel and zinc were found to replace magnesium (Mg^{+2}) in the catalysis of DNA formation by DNA polymerases of several species (278), and that the quantity and fidelity of

DNA synthesized may be altered in the presence of metal ions other than magnesium. Since lead has been reported to produce tumours in kidney and lung (135-137,279), the possibility exists that the lead-stimulated increase in DNA formation may reflect the synthesis of an abnormal species of DNA. In recent reports, Choie and Richter (16,139,280,281) also demonstrated that in mice and rats given a single injected dose of lead or prolonged oral metal administration, a marked elevation resulted in the biosynthesis of renal DNA, RNA and protein. A similar augmentation in the incorporation of thymidine into mouse kidney DNA was noted by Cihak and Seifertova (223) in mice administered lead acetate by the intracardiac route. In contrast, treatment with the heavy metal, silver, failed to produce any significant alteration in the incorporation of thymidine into mouse renal DNA (223). Although subacute cadmium administration resulted in a marked reduction in the capacity of kidney and liver to incorporate thymidine into DNA, significant augmentation in pulmonary DNA synthesis was found in metal-treated rats (232,282). The phenomenon of heavy metal-induced stimulation in the incorporation of thymidine into tissue DNA is by no means unique to lead and has been reported in the case of kidneys obtained from folic acid-treated rats (233); in livers of animals given azacytidine or a mixture of triiodothyronine, amino acids, glucagon and heparin (221,223); in parotid glands of rats administered isoproterenol (224); as well as in lungs of animals exposed to cadmium, paraquat, butylated hydroxytoluene, nitrogen dioxide and ozone (225,226,228-232). It is of interest that cell proliferation following surgical removal of one kidney or a portion of liver also results in enhancement in the incorporation of thymidine into hepatic and renal DNA, respectively (212,213). Our data indicate that lead is indeed capable of initiating renal, hepatic and pulmonary DNA synthesis and that the presence of metal in lung is not an essential factor for the observed response.

It is conceivable that the initial depression in the incorporation of thymidine into liver and kidney DNA, seen at 2 and 4 weeks respectively, may be associated with a local reduction of blood flow to these organs.

Although the precise biochemical mechanism(s) underlying mammalian cell growth remains to be elucidated, evidence suggests that modulation of polyamine metabolism may be an essential prerequisite to trigger tissue development (17,206). Administration of either testosterone or estradiol to castrate male and female rats, respectively, produced a rise in the endogenous levels of putrescine, spermidine and spermine as well as in the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase, two rate-limiting enzymes involved in polyamine synthesis, prior to the increase in accessory sex gland size and nucleic acid biosynthesis (200,201). It is worthwhile to note that the partial hepatectomy- or unilateral nephrectomy-induced stimulation of DNA synthesis in liver and kidney, respectively also was accompanied by augmentation in polyamine formation (218,219). A similar positive correlation between elevated incorporation of thymidine into DNA and an increase in the levels of putrescine, spermidine and spermine was found by Kacew et al. (232) in lungs of rats administered cadmium. In contrast, Kacew et al. (232,283) reported a lack of any positive relationship between the effects of subacute cadmium on pancreatic and kidney DNA synthesis and polyamine metabolism. In the present study, while hepatic putrescine and spermidine levels were not noticeably altered, the concentration of spermine rose after two weeks of lead exposure. Although liver spermine levels subsequently fell below controls after 4 weeks, and were restored to control values after 2 months, it was not until the end of the 8 week treatment period that an elevation was noticed in thymidine incorporation into DNA suggesting that alterations in polyamines may precede the observed increase in DNA synthesis. In kidney, the reduction

in the incorporation of thymidine into DNA noted after 4 weeks was accompanied by a fall in tissue levels of putrescine and spermidine; however, the stimulation of renal DNA formation seen at 8 weeks was associated with an elevation in the concentration of spermidine. Although the correlation between alterations in renal nucleic acid synthesis and polyamine levels is not clear-cut, it would appear that changes in spermidine may play a role in DNA formation. In the lung, the concentrations of putrescine, spermidine and spermine were reduced after 4 weeks, without any concomitant depression in DNA synthesis. In contrast, pulmonary DNA synthesis was markedly stimulated after 8 weeks, at which time the polyamine levels were insignificantly different from controls, again indicating a lack of any apparent correlation between polyamine changes and nucleic acid synthesis in lead-treated rats. It is of interest, that changes in polyamine biosynthesis may be more closely related to the formation of RNA and protein in growing tissues (17,206), and this may, in part, explain the lack of correlation between polyamine levels and DNA synthesis in liver and lung. Thus, in the case of lead-stimulated DNA synthesis of the three tissues examined, alterations in polyamine levels need not necessarily play a role in the observed response.

In recent years, attention also has been focussed on the role of the adenylate cyclase-cyclic AMP system in the drug- or surgical-induced model of cell growth (18). In regenerating rat liver following partial hepatectomy, the observed increase in DNA synthesis was preceded and accompanied by elevation in the endogenous amounts of cyclic AMP (220). Similarly, Comber and Taylor (233) demonstrated that the folic acid-stimulated enhancement in the incorporation of thymidine into kidney DNA was associated with a rise in tissue cyclic AMP. Further, administration of isoproterenol to rats produced an augmentation in the concentration of cyclic AMP and the activity of adenylate cyclase of the parotid gland prior to initiation of enhanced DNA synthesis (224,237). In

addition, Kacew et al. (232) found that the cadmium-induced elevation in the incorporation of thymidine into pulmonary DNA was preceded and accompanied by stimulation of the adenylate cyclase-cyclic AMP system. Results of this investigation show that the lead-inflicted increase in the incorporation of thymidine into renal and hepatic DNA also was associated with elevation in tissue cyclic AMP and adenylate cyclase activity. It is of interest that in kidney, the reduction in DNA synthesis seen after one month was preceded by a decrease in both cyclic AMP and adenylate cyclase at 2 weeks. Although a positive correlation between enhanced DNA synthesis and cyclic AMP levels was noted in lungs of lead-treated animals, an unexpected fall in adenylate cyclase activity occurred at 2 and 8 weeks. Since pulmonary cyclic AMP content was significantly greater than controls only after 1 month of lead treatment, it is conceivable that during the period between 2 weeks to 1 month, a rise in the activity of adenylate cyclase or a fall in phosphodiesterase activity or a combination of both might have taken place. In general, administration of lead enhanced the capacity of liver, kidney and lung to incorporate thymidine into DNA, produced stimulation in the adenylate cyclase-cyclic AMP system and the observed metal-inflicted responses appeared to be unrelated to alterations in polyamine metabolism.

Acute treatment with lead has been employed as an experimental tool for investigating the effects of this metal on growth processes in mouse kidney. Choie and Richter (16,139,280) demonstrated that administration of lead resulted in an elevation of the synthesis of RNA, DNA and protein in mouse and rat kidney. Similar results were obtained by Cihak and Seifertova (223), where a single intracardiac injection of lead acetate produced an enhancement in mitotic activity and DNA synthesis of mouse kidney. In view of these findings, studies were undertaken to compare the influence of chronic exposure

with acute lead treatment on renal, hepatic and pulmonary metabolism. A single intraperitoneal injection of lead chloride also was found to increase the incorporation of thymidine into renal DNA and this observed elevation was preceded by a rise in the incorporation of orotate into RNA. Although acute lead administration initially enhanced the formation of RNA in rat liver and lung, the incorporation of thymidine into hepatic and pulmonary DNA was markedly reduced after metal treatment. In addition, the initial increase in the incorporation of orotic acid into hepatic and pulmonary RNA was followed by a fall in RNA synthesis on the 5th and 7th day after lead treatment, respectively. It is possible that the observed reduction in hepatic and pulmonary DNA formation is associated with a decrease in local circulation to these organs or to a decreased capacity of the cells to incorporate thymidine into DNA.

In order to examine the role of the adenylate cyclase-cyclic AMP system in the initiation of renal nucleic acid synthesis, the influence of lead was examined on the concentration of this cyclic nucleotide. In the present study, we were unable to observe any correlation between lead-inflicted alterations in DNA synthesis and the endogenous concentration of cyclic AMP in kidney as well as lung. Whereas the incorporation of thymidine into renal DNA was elevated after 3 days, the concentration of cyclic AMP in this tissue remained depressed from the 3rd through the 7th day of the experiment. Similarly, while pulmonary cyclic AMP levels were elevated 24 hours after lead administration, the formation of DNA in this organ was reduced throughout the treatment period. Since various investigators have reported that alterations in the adenylate cyclase-cyclic AMP system may not necessarily act as a trigger for DNA synthesis (230,239), it can be concluded that changes in nucleic acid formation in kidney and lung may be independent of modulation in the adenylate cyclase-cyclic AMP system. In contrast, the observed

reduction in the incorporation of thymidine into hepatic DNA was preceded by a fall in the endogenous concentration of cyclic AMP in this tissue, which indicates that the effects of a single treatment with lead on hepatic cell proliferation may be associated with alterations in the cellular levels of cyclic AMP. It is of interest that this heavy metal was found to inhibit the activities of hepatic adenylate cyclase and cyclic AMP phosphodiesterase at very low concentrations (I_{50} < 3 μM) in vitro (284). In contrast, acute intravenous administration of lead failed to produce any apparent change in the endogenous concentration of cyclic AMP in rat liver in vivo for as long as 4 hours (285). Similarly, our data show that hepatic and renal cyclic AMP levels were not reduced until the 3rd day after lead administration. In contrast, the concentration of pulmonary cyclic AMP rose markedly one day after lead treatment. It seems evident that the varying influence of lead on the adenylate cyclase-cyclic AMP system of different tissues may be more complex than initially proposed.

More recently, a model has been suggested which indicates that the process of cellular growth in mammals may involve stimulation of both the adenylate cyclase-cyclic AMP system and polyamine formation. In the present study, the lead-inflicted reduction in hepatic and pulmonary nucleic acid synthesis was preceded and accompanied by a fall in the endogenous concentration of putrescine, spermidine and spermine suggesting that a positive correlation may exist between DNA formation and polyamine levels. In contrast, the polyamine levels remained unaltered in kidney except for a slight yet significant elevation in spermine content which did not occur until after the lead-induced stimulation in RNA (1 day) and DNA synthesis (3 days), indicating that the observed alterations in renal nucleic acid metabolism may be independent of changes in polyamine content. In view of the fact that lead treatment enhanced the formation of a specific binding protein in mouse kidney

(131), and that polyamine metabolism is predominantly related to protein synthesis (17,206), it is possible that the observed elevation in spermine may reflect increased formation of this metal-binding protein in kidney tissue. Indeed, lead administration produced an increase in renal protein levels from the 3rd through the 7th day of the experimental period. It is of interest that other heavy metals, such as mercury and cadmium, also enhanced the synthesis of a specific metal-binding protein (metallothionein) in rat liver and kidney (286-288).

6. SUMMARY

Environmental contamination arising from the emission of heavy metals such as lead into our biosphere has been considered to result in a variety of health hazards in mammalian species. Since the utilization of lead in various industries and its resultant release into the environment as a by-product has markedly increased, considerable interest has been stimulated in studying the long-term consequences of exposure to this heavy metal. The aim of the present investigation was to examine the effects of acute and chronic lead treatment on carbohydrate metabolism in rats and to elucidate the possible mechanism(s) by which this environmental contaminant exerts its adverse influences on glucose homeostasis. In addition, studies on the effects of prolonged low level administration of lead on certain metabolic parameters associated with growth were undertaken in an attempt to compare the observed manifestations of metal intoxication with those which may occur in exposed children.

The present study shows that daily intraperitoneal administration of lead chloride (0.2 or 1.0 mg/kg) for 45 days increased the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase, the quartet of key gluconeogenic enzymes, in rat liver and kidney cortex. In addition, prolonged lead treatment produced an elevation in blood glucose and serum urea as well as a reduction in the concentration of hepatic glycogen. Chronic lead administration also was found to produce a marked rise in liver cyclic AMP and in the activity of adenylate cyclase. These results indicated that the observed hyperglycemia inflicted by prolonged exposure to lead may be related to an enhanced capacity of hepatic tissue to synthesize glucose from non-carbohydrate precursors and that these alterations of carbohydrate metabolism may be related to changes in the

adenylate cyclase-cyclic AMP system. Discontinuation of lead administration for 28 days in rats pretreated with lead chloride (1.0 mg/kg) for 45 days failed to reverse the observed changes in hepatic cyclic AMP or carbohydrate metabolism. As in the case of liver, withdrawal from heavy metal treatment for 28 days failed to restore the observed biochemical alterations in rat kidney cortex. However, in contrast to liver, lead treatment produced a significant decrease in adenylate cyclase activity of renal cortex, indicating that the lead-inflicted alterations in carbohydrate metabolism in this tissue may not be related to modulation in the adenylate cyclase-cyclic AMP system.

Subacute exposure to lead chloride (5 mg/kg, intraperitoneally, twice daily for 7 days) produced changes in renal metabolism which were qualitatively similar to those noted after chronic treatment. Data demonstrate that although the activity of adenylate cyclase remained insignificantly different from controls, the concentration of cyclic AMP was reduced in rat kidney cortex. The possibility remains that the observed decrease in the concentration of cyclic AMP in kidney cortices of metal-treated rats may be due to either enhanced breakdown by cyclic nucleotide phosphodiesterase or to increased urinary excretion of this nucleotide arising from tubular damage. Whereas subacute lead treatment failed to significantly alter the concentration of blood glucose or serum insulin, administration of a glucose load to metal-treated animals resulted in decreased glucose tolerance which was associated with a marked suppression in glucose-stimulated insulin release.

In order to simulate a more physiological mode of lead exposure for humans, neonatal rats were given this heavy metal either via the mothers' milk or by gastric intubation. Data demonstrate that administration of a low dose of lead for 56 days failed to appreciably alter the growth rate of new-born rats. Oral administration of lead also resulted in hyperglycemia, hypoinsulinemia, uremia and diminution of hepatic glycogen. In addition, ingestion of lead

produced an elevation in the activities of the quartet of renal gluconeogenic enzymes, cyclic AMP levels and in the activity of adenylate cyclase. In general, lead ingestion for 2 months enhanced the incorporation of thymidine into renal, hepatic and pulmonary DNA. In contrast, exposure to lead for 2 weeks produced a significant decrease in the incorporation of thymidine into hepatic DNA, and a similar reduction in DNA formation was noted in kidneys of 4 week-intubated animals. The observed alterations in DNA synthesis were preceded in all cases by corresponding changes in the endogenous concentration of cyclic AMP in kidney, liver and lung. In general, the levels of putrescine, spermidine and spermine were unaltered or decreased in the three tissues examined except in the case of hepatic spermine at 2 weeks and renal spermidine at 2 months. In liver and kidney, the lead-inflicted biochemical disturbances were associated with a significant retention of this heavy metal, with maximal accumulation being noted 2 weeks after exposure. In contrast, lead treatment produced a variety of metabolic alterations in rat lung in the absence of any apparent retention of the metal by this organ, suggesting that the presence of this non-essential element in pulmonary tissue need not necessarily constitute an absolute requirement for lead to inflict metabolic disturbances.

Since various investigators had previously reported that acute injection of lead also produces alterations in mammalian growth processes, the influence of a single injection of metal was examined on nucleic acid levels and synthesis, as well as on the endogenous concentrations of cyclic AMP and polyamines in liver, kidney and lung. Whereas lead increased the incorporation of thymidine into renal DNA, a significant reduction in DNA formation was seen in hepatic and pulmonary tissue. The incorporation of orotic acid into renal, hepatic and pulmonary RNA was markedly elevated after one day, and subsequently fell below control levels after 5 and 7 days. Although metal administration generally failed to alter kidney polyamine levels, the

concentrations of putrescine, spermidine and spermine fell in lungs and livers of lead-treated animals throughout the experimental period. Whereas the reduction in the incorporation of thymidine into hepatic DNA was associated with a decrease in the concentration of cyclic AMP, lead-induced alterations in the formation of renal and pulmonary DNA appeared to be independent of changes in cyclic nucleotide levels.

In short, the present investigation demonstrates that chronic and subacute lead administration produces an elevation in the concentration of blood glucose and serum urea and reduces the level of serum insulin and hepatic glycogen. In addition, metal treatment enhances the potential of renal and hepatic tissues to synthesize glucose. Since these alterations in carbohydrate metabolism resemble those produced by cyclic AMP, it is possible that the lead-inflicted metabolic alterations may be associated with and/or triggered by stimulation of cyclic AMP synthesis. In addition, lead was found to reduce the release of insulin into the blood in response to an injected glucose load. Neonatal exposure to low amounts of lead in the diet produced similar effects on carbohydrate metabolism as compared to the changes observed after intraperitoneal injection of the metal. In addition, the incorporation of thymidine into renal, hepatic and pulmonary DNA was elevated after 2 months of lead ingestion and these alterations in nucleic acid formation were preceded by a rise in the concentration of cyclic AMP in the three tissues. In contrast, polyamine levels in liver, kidney and lung were in general unaltered or depressed, and appeared to be unrelated to changes in DNA synthesis. In acute studies, the metal-induced reduction in hepatic and pulmonary DNA biosynthesis was associated with a fall in polyamine levels; however, the stimulation of DNA and RNA synthesis in rat kidney did not appear to be related to alterations in the endogenous concentration of cyclic AMP or polyamines. The present investigation indicates that the prolonged

administration of lead to young animals may provide a useful experimental tool for further elucidation of the effects of this heavy metal on mammalian energy production, growth and development.

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A.J. Stevenson, S. Kacew and R.L. Singhal. Influence of lead on hepatic, renal and pulmonary nucleic acid, polyamine and cyclic adenosine 3':5'-monophosphate metabolism in developing neonatal rats. Toxicol. Appl. Pharmacol. In press.

A.J. Stevenson, S. Kacew and R.L. Singhal. Reappraisal of the use of acute lead for the study of cell proliferation in rat kidney, liver and lung. Submitted for publication.

T.C. Dubas, A. Stevenson, R.L. Singhal and P.D. Hrdina. Alterations of brain biogenic amines in young rats following chronic low-level lead exposure. Submitted for publication.

(b) Abstracts

A.J. Stevenson, S. Kacew and R.L. Singhal. Subacute lead intoxication: influence on nucleic acid, polyamine and cyclic AMP metabolism. Soc. Exp. Biol. Med. p. 6 (May, 1976).

A. Stevenson, S. Kacew and R.L. Singhal. Influence of lead treatment on nucleic acid and polyamine metabolism in rats. Proc. Can. Fed. Biol. Soc. 19, 103 (1976).

A.J. Stevenson, S. Kacew and R.L. Singhal. Prolonged administration of lead to developing neonatal rats as an experimental model of plumbism. Toxicol. Appl. Pharmacol. In press.

ABSTRACT

Administration of lead chloride (0.2 or 1.0 mg/kg, i.p.) for 45 days produced an elevation in blood glucose, serum urea and the activities of the four key gluconeogenic enzymes, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase and glucose 6-phosphatase in rat liver and kidney cortex. In contrast, metal administration produced a reduction in serum immunoreactive insulin and hepatic glycogen levels. Whereas prolonged lead treatment increased hepatic cyclic AMP content and the activity of adenylate cyclase, renal cyclic nucleotide synthesis was markedly reduced in these animals. The results indicate that the observed hyperglycemia produced by chronic exposure to this heavy metal may be related to an enhanced capacity of hepatic and renal tissue to synthesize glucose from non-carbohydrate precursors, and that, in hepatic tissue at least, the alterations in carbohydrate metabolism may be associated with modulation of the adenylate cyclase-cyclic AMP system. It is interesting that discontinuation of lead administration for 28 days failed to restore the observed changes in cyclic AMP or carbohydrate metabolism to control levels in liver and kidney cortex. Subacute exposure to lead chloride (5 mg/kg, i.p., twice daily for 7 days) produced changes in renal carbohydrate and cyclic AMP metabolism which were qualitatively similar to those noted after chronic treatment. Whereas subacute lead treatment failed to significantly alter the concentration of blood glucose or serum insulin, administration of a glucose load to metal-treated animals resulted in decreased glucose tolerance which was associated with a marked suppression in glucose-stimulated insulin release.

Oral administration of low levels of lead (20, 40 and 80 ppm) also resulted in hyperglycemia, hypoinsulinemia, uremia and a reduction in hepatic glycogen. In addition, the ingestion of lead elevated renal cyclic AMP levels

as well as the activities of the quartet of gluconeogenic enzymes and adenylate cyclase. In general, although oral treatment with lead failed to significantly alter whole body growth, ingestion of this metal for 2 months enhanced the incorporation of thymidine into renal, hepatic and pulmonary DNA. In contrast, exposure to lead for 2 weeks produced a significant decrease in the incorporation of thymidine into hepatic DNA, a similar reduction in DNA formation was noted in kidneys of 4 week-intubated animals.

Whereas the levels of putrescine, spermidine and spermine were generally unaltered or decreased in each of the three tissues examined, the observed lead-induced alterations in DNA synthesis were preceded in all cases by corresponding changes in the endogenous concentration of cyclic AMP. In liver and kidney, the lead-induced biochemical changes were associated with a significant retention of this heavy metal. In contrast, lead treatment produced a variety of metabolic alterations in lung tissue even in the absence of any apparent retention of the metal by this organ. Administration of a single dose of lead (10 mg/kg $PbCl_2$, i.p.) also was found to alter certain parameters associated with growth in renal, hepatic and pulmonary tissue. Whereas lead increased the incorporation of thymidine into renal DNA, a significant reduction was seen in DNA formation of hepatic and pulmonary tissue. The incorporation of orotic acid into RNA was markedly elevated after one day which subsequently fell below control levels after 5 and 7 days. Although metal administration generally failed to alter kidney polyamine levels, the concentrations of putrescine, spermidine and spermine fell in lungs and livers of lead-treated animals throughout the experimental period. Whereas the reduction in the incorporation of thymidine into hepatic DNA was associated with a decrease in the concentration of cyclic AMP, the lead-inflicted alterations in the formation of renal and pulmonary DNA appeared to be independent of changes in cyclic nucleotide levels. Results presented in this

dissertation suggest that the prolonged administration of lead to young animals may provide an appropriate experimental tool for further elucidation of the effects of this heavy metal on mammalian energy production, growth and development.

TABLE 29

Summary of the Biochemical Effects of Lead

Neonates	Adults	
(A) Chronic Oral	(B) Subacute i.p.	(C) Chronic i.p.
No Δ body weight	↓ body weight	no Δ body weight
↓ hepatic glucogen		↓ hepatic glycogen
↑ serum urea		↑ serum urea
↑ blood glucose	↓ glucose tolerance	↑ blood glucose
↓ serum IRI		↓ serum IRI
↓ insulinogenic index		↓ insulinogenic index
↑ PEPCK, ↑ PC, ↑ G6-Pase, ↑ FdPase — Kidney	↑ PEPCK, ↑ PC, ↑ G6-Pase, ↑ FdPase — Kidney	↑ PEPCK, ↑ PC, ↑ G6-Pase, ↑ FdPase — Liver & Kidney
↑ AC, ↑ cAMP — Kidney	no Δ AC, ↓ cAMP — Kidney	↑ AC (basal & GLN), ↑ cAMP — Liver
↑ AC, ↑ cAMP — Kidney		↓ AC, no Δ cAMP — Kidney
		↑ glucose & protein in urine

(A) 2% Pb to lactating females
21 days from birth
21-56 days 20, 40, 80 ppm in water

(B) 2 x 5 mg/kg i.p., 7 days

(C) 0.2 or 1.0 mg/kg i.p., 45 days

no Δ no change
↑ increase
↓ decrease

TABLE 30

Summary of the Biochemical Effects of Lead

	Neonates	Adults
	(D) Chronic Oral	(E) Acute i.p.
Liver	↑ ^{14}C-TdR into DNA ↓ DNA content ↓ RNA content ↓ RNA/DNA ratio ↓ protein content ↓ polyamines (transient) ↑ cAMP ↑ AC	↑ then ↓ ^{14}C-orotic acid into RNA ↓ ^{14}C-TdR into DNA ↑ DNA content ↑ RNA content ↓ RNA/DNA ratio ↓ polyamines ↓ cAMP
Kidney	↑ ^{14}C-TdR into DNA ↓ DNA content ↓ RNA content ↑ RNA/DNA ratio ↑ protein content ↓ polyamines (transient) ↑ cAMP ↑ AC	↑ then ↓ ^{14}C-orotic acid into RNA ↑ ^{14}C-TdR into DNA ↑ DNA content ↑ RNA content ↓ RNA/DNA ratio ↑ protein content ↓ cAMP
Lung	↑ ^{14}C-TdR into DNA ↓ DNA content ↓ RNA content ↑ protein content ↓ polyamines (transient) ↑ cAMP ↓ AC	↑ then ↓ ^{14}C-orotic acid into RNA ↓ ^{14}C-TdR into DNA ↑ DNA content ↓ RNA content ↓ RNA/DNA ratio ↓ polyamines ↑ cAMP

(D) p.o. 50 µg/day, 21 days from birth
21-56 days 80 ppm in water

(E) 10 mg/kg i.p., single dose

↑ increase
↓ decrease