concomitant nephrectomy. However, the endogenous parathyroid stimulation produced by nephrectomy also resulted in an increase in bound hydroxyproline which is detectable only after hydrolysis of the lavage fluid or plasma. The results indicate that hydroxyproline levels in plasma and lavage fluid are parathyroid-dependent and can be correlated with calcium removal under the conditions of parathyroidectomy and/or nephrectomy. These studies further suggest that the breakdown of bone under the influence of parathyroid hormone includes both its organic and inorganic components.

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Received October 22, 1963. P.S.E.B.M., 1964, v115.

Effect of Zinc on Cancerogenesis by Cadmium.* (28996)

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Previous experimental reports have brought out that in some species the metals chromium (1,2), cobalt(1), gold(3), nickel(1,2,4,5), platinum(3), silver(2,3) and tantalum(2) are capable of inducing tumors when introduced into femur, pleura, nasal sinus, intramuscular or subcutaneous tissue sites. Heath (6-8) also investigated the cancerogenic properties of cobalt and found that the metal powder, suspended in fowl serum, produced rhabdomyosarcomas when injected intramuscularly into rats. Metabolic studies had revealed that one of the effects of cobalt was inhibition of the enzymatic oxidation of *a*-ketoglutarate(9). Since cadmium was also known to be an inhibitor of keto-acid oxidation(10), Heath and co-workers(11) investigated whether cadmium, like cobalt, was also cancerogenic. From 14 to 28 mg of a powdered form of cadmium metal, suspended in fowl serum, was injected intramuscularly in rats in a similar manner as in the studies with cobalt. These investigators did, indeed, find that cadmium produced tumors, chiefly rhabdomyosarcomas, at the site of injection. Zinc and tungsten, similarly administered were not tumorigenic(7).

In recent years there has been renewed interest in cadmium, particularly since Parizek and Zahor(12) called attention to the fact that subcutaneously administered cadmium caused destruction of the rat and mouse testis and that these acute effects could be prevented by simultaneous administration of large amounts of zinc. These observations have since been confirmed by others(13-16). A recent report from this laboratory(17) brought out that, in both rats and mice, interstitial cell tumors of testis resulted from a single injection of cadmium chloride (0.03 mmole/kg) administered subcutaneously in the interscapular region and that this tumor formation could be prevented by simultaneous administration of zinc acetate (3.0 mmole/kg).

* Supported in part by a U. S. Public Health Service research grant from Nat. Cancer Inst., and in part by a research grant from U. S. Atomic Energy Commission.

The report by Heath and co-workers(11) on the local cancerogenic effect of cadmium powder led us to question whether (a) the subcutaneous administration of cadmium chloride we had used for testicular effects would produce local tumors at site of injection, (b) the subcutaneous injections of zinc acetate used to protect against the injurious effects of cadmium on the testis would also protect against possible local tumor development by cadmium and (c) whether zinc acetate injections *per se* would induce tumors.

Materials and methods. An experiment, consisting of fifty-seven 14-month-old male Wistar rats, was on hand which had been originally designed for further studies on induction of interstitial cell tumors of testis by cadmium and their prevention by zinc(17). This experiment had been initiated 10 months previously, when these rats were 4 months old, body weight approximately 400 g. At that time, 22 of these rats, comprising Group I, each received a single injection (0.4 ml) of cadmium chloride (0.03 M) subcutaneously in the interscapular region. Each rat thereby received 0.03 mmole/kg of cadmium chloride, equivalent to 1.35 mg of cadmium ion.

At the same time, 17 other rats, comprising Group II, each received the same type of cadmium injection as described above; in addition, however, they were given zinc injections. Ten of these rats received a schedule of zinc treatment which had been designed to protect the testis from the acute destructive effects of cadmium(12,13,16). In this treatment schedule, zinc acetate was given subcutaneously in 3 divided doses, each dose consisting of 0.4 ml of zinc acetate (1.0 M). The first zinc injection was given in the right lumbosacral area 6 hours prior to the cadmium injection; the second zinc injection was given in the left lumbosacral area simultaneously with the cadmium administration in the interscapular region; and the final zinc injection was given in the mid-dorsal line 19 hours following the cadmium injection. The zinc injections to the lumbosacral areas were each approximately 14 cm from the interscapular injection site of cadmium; the final zinc injection in the mid-dorsal line was approximately 6 cm from the cadmium injection. Each rat thereby received, in addition to cadmium, a total dose of 3.0 mmole/kg of zinc acetate in 3 divided doses, equivalent to 26.15 mg of zinc ion in each of 3 sites, or 78.45 mg of zinc ion per rat. The remaining 7 rats of Group II, which had also received cadmium at 4 months of age, were given the same type of zinc treatment (3.0 mmole/kg in 3 divided doses), except that zinc was not administered until 4½ months following the cadmium injection.

Group III, consisting of 18 Wistar males of the same age and body weight, housed under identical conditions, were not given injections and served as controls. All 3 groups of rats received Purina Laboratory Chow and drinking water *ad libitum*.

Ten months following injection of cadmium, the rats were examined for tumors at the 4 subcutaneous injection sites, one for cadmium and 3 for zinc. Subcutaneous tumor tissue taken for microscopic study was fixed in 10% buffered neutral formalin, stained with hematoxylin and eosin and Mallory's phosphotungstic acid hematoxylin. Testes of all rats were taken for microscopic study; they were fixed in Bouin's fluid, serially sectioned and stained with hematoxylin and eosin.

Results. Of the 22 rats which received cadmium only (Group I) 9 had subcutaneous tumors in the interscapular region, the area of cadmium injection. Tumors in 3 of the rats measured 4.0 × 4.0 × 2.5 cm. These rats were killed; subcutaneous tumor tissue and testes were fixed for microscopic study. Another rat bore a subcutaneous tumor measuring 3.5 × 2.5 × 1.5 cm; under ether anesthesia the tumor was cut back to 0.5 × 0.5 × 0.5 cm. The excised portion was fixed for microscopic study; the portion of the original tumor left *in situ* grew, approximating its original size within a 3-week period. In the 5 other rats each bearing a subcutaneous tumor, one measures 3.0 × 2.5 × 1.5 cm, 2 measure 1.0 × 1.0 × 1.0 cm and 2 measure 0.5 × 0.5 × 0.5 cm.

Microscopic examination of the subcutane-

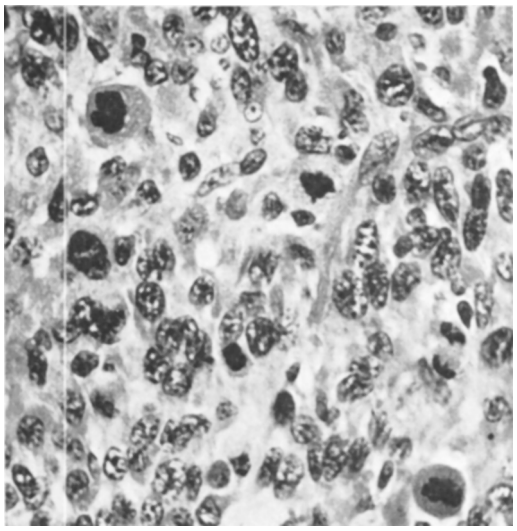

FIG. 1. Photomicrograph of a section of pleomorphic sarcoma from a rat injected subcutaneously with cadmium chloride (0.03 mmole/kg) 10 months previously. Nuclei are markedly hyperchromatic and vary in size and shape; bizarre mitotic figures are present. Hematoxylin and eosin × 438.

ous tumors removed from 4 of the tumor-bearing animals shows the identical histologic picture of a marked pleomorphic sarcoma. The tumor is composed of cells tightly packed, forming sheets, interlacing bundles and whorls. The tumor cells are usually elongated and spindle-shaped. Fascicles of spindle-shaped cells are interspersed with areas of loosely arranged cells. The spindle-shaped cells often resemble fibroblasts. The fasciculi of spindle-shaped cells divide the tumor into irregular compartments containing bizarre tumor cells. These cells are pleomorphic and have no special arrangement. Some of the cells have multiple nuclei. Phosphotungstic acid hematoxylin stain shows fine fibroglial fibers present. No beading or cross-striations are seen. Fig. 1 is typical of the tumors sectioned. It shows that the neoplastic cells vary markedly in size, shape and chromatin distribution; many bizarre mitotic figures are evident.

In the 17 rats of Group II, only 2 bore small subcutaneous nodules (0.5 × 0.5 × 0.5 cm) at the site of cadmium injection. There was no tumor formation at any of the 3 zinc-injection sites in any of the cadmium-zinc treated animals. No subcutaneous tumors were detectable in any of the 18 control rats of Group III.

In addition to examination for subcutaneous growths, each rat was examined for interstitial cell tumors. A testis was removed from each rat, either when the rat was killed, or by unilateral castration under ether anesthesia. In Group I, cadmium-treated rats, 19/22 rats had interstitial cell tumors as described previously(17). (An additional 2 rats in this series, which had died a few weeks earlier before subcutaneous palpations were initiated, had interstitial cell tumors.) Only 3/17 of the cadmium-zinc treated rats, Group II, showed evidence of interstitial cell tumors. The possibility exists that these 3 animals did not absorb their full quota of zinc acetate. No interstitial cell tumors occurred spontaneously in any of the 18 controls of Group III.

Table I summarizes the distribution of both subcutaneous and interstitial cell tumors in the 3 experimental groups.

Periodically throughout the 10-month experiment, the rats had been weighed and checked for signs of debilitation from cadmium or cadmium-zinc injections. The initial reaction approximately 24 hours following cadmium administration was a localized edema at site of injection, which subsided in a few days. The animals were left with no signs of physical impairment other than a small scarred area at the local site of injection. Body weights of the cadmium-treated rats, Group I, did not differ significantly from the controls, Group III, at 1 week, 4 and 10 months post-injection (Table II). Im-

TABLE I. Distribution of Local and Systemic Tumors in Wistar Rats 10 Months After Cadmium or Cadmium-Zinc Injections.

	Local tumors		Systemic tumors	
	Subcutaneous Pleomorphic sarcomas		Testis Interstitial cell tumors	
Experimental	Incidence	%	Incidence	%
I Cd injected	9/22	41	21/24*	88
II Cd-Zn "	2/17	12	3/17	18
III Control	0/18	0	0/18	0

* Two rats in this series, which had died a few weeks before initiation of palpation for subcutaneous growths, had interstitial cell tumors of testis.

TABLE II. Body Weights of Wistar Rats 10 Months After Cadmium or Cadmium-Zinc Injections.

Experimental	Body wt in g* 1 wk	4 mo	10 mo
I Cd injected	416 ± 14	509 ± 28	546 ± 15
II Cd-Zn "	410 ± 15	510 ± 18	567 ± 21
III Control	434 ± 19	470 ± 9	540 ± 20

* Mean ± S.E.

mediately following the zinc injections, there was an intense local inflammatory response. In 2 to 3 weeks, there was ulceration of the overlying skin followed by healing with scarring. Throughout the experiment, there were no signs of systemic damage from the zinc. Body weights of the cadmium-zinc treated rats, Group II, were within control range (Table II). Furthermore, it was previously shown(16) that rats which received cadmium-zinc injections showed normal fertility records up until the time the zinc protection to the testis wore off and the damaging effects of cadmium to the testis evoked sterility. In that same study it was also shown that administration of this same dose of zinc acetate (3.0 mmole/kg) alone to male rats did not impair fertility in rats bred steadily over a period of 12 weeks.

Discussion. Most of the studies on the cancerogenic effect of non-radioactive metals concerns the development of tumors by relatively large doses of metal foil or powder at the local site of application. Only beryllium (18-21 and others) and possibly chromium and cobalt(1) have been reported to produce tumors at a site remote from injection. Cadmium now appears as another element which is cancerogenic, not only locally, but systemically. The previous observation that interstitial cell tumors of testis develop in rats following a single subcutaneous injection of cadmium chloride, and that the occurrence of these tumors is inhibited by zinc acetate (17), has been confirmed in the study reported here. This cancerogenic action of cadmium on the testis occurs by systemic selectivity. As a result of the specific damage cadmium causes to the internal spermatic artery and pampiniform plexus(22), the testis undergoes complete destruction of both interstitial and tubular elements(12-16). The Leydig cells eventually regenerate(13,15,16) and go on to tumor formation(17). Protection against the immediate destructive effects of cadmium on the testis(12,13,16) and against interstitial cell tumor formation(17) is brought about by flooding the body with zinc, in the molar ratio of 100:1 (zinc-cadmium).

In the experiments reported here, in which rats were observed 10 months following injection of cadmium chloride, 9/22 animals had developed pleomorphic sarcomas at the interscapular subcutaneous site of injection. This cancerogenic effect of cadmium occurred following injection of 1.35 mg of cadmium ion in solution. Using 10 to 20 times this amount of cadmium metal powder in an intramuscular site, Heath and co-workers(11) reported the development of tumors which were mainly rhabdomyosarcomas with some showing evidence of concurrent fibrosarcomas.

In contrast to the local cancerogenic effect of cadmium, zinc, a physico-chemically related element and severe escharotic, failed to produce any local or systemic tumors when given in molar concentrations 33 times greater than that of cadmium. This is in agreement with the studies of Heath and co-workers(11) who reported that there was no tumor formation following the intramuscular injection of zinc powder. However, zinc has been shown, under certain conditions, to have cancerogenic activity when injected as a 5% solution of the chloride(23-28) or sulfate(29) directly into the testes of mature roosters; teratomas resulted if zinc injections were made in the spring period of high gonadal activity, or if roosters were geared to high gonadal activity by injections of gonadotrophins. Although several investigators have not been successful in producing such tumors in the mammal, Riviere, Chouroulinkov and Guerin(30,31) have recently reported that in Wistar rats testicular tumors develop following intratesticular injection of 0.025 to 0.10 ml of 5% zinc chloride. In a series of 125 animals, these investigators found 9 interstitial cell tumors, 1 seminoma and 1 teratoma.

There was a long latent period for tumor development; tumors did not appear until 15-26 months following injection.

What appears to be significant in the experiments reported here is that cadmium produces tumors locally at the site of injection and systemically in the interstitial tissue of testis, far removed from the site of injection. Another important observation is that the development of both of these cadmium-induced tumors appear to be inhibited by systemic flooding with zinc. Bischoff and Long(32) have reported that injections of 0.05 mg doses of zinc sulfate, in the area of the mammary glands, significantly delayed the appearance of mammary adenocarcinoma in virgin mice of the Marsh-Buffalo strain. In our experiments the fact that the 3 zinc injections were 14 cm, 14 cm and 6 cm, respectively, removed from the site of the cadmium injection, reduced the implication that the inhibitory effect of zinc on the development of sarcomas involved local mechanisms. In spite of the local ulceration initially caused by zinc at the site of injection, there was no evidence that the anti-cancerogenic effects of zinc could be attributed to general debilitation. The doses of zinc employed did not alter general physical appearance, body weight or breeding capacity.

It is known that zinc is an essential component of many enzyme systems and that deficiency, as well as excess, of zinc interferes with normal growth and development (33). It is thus tempting to postulate that cadmium may injure some growth-regulating mechanism of cells by deleting an essential zinc complex; and by flooding the body with excessive zinc, the cadmium effect is prevented or retarded.

Summary. A single injection of cadmium chloride (0.03 mmole/kg) to male Wistar rats has been shown to be cancerogenic both locally, by production of pleomorphic sarcomas at the subcutaneous site of injection, and systemically by production of interstitial cell tumors of testis. The development of both types of cadmium-induced tumors is inhibited by subcutaneous administration of zinc acetate (3.0 mmole/kg).

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Received October 23, 1963. P.S.E.B.M., 1964, v115.