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EXPERIMENTAL ZIRCONIUM GRANULOMAS AND CHONDROMAS IN CBA MICE*

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ABSTRACT

The intradermal as well as intraperitoneal injection of zirconium salts in CBA/J mice produced local foreign body type granulomas which regularly persisted for over eight months.

None of the animals exhibited evidence of the late delayed immune type of epithelioid cell granulomatous hypersensitivity such as has been induced experimentally in man.

Benign chondromas developed locally in the ear cartilage plate in half of the mice who had received zirconium injections in this area.

Although immune epithelioid cell granulomas have been experimentally induced in man by the intradermal injection of zirconium (1, 2) they have never been produced in animals. In view of the potential significance of an animal model for the study of this unique late delayed hypersensitivity state (3), repeated attempts have been made to induce granulomatous hypersensitivity in guinea pigs, mice, rabbits, hamsters, and rats. All have been unsuccessful (4, 5). The recent report that late delayed granulomatous hypersensitivity to the Kveim antigen could be specifically induced in CBA mice by the injection of sarcoid tissue (6) gave us new hope. The fact that the sarcoid response is closely analogous both clinically and histologically to that of the zirconium granuloma appearing in sensitized man (7) suggested that possibly this strain of mice might be uniquely capable of being sensitized to zirconium. Accordingly the present series of experiments was undertaken.

METHODS AND MATERIALS

All of the studies described herein were done on two hundred inbred female CBA/J strain agouti mice (Jackson Laboratories, Bar Harbor, Me.) approximately 4 months of age. Injections (0.02–0.05 ml.) were made either intradermally in the foot pads and the pinnae, or intraperitoneally. Biopsy specimens of the foot pads and ears as well as autopsy material from the liver, kidney, spleen, peritoneal wall, fat and lung were formaldehyde fixed, serially sectioned, and stained with hematoxylin and eosin.

Zirconium was introduced as: 1) zirconium lactate, an insoluble salt in 10% suspension in physiological saline solution, and 2) sodium zirconium lactate, a water soluble complex salt prepared by adding 1 mol of zirconium oxychloride to 3 mols of lactic acid, followed by adjustment of the pH to 7.5 by adding sodium hydroxide. Such a solution (40 to 50% concentration) was diluted to 10% with saline solution. It was then added in appropriate amounts to a 0.2 M sodium stearate solution to produce suspensions containing 8, 14 and 20% sodium zirconium lactate (7), and 3) zirconyl chloride, a soluble salt in 0.1% solution in physiological saline solution.

Control injections were made using a mixture of sodium stearate (0.2 M) and lactic acid adjusted to a pH of 7.5 by the addition of sodium hydroxide (5N).

RESULTS

The local intradermal injection of both soluble and insoluble zirconium salts into the mouse foot pad produced gross swelling and persistent enlargement. Biopsy at one week showed a nonspecific inflammatory round cell infiltrate. However, serial histologic study over a half year disclosed in each instance varying degrees of granulomatous response, consisting largely of macrophages (Figs. 1, 2). On autopsy no other gross or histologic changes were found in the organs studied. The foot pads of the mice receiving a control injection of sodium stearate showed no evidence of granuloma after 5 weeks.

The injection of a suspension of insoluble zirconium lactate routinely produced the largest most uniform sheets of macrophages. In the case of the soluble sodium zirconium lactate in a

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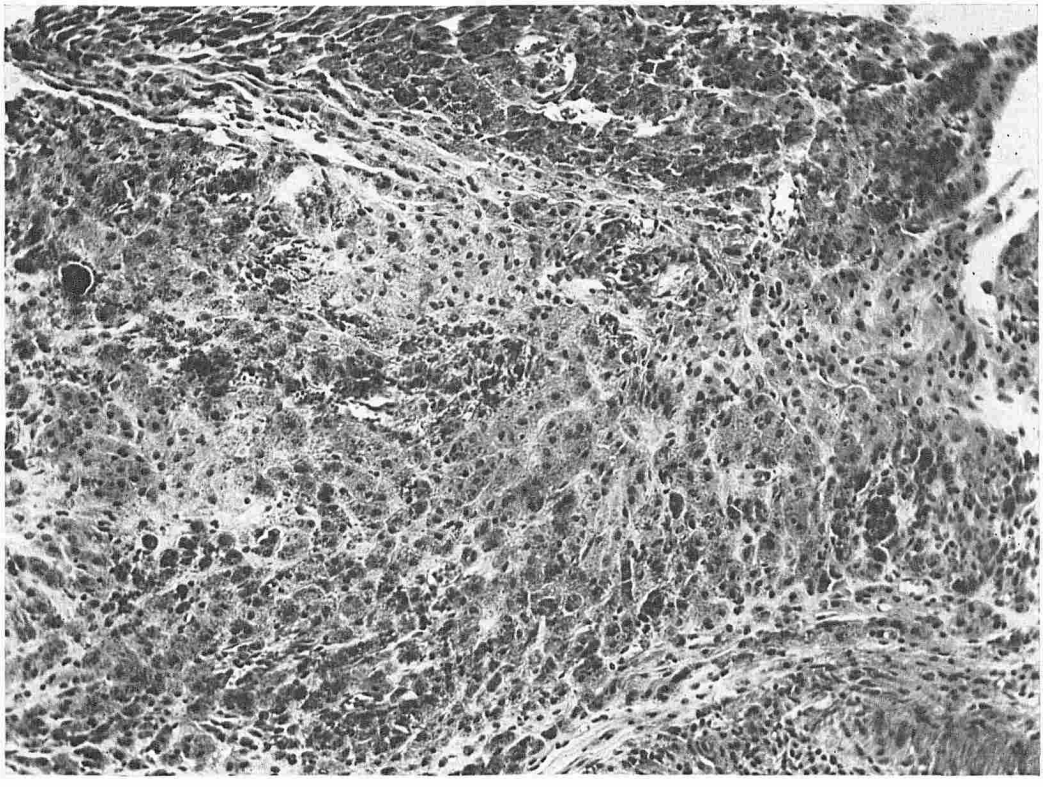

FIG. 1. Chronic foreign body granuloma in skin of foot pad of CBA/J mouse six months after injection of 0.03 ml. of 10% suspension of zirconium lactate. Note replacement of dermis by sheets of macrophages containing particulate matter.

stearate base, large masses of the foreign material could be detected indefinitely. Only minor foreign body granulomas developed at the dermal interface of these deposits. In all instances of the zirconium granulomas, multinucleated giant cells and fragments of foreign material were seen. There was no caseation necrosis and lymphocytic infiltration was minor. However, the appearance of islands and sheets of rather pure large pale stippled phagocytic type cells was the central predominant histologic finding. There was no organization or patterning into nests.

Skin testing of the mice who had developed granulomas was done with zirconyl chloride (1/1000). At six weeks there was no gross or histologic evidence of granuloma formation at the test sites on the ear. However, in over twenty per cent of the animals a small firm papule appeared after two months (Fig. 3). Biopsy of 182 ears both with and without clinical evidence of such change revealed that in half of the specimens an encapsulated benign chondroma had grown out from the ear plate cartilage at the exact site of the zirconyl chloride injection in the ear (Fig. 4).

Control sites in the ear injected with saline showed no change. Although the initial observations were made on animals previously injected in the foot pads with zirconium lactate, the chondromas could also be produced by the injection of zirconyl chloride into the ears of previously untreated animals.

The intraperitoneal injection of sodium zirconium lactate-stearate mixture in varying concentrations was regularly followed by the development of small local granulomatous infiltrates in the peritoneal fat (Fig. 5). In half of the mice discrete white lesions were seen on the surface of the liver (Fig. 6). These proved to be gross masses of macrophages (Fig. 7). They were of variable size, and in sharply defined, well orga-

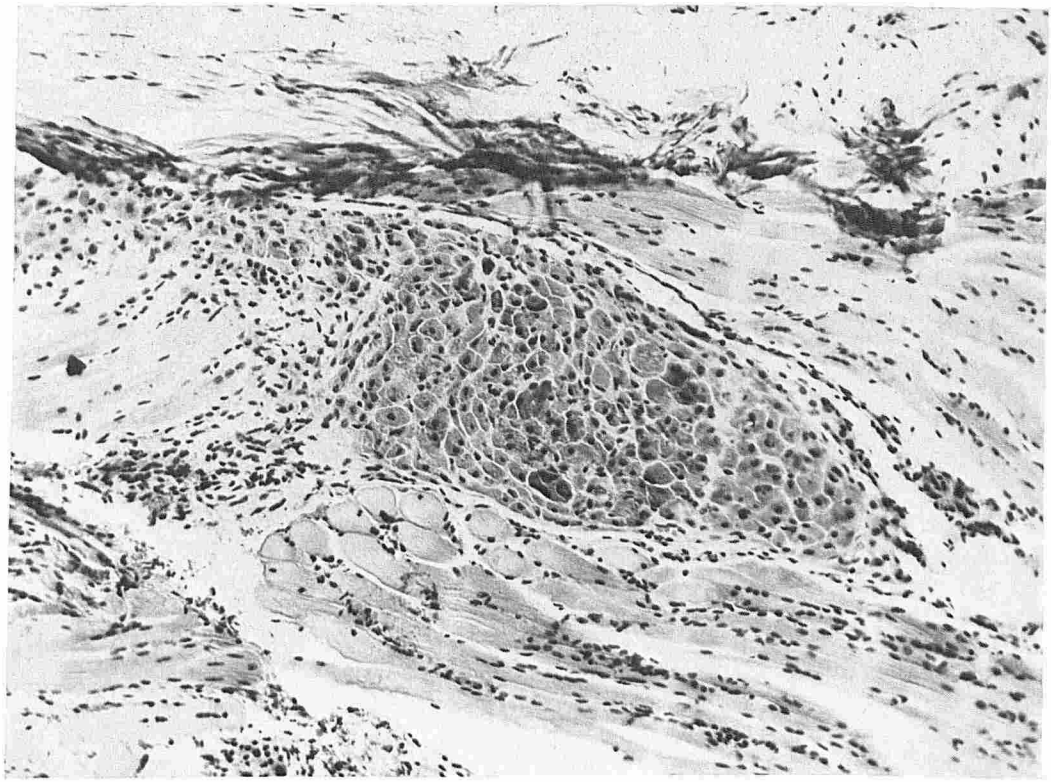

FIG. 2. Island of chronic foreign body granuloma in skin of foot pad of CBA/J mouse six months after injection of 0.03 ml. of a 20% suspension of sodium zirconium lactate in sodium stearate mixture. Note reaction to soluble sodium zirconium lactate is less pronounced than to insoluble zirconium lactate. × 150.

nized aggregates. Langerhans giant cells and occasional small foci of lymphocytes were present. Three of the thirteen mice showed the same change on the surface of the spleen. Autopsy studies did not disclose granulomas at a distance. The control animals receiving sodium stearate showed no lesions or granulomas histologically at six months. See Tables I–III for summary of results.

DISCUSSION

Zirconium and beryllium salts are uniquely capable of producing both foreign body and immune granulomas in man (3). However, in animals these compounds, to date, have been associated only with the induction of the non-allergic foreign body granulomas. Most of the studies in animals have been concerned with beryllium (8–10), although some observations are recorded on zirconium granulomas (4, 8). In all of these studies foreign body granulomas were regularly produced. In none was there evidence of an altered reactivity such as is characteristic of the

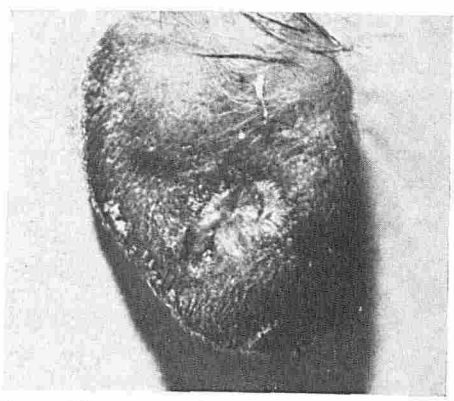

Fig. 3. Firm tumor in center of ear pinna. This developed several months following the injection of 0.03 ml. 1/1000 zirconyl chloride solution. See Figure 4.

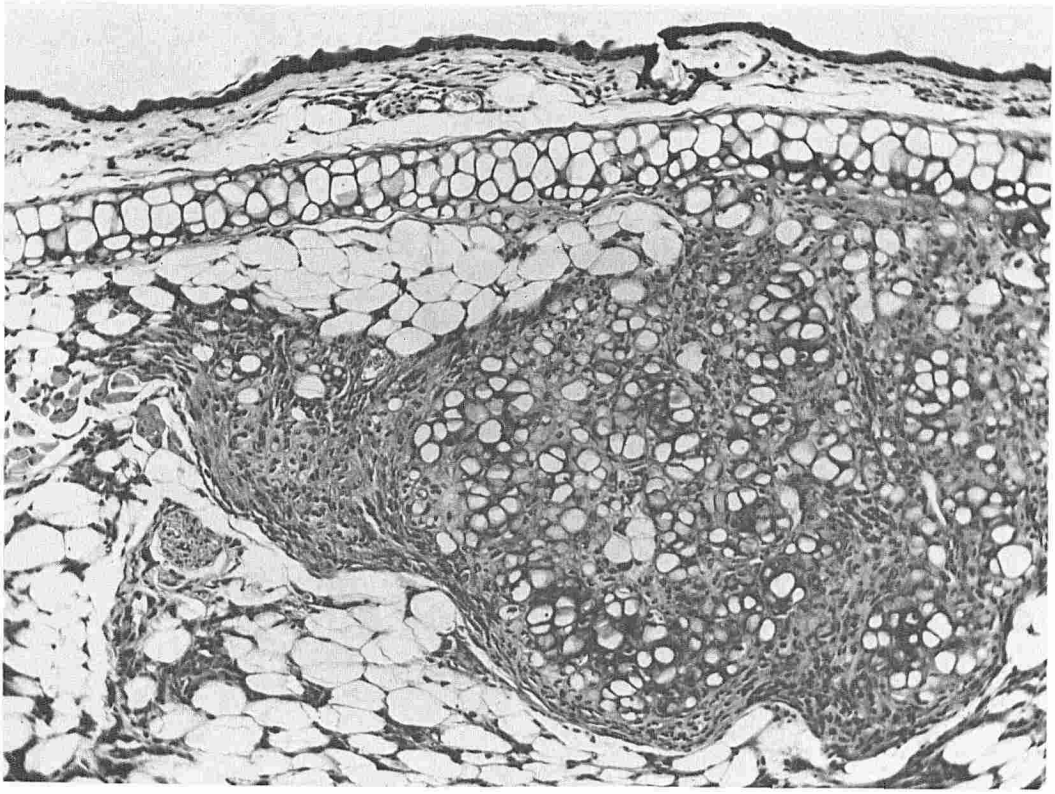

FIG. 4. Experimental zirconium chondroma. Large benign encapsulated chondroma arising from ear plate cartilage of CBA/J mouse, following zirconyl chloride injection. Biopsy and clinical photograph (Fig. 3) taken at 4 months. × 150.

immune state. The present study was an attempt to induce such an altered reactivity to zirconium.

On the basis of our observations in man, we would postulate that an immune granulomatous hypersensitivity state could be identifiable by the following criteria:

1. Specific elicitation of patterned epithelioid cell granuloma at site of dilute zirconyl chloride skin test at 6 weeks in a *limited* number of animals previously injected with zirconium salts.

2. Appearance of enlarging granulomatous masses at site of original zirconium injections.

3. Appearance of granulomas at sites distant to injection areas.

4. Persistence of granulomas for months to years at all sites of zirconium injection.

On the basis of these criteria none of the animals studied had an immune response.

It was felt that the experimental design we employed offered maximal opportunities for the development of an immune state. We used a mouse strain in which a sarcoid granuloma had been induced. We gave the zirconium 1) to relatively large numbers of animals, 2) in high concentrations, 3) in the form of a variety of salts, both soluble and insoluble, 4) with and without a stearate vehicle (originally associated with the deodorant granuloma in man), and 5) in all four foot pads as well as intraperitoneally. Our failure would seem to underscore the unusual capacity of man to react immunologically to zirconium. Admittedly the percentage of individuals capable of this altered response is small. Nonetheless at present man stands separate in that he can be experimentally sensitized to both zirconium and beryllium.

The granulomas produced in the present study are remarkably consistent in morphology and are long lasting, similar to the experimental silica granuloma in man (11). The inability of the skin to metabolize and eliminate either zir-

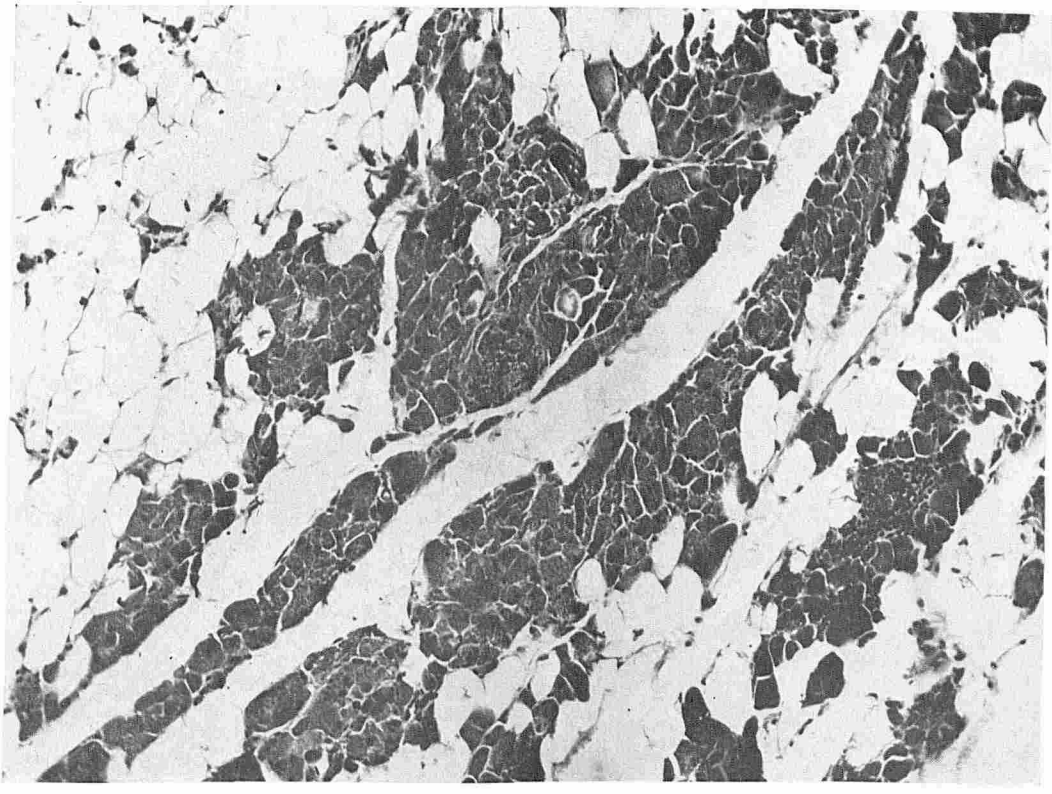

FIG. 5. Focal foreign body granuloma of peritoneal lining in CBA/J mouse 7½ months after intraperitoneal injection of 0.03 ml. of 20% sodium zirconium lactate-stearate mixture. × 150.

conium or silicon presumably accounts for the persistence of the granulomas. It should be pointed out that zirconium is a trace metal of no known function. Our daily intake in food and water is only about 3 milligrams (12). In these experiments the body sequestered and held the large quantities of injected zirconium as a foreign body for months and probably years.

The discovery that zirconium can induce benign chondromas to grow out of the normal ear cartilage was serendipitous, reflecting the use of the ear in these tests. Zirconium has never been associated with tumor formation, although beryllium, cadmium, chromium, cobalt and nickel are well known to be experimental carcinogens (13, 14). Beryllium for instance produces sarcomas in rabbits (15).

Interestingly the amount of zirconium required to elicit the chondroma is small since zirconyl skin tests (0.02 ml. 1/1000) was effective. When higher concentration of zirconium (10% sodium

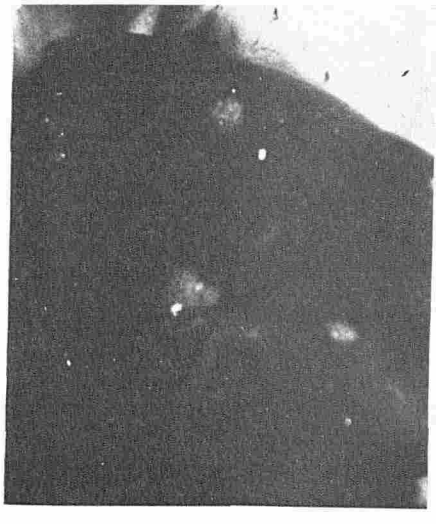

FIG. 6. Gross appearance of focal granulomata of liver of CBA/J mouse at 7½ months after intraperitoneal injection of 0.03 ml. of 14% sodium zirconium lactate-stearate mixture. See Figure 7.

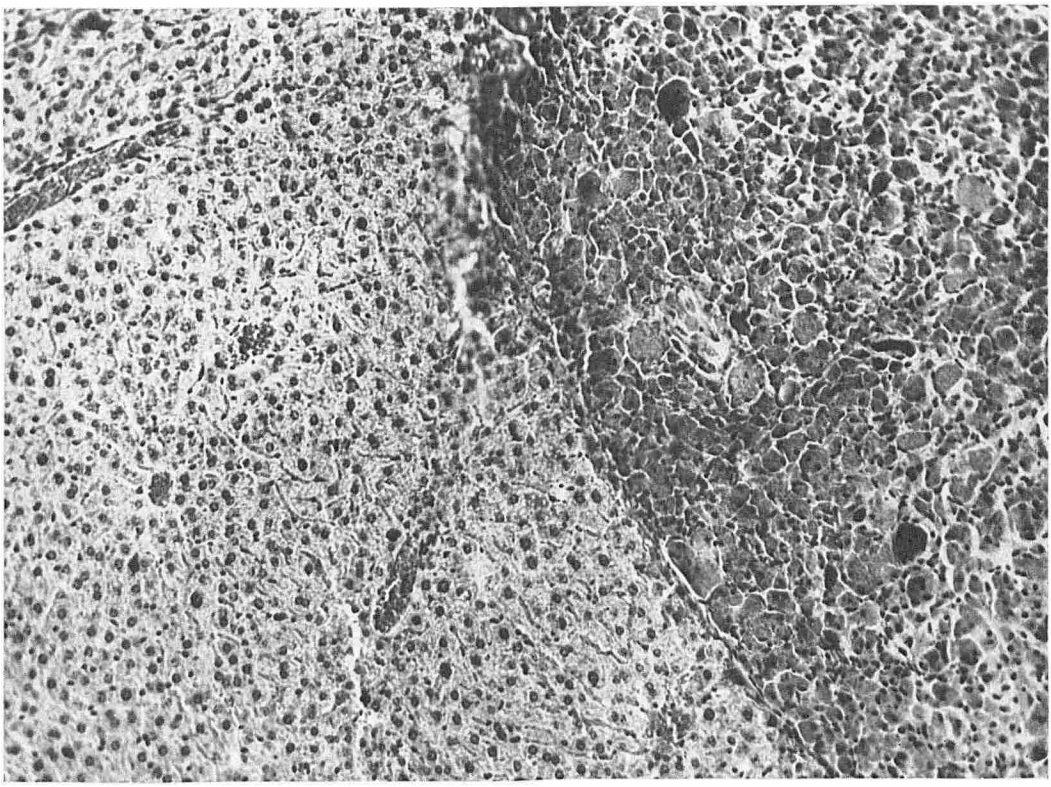

FIG. 7. Histologic findings in chronic granuloma of liver seen clinically in Fig. 6. Note zirconium foreign body granulomatous structure on right. Unaffected hepatic parenchyma is seen on the left. × 150.

TABLE I

Foreign body zirconium granulomas in the skin of CBA mice

Injectant	Site	# Mice	# of mice with foreign body granuloma at 5–7 months
Zirconium lactate suspension 10%	All four foot pads	48*	48 (Fig. 1)
Sodium zirconium lactate in sodium stearate suspension (8–20%)	All four foot pads	58	47 (Fig. 2)
Sodium stearate—sodium lactate 0.2 Molar	All four foot pads	6	0
Sodium zirconium lactate 10% solution	Both ears	12	12

* Each of these 48 mice were skin tested to zirconyl chloride (1/1000) in both ears at 6 weeks. All were histologically negative for granuloma formation at 3½ to 5½ mos.

TABLE II

Foreign body granulomas following intraperitoneal injection of 0.5 ml. of sodium zirconium lactate-stearate mixture in CBA mice

Concentration %	No. mice	No. of mice with foreign body granuloma in liver or peritoneum at 7½ months
0*	6	0
8	6	5†
14	3	2 (Fig. 6, 7)
20	4	3 (Fig. 1, 2, 5)

* Control injection of sodium stearate, sodium lactate (0.2 M).

† Each of these animals was skin tested in the food pad with zirconyl chloride (1/1000) at 6 months. All histologically negative 6 weeks later.

zirconium lactate) was employed, not only the chondroma evolved, but a granuloma was seen as well. It should be pointed out that only half of the mice showed this change, and that an induction period of several months was required.

TABLE III

Chondromas of ear cartilage of CBA mice following local intradermal injection of zirconyl chloride

Injectant in ear	No. mice ears injected	No. of mice ears showing chondroma at 2 to 5½ mos.	
		Grossly	Histologically
Zirconyl chloride 1/1000 in mice previously injected with zirconium in food pads (Table I)	182	46 (Fig. 3)	87 (Fig. 4)
Sodium zirconium lactate 10%	24	4	12
Physiological Saline	18	0	0
Zirconyl chloride 1/1000 in previously untreated mice	18	5	8

To our knowledge, this is the first technique of experimentally producing chondromas.

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