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Growth and S^{35} Uptake of Cotton Granulomas in Rats. (24927)

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The cotton pellet granuloma technic of Meier, *et al.*(1), has provided a new approach to studies of inflammatory potentials of animals under various physiological conditions(2-5). In addition it has provided the basis for development of several tests for anti-inflammatory materials(6-11). This is a study of long term growth of granulomas around cotton pellets and incorporation of labeled sulfate into the tissue in early stages of granuloma formation.

*Methods.** Male Sprague-Dawley rats averaging 175-200 g in weight were adrenalectomized 2 weeks after arrival and maintained on saline and Purina laboratory chow. The saline was supplemented with 0.5% glucose for 24 hours after adrenalectomy. In an experiment designed to study granuloma growth and histology, 4 cotton pellets averaging 6 mg in weight were implanted subcutaneously(11) into each rat the day following adrenalectomy, and the granulomas were removed at autopsy 2-100 days later. In a second experiment in which sulfate uptake in granuloma tissue was measured, each rat was implanted with a 6 mg pellet 12, 6, 4 and 2 days before autopsy. These animals were adrenalectomized on day before first pellet was implanted, and placement of pellets was varied to allow for possible position effects. Twenty-four hours before sacrifice all animals received 150 microcuries of S^{35} labeled sodium sulfate subcutaneously. In both experiments test compounds were administered subcutaneously as solutions or suspensions in oil beginning first day of implantation. At autopsy animals were sacrificed with ether, the granulomas removed, and those granulomas used for growth curve or S^{35} studies were trimmed of extraneous connective tissue. Granulomas used for histological purposes were fixed in Zenker's solution untrimmed. As control tissue for the S^{35} data normal connective tissue was removed from subcutaneous area of back according to method of Boas and Foley(12). Trimmed granulomas and normal connective tissue were dried to constant weight at 72°C. Granuloma weights were expressed as total granuloma weight/rat by subtracting original weight of the 4 cotton pellets. For radioactivity measurement individual granulomas and tissue samples were weighed and wet-ashed in perchloric acid. Aliquots of each tissue digest were mounted directly on lens paper discs supported by copper planchets and counted with end-window Geiger counter. In calculating percentage of administered S^{35} incorporated/g of tissue, corrections were made for self-absorption, radioactive decay, and variations in body weights of animals. Granulomas for histological examination were fixed in Zenker's solution 24 hours, then cut in half and washed in tap water. They were dehydrated with S-39 Technicon dehydrant, cleared with butyl acetate, infiltrated 3 or 4 days in paraffin and embedded. The paraffin blocks were trimmed to the tissue and soaked in detergent for several days to soften the cotton fibers for cutting. Chilled blocks

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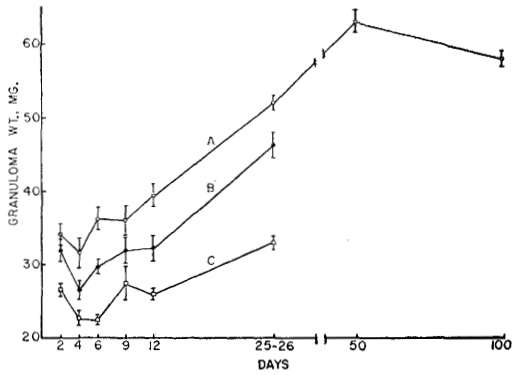

FIG. 1. Growth of cotton granulomas in adrenalectomized rats treated with oil (A), or cortisol at .25 mg (B) and 1 mg (C) per day. Vertical bars show stand. errors of means.

were cut at 6 μ, and sections stained with hematoxylin and eosin or Mallory's stain.

Results. Fig. 1 shows that cotton pellet granulomas increased in weight for 50 days after implantation. Rate of growth decreased after 25 days, and granuloma weights either remained constant or fell off between 50 and 100 days. The apparent fall in granuloma weight at 4 days was not statistically significant ($P>.05$). Daily cortisol treatment retarded granuloma growth markedly but did not completely block growth at dose levels used (Table I).

Histological studies of early granulation tissue encapsulating 2 day pellets showed many phagocytes, polymorphonuclear neutrophils, and immature connective tissue cells, while few mature fibroblasts and collagen fibers were seen. Little tissue grew into the cotton pellet although many degenerating polymorphs were observed among the cotton fibers. Granulomas encapsulating pellets implanted for 6 days showed more fibroblasts and fibers than those implanted for 2 days although many macrophages were still present. At the 6 day stage macrophages and fibroblasts started to penetrate into the pellet between cotton fibers while degenerating polymorphs were still found in center of pellet. The capsules of 25 day granulomas were dense with collagen fibers and elongated fibroblasts. These elements, vascular channels, and some macrophages penetrated all the way to a small tissue-free region at center of pellet. Granulomas of 50 and 100 days of age were somewhat more fibrous than younger tissues; and in addition, granulation tissue had penetrated throughout the center of pellet. Grossly, all granulomas were approximately the same diameter with later growth progressing to center of pellet rather than peripherally.

Incorporation of S^{35} into 4, 6 and 12 day granulomas was significantly higher ($P<.01$) than that exhibited by normal connective tissue (Fig. 2). Cortisol treatment, however, significantly decreased the labeled sulfate incorporation into normal tissue as well as into granulomas of all ages ($P<.01$). Similarly, phenylbutazone decreased S^{35} uptake in the granuloma tissue ($P<.01$ at 2, 4, and 6 day; $P<.05$ at 12 day); but its effect on normal connective tissue was negligible ($P>.05$). 17α-ethyl-19-nortestosterone (Nilevar) exhibited no effect upon sulfate incorporation into either normal tissue or granulomas.

Discussion. In the present report we assumed that S^{35} found in tissues studied was present primarily as sulfated mucopolysaccharides(13). As granulomas aged, rate of incorporation of S^{35} into these mucopolysaccharides exceeded rate of tissue growth. Thus, control granuloma weights increased but 5% between 2 and 6 days (Table I), while the

TABLE I. Growth of Cotton Granulomas in Adrenalectomized Rats.

	Oil control		Cortisol, 0.25 mg/day		Cortisol, 1 mg/day	
Days	Rats	Granuloma wt, mg	Rats	Granuloma wt, mg	Rats	Granuloma wt, mg
2	41	34.1	41	31.9	41	26.6
4	20	31.7	20	26.5	20	22.7
6	26	36.2	26	29.7	26	22.4
9	12	36.0	12	32.0	12	27.4
12	27	39.4	7	32.3	7	25.9
25–26	37	52.0	14	46.2	16	33.1
50	20	63.0				
100	19	58.1				

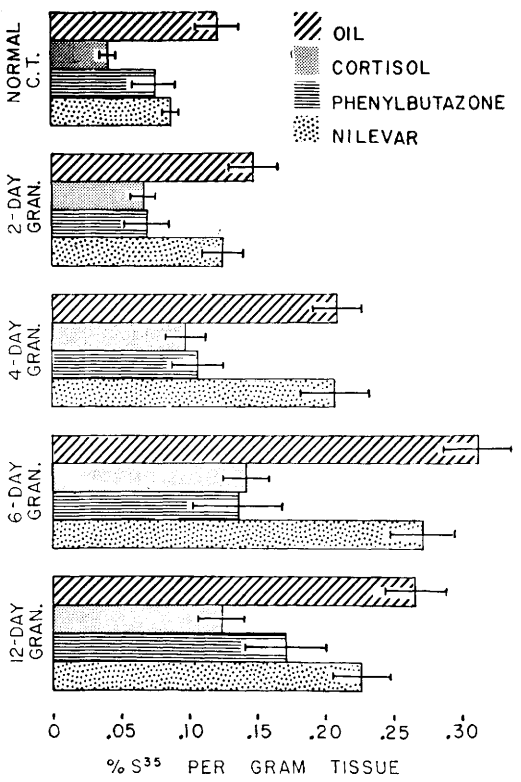

FIG. 2. Incorporation of labeled sulfate into normal connective tissue and cotton granulomas in adrenalectomized rats. Rats (11-15/group) were treated daily with oil; cortisol, 1 mg; phenylbutazone, 20 mg; or Nilevar, 1 mg; for 12 days. Small bars represent stand. errors of means.

amount of S^{35} incorporation into this tissue nearly doubled in the same time (Fig. 2). This coincided with a shift in cell population from undifferentiated cells, polymorphs, and macrophages to a greater proportion of fibroblasts as well as increase in collagen fibers. The inhibitory effect of cortisol on S^{35} uptake was consistent with the observation that anti-inflammatory corticoids inhibit synthesis of chondroitin sulfate(14). Seemingly, phenylbutazone was more active in inhibiting incorporation of S^{35} into forming granuloma mucopolysaccharide than in affecting normal tissue metabolism. While we found negligible effects of 17α-ethyl-19-nortestosterone upon S^{35} uptake in granuloma tissue, Kowalewski and Morrison found this substance to enhance incorporation of labeled sulfate into bone mucopolysaccharide(15) suggesting that 17α-ethyl-19-nortestosterone may have a greater effect upon S^{35} incorporation in bone than in granuloma tissue.

Summary. 1) Cotton pellets implanted subcutaneously in rats induced growth of foreign body granulomas that did not completely penetrate the pellet or reach maximal weight until 50 days. As granulomas aged, shifts in connective tissue elements coincided with increases in uptake of labeled sulfate. 2) Cortisol treatment decreased S^{35} incorporation into both granuloma and normal connective tissue. Phenylbutazone reduced labeled sulfate uptake by granulomas, but not normal connective tissue, while 17α-ethyl-19-nortestosterone had no effect on either.

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