into thyroxine and its precursor analogs if, indeed, any such incorporation occurred.

Summary. 1. Beef thyroid tissue slices were incubated in the presence of 100 microcuries UL-C^{14}-tyrosine with 3 variations: a) no additions, b) propylthiouracil addition, c) nonradioactive thyronine. 2. Following digestion, analysis proceeded through counter current, paper and thin layer chromatography. The results for each of the experimental designs were the same. 3. Of the 3 major areas of radioactivity seen after counter current, only one could be further subdivided by the systems usually used for separation of iodotyrosines, and iodothyronines, and this yielded at least 7 peaks in the systems used. 4. No evidence was found for *in vitro* formation of thyroxine or any of its precursors using tyrosine as the labeled substrate.

Since this work was completed, Nunez, Mauchamp, Macchia and Roche (Biochim. Biophys. Acta, 1965, v107, 247) have also reported that they were unable to demonstrate incorporation of tritiated tyrosine into the iodotyrosines of thyroglobulin formed by sheep thyroid slices. Since the tritium label of tyrosine is stable only on the alanine sidechain, this observation is specific only for the alanine chain on the tyrosyl residue attached to the thyroglobulin skeleton. The use of UL^{14}C-tyrosine in our experiments also affords information about the ether-linked distal iodophenol.

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Collagen and Hexosamine Changes in Subcutaneous Granuloma Irradiated Locally with a Co^{60} Source.* (31072)

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We have recently reported that γ-radiation from sealed sources of Co^{60} embedded inside developing granulomas was able to decrease the amount of soluble collagen at this site (1). Although this reflected a drop in collagen synthesis it did not seem to affect the amount of insoluble collagen present, presumably because the level of radioactivity used was relatively low (55 and 180 μc of Co^{60}).

In the present series of experiments we intended to pursue this study using a more radioactive source of Co^{60}, and in addition to investigate changes in the skin just above the subcutaneous granulomas as well as remote from the area of implantation.

Methods. Radiation source. Stainless steel capsules, 6 mm long and 1.5 mm in diameter, were loaded with 1 mC of Co^{60}. Fig. 1 shows diagrammatically the radioactive capsule positioned in the center of a plastic sponge. The isodose radiation curves were calculated on the basis of theoretical attenuation and experimental data compiled by Fletcher *et al* (2). Empty capsules were used as controls.§

Experimental procedure. Adult Holtzman male rats weighing 360 to 400 g were implanted subcutaneously with polyvinyl sponges (Ivalon) weighing 100 mg ± 2 mg. The sponges were sterilized by autoclaving, allowed to dry and at this time a small hole

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§ Capsules supplied by U.S. Nuclear Corp., Burbank, Calif.

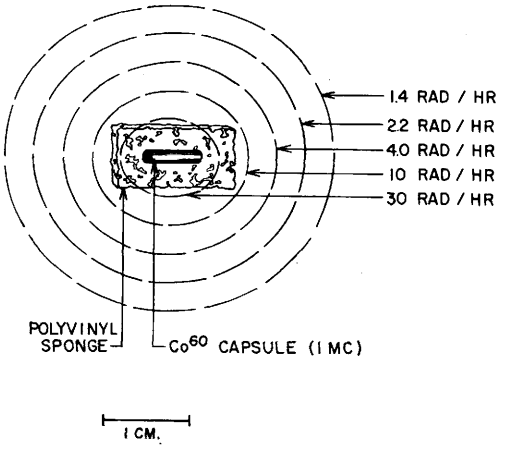


FIG. 1. Stainless steel capsule containing 1 mC of Co^{60} imbedded in a polyvinyl sponge. Ellipsoid curves represent approximate isodose radiation exposure at different distances from radiation source.

was made using a blunt probe. The radioactive and control capsules were inserted into this opening in the sponge using a forceps. The sponge was then soaked in normal saline, and when soft implanted subcutaneously in the dorsum of the animal.

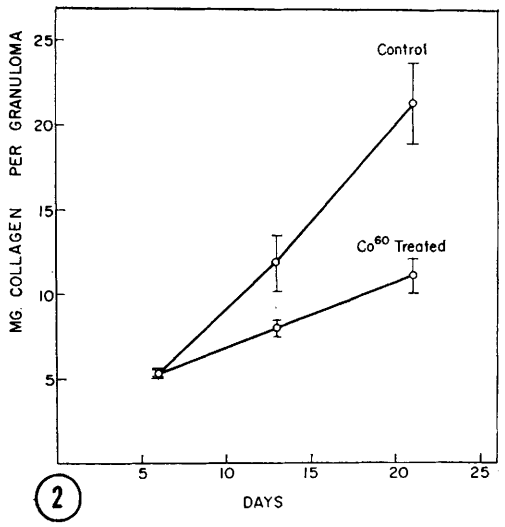
Animals were fed Purina Chow as well as water *ad lib,* and weighed every other day. They were then sacrificed at different time intervals and the subcutaneous granuloma carefully dissected from the surrounding tissue. The dorsal skin adjacent to the radiation source and remote from this area was removed for analysis. All samples were immediately weighed and maintained frozen.

Analysis. The granuloma and skin were fractionated for collagen by a previously described procedure(3). For this purpose they were sequentially extracted with 0.15 M NaCl, 0.5 M NaCl, 0.5 M citric acid pH 3.6 and the final residue autoclaved with water for conversion of the insoluble collagen into gelatin. All fractions were dialyzed against running tap water in the cold. Total collagen was determined in some specimens by autoclaving the sample with water for gelatinization. An aliquot was hydrolyzed and analyzed for hydroxyproline(4) using an automated method of analysis (Technicon Autoanalyzer). The collagen content of the sample was calculated on the basis of its hydroxyproline content. Hexosamine in sponge granuloma was determined by the procedure of Rondle and Morgan(5). Each value plotted in the Figure or presented in the Tables represents an average of 5 animals.

Results and discussion. All the animals in these experiments showed a normal growth rate. The cellular elements infiltrating the plastic sponge as well as those on its surface were exposed to radiation dosages ranging from 10 to 30 Rads per hour. The skin specimens obtained immediately above the subcutaneously implanted sponge were exposed to approximately 10 Rads per hour at the center, diminishing to 6 Rads per hour at the periphery of the skin sample. The skin specimen remote from the radioactive capsule received an average radiation exposure of 0.15 Rad per hour. The changes in total collagen content of the subcutaneous granulomas removed following 7, 14, and 21 days of sponge implantation are shown in Fig. 2.

Both groups accumulated the same amount of collagen in the first week of granuloma development. However, the synthesis of collagen by the granulation tissue began to drop significantly between 1st and 2nd week in those animals with implanted Co^{60} source. At the end of the experimental period (21 days) the untreated animals accumulated almost twice as much collagen as the irradiated ones. The hexosamine content of the developing granulomas was also determined and the results are summarized in Fig. 3. It is of interest that the concentration of amino-sugar was not affected to any great extent by radiation. At no time interval did the values differ with any degree of statistical significance.

The changes in distribution of collagen among the different solubility fractions of 24-day-old granulomas isolated from control and treated rats are shown in Table I. The significant drop in soluble collagen caused by irradiation reflects a decrease in synthesis. This is further substantiated by the lesser amount of insoluble collagen isolated from these same animals. The skin area immediately above the granulomas that contained Co^{60} capsules showed signs of inflammation and necrosis. Histologically the damaged skin exhibited atrophy of the dermis, an increased number of inflammatory cells, particularly in regions devoid of epidermis. In the deeper



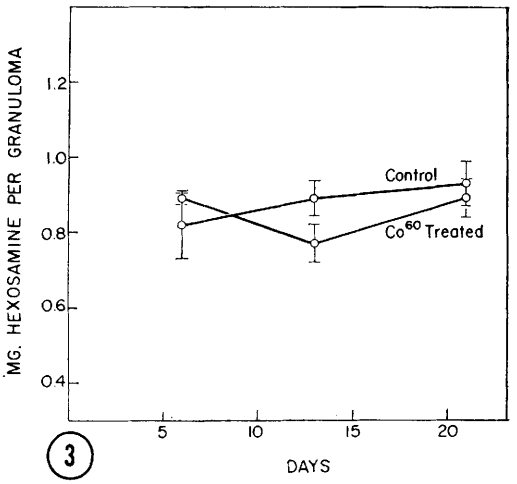


FIG. 2. Collagen content of subcutaneously induced granulomas in rats implanted with control and radioactive capsules, and sacrificed at different time intervals.

FIG. 3. Hexosamine content of subcutaneous granuloma of control and Co^{60} treated animals.

layers there could be seen numerous young mast cells. The subjacent striated muscle in the treated animals underwent moderate damage with some fibrous replacement and atrophic changes.

Table II summarizes the results obtained when skin from control and locally irradiated rats was analyzed for collagen. Skin specimens were obtained from areas above and remote from the granuloma. In the non-irradiated animals the skin above and remote from the granuloma yielded similar values, so the data represent an average of all determinations at both sites.

The skin area above the irradiated granuloma exhibited a decreased rate of synthesis as judged by its lower content of 0.15 M NaCl soluble collagen. On the other hand this same area showed the highest concentration of mature collagen (citrate extractable and insoluble). This could represent a relative increase due to loss of non-collagenous components. This area was necrotic and presumably has retained the more metabolically inert constituent, collagen, at the expense of losing the more easily degraded soluble components. The area remote from the radioactive capsules exhibited values for insoluble collagen, slightly higher than the controls, a probable indication of the known "aging effect" of ra-

TABLE I. Soluble and Insoluble Collagen Fractions Isolated from 24-Day-Old Subcutaneous Granulomas With and Without Imbedded Capsules Containing Co^{60}.

Treatment	Soluble collagen (mg/granuloma) .15M NaCl	.5M NaCl	.5M citrate, pH 3.6	Insoluble collagen (mg/granuloma)
None	1.25 ± .12	.55 ± .06	1.55 ± .39	24.4 ± 3.5
1 mC Co^{60}	.46 ± .03	.15 ± .01	.54 ± .02	12.6 ± 1.3

TABLE II. Soluble and Insoluble Collagen Fractions Extracted from Skin of Control and Locally Irradiated Animals.

Skin specimen	Soluble collagen (mg/100 mg fresh skin) .15M NaCl	.5M NaCl	.5M citrate, pH 3.6	Insoluble collagen (%)
Control	.88 ± .08	2.29 ± .4	.53 ± .2	12.7 ± 1.3
Above Co^{60} capsule	.64 ± .04	1.96 ± .02	.96 ± .3	16.9 ± 1.1
Remote from Co^{60} capsule	1.04 ± .08	2.17 ± .09	.38 ± .1	14.8 ± .3

diation. This concept is presently being further investigated for it may demonstrate that it is possible to have a local aging of tissues as contrasted to the generalized effects. This accumulation could be caused by an increased synthesis, or by a decrease in turnover rate caused by the ionizing radiation. In either circumstance the net result would be an increase in fibrous material.

Summary. Radioactive capsules containing 1 mC of Co^{60} when imbedded in subcutaneous induced granulomas, significantly decreased the amount of collagen present. This decrease is due mainly to a change in the rate of synthesis, as judged by the considerable drop in the amount of soluble collagen precursors present in the irradiated granulomas. Hexosamine concentration in developing granuloma was not significantly affected by irradiation. The necrotic skin above the granulation tissue in the irradiated animals showed an increase in total collagen, presumably of a relative nature due to a depletion of soluble tissue constituents. The rate of synthesis at this site was decreased as judged by a drop in soluble collagen precursors extractable by isotonic NaCl solution.

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Measurement of Non-Crystalline Calcium Phosphate in Bone Mineral.* (31073)

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Bone is a connective tissue with extracellular substance consisting of a calcium phosphate mineral dispersed in what is essentially a hydrated collagen matrix. It is well known that the X-ray diffraction pattern of bone mineral is characteristic of an apatite structure. On the basis of this evidence and chemical analysis, it is thought that bone mineral consists of a crystalline phase similar to, but not identical with, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The purpose of this paper is to show that the periodic structure giving rise to the X-ray diffraction peaks does not account for all of the mineral present in bone and that, in fact, there must be at least a single major calcium phosphate phase present which is non-periodic, or amorphous, in nature. This investigation has been stimulated by the recent finding that a non-crystalline calcium phosphate is the precursor phase in the preparation of hydroxyapatite under basic conditions(1,2).

Materials and methods. All materials studied were ground to 300 mesh, air dried and examined by standard X-ray diffraction techniques in a *Siemens* scintillation counter, recording diffractometer. The operating conditions were: 37 Kv, 38 ma, Copper target, nickel filter, percentage counting error 0.5%, $1/8°2\theta$ per minute diffractometer scan speed, 4 mm exit slit, 1.2 mm entrance slit for lower angle region and 2.4 mm slit for higher angle region. The X-ray powder data for each sample were obtained in 2 regions, peak No. 1 between $27.25°2\theta$, and $24.50°2\theta$ and Peak No. 2 between $41.25°2\theta$ and $37.50°2\theta$ (Fig. 1).

The moisture content of each sample was determined by the difference in weight after air drying and drying for 18 hours at 110°C. The ash weight was determined by the difference in weight between the air dried sample and the sample heated for 18 hours at 600°C.

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