cantly after 60 hours of oxygen breathing from a control value of 0.113 to a value of 0.198, an increase in the ratio of approximately 75%. The increase in SPN/IPN from the control value of 0.91 to 1.71 represents an increase of soluble protein in the lung to a value almost double the normal value.

Although most observers report "bloody lungs" as a result of oxygen poisoning, the results obtained in this present study do not indicate a pulmonary hemoglobin significantly higher than normal. The mean values of pulmonary hemoglobin in 3 of the 4 experimental groups increase, but the variability, both in controls and in oxygen poisoning groups, is so great that the changes are without significance. In one group, namely, the group subjected to pure oxygen for 60 to 83 hours, the Hb/IPN value was 1.92, this being lower than the control mean of 2.47.

Histological examination of the sections stained for erythrocytes revealed in some cases extensive invasion of the alveoli and interstitial spaces with erythrocytes. This would indicate that in some lungs or parts of lungs there is extensive congestion. When, however, the entire lung is analyzed, the pulmonary hemoglobin is not significantly raised. This would lead to a conclusion, supported also by visual examination of the lungs, that congestion was not uniform throughout the lungs, but localized in regions. An interesting observation also noted by Karsner(2) and Barach(7) was the finding of an increase in "fibrinoid" material after oxygen poisoning.

Conclusion. When guinea pigs are placed in an environment of 95 to 99% oxygen at ground level barometric pressure 73 to 75 cm Hg, the animals will develop pulmonary edema as a symptom of oxygen poisoning after 48 hours. The condition becomes progressively worse, and the animals die after 4 to 6 days in the oxygen atmosphere. Lung analyses during severe oxygen poisoning indicate an approximate doubling in lung weight and soluble protein nitrogen. Pulmonary hemoglobin, while quite variable in amount in different lungs, does not increase significantly in the entire lung during oxygen poisoning, but local regions, as shown by histological examination, may be intensely congested.

1. Smith, J. Lorrain, *J. Physiol.*, 1899, v24, 19.
2. Karsner, H. T., *J. Exp. Med.*, 1916, v23, 149.
3. Paine, J. R., Lynn, David, and Keys, Ancel, *J. Thor. Surg.*, 1941-42, v11, 151.
4. Hemingway, Allan, *J. Lab. Clin. Med.*, 1950, v35, 817.
5. ———, 1951, v37, 143.
6. Snedecor, G. W., *Statistical Methods,* 1946, Iowa State College Press.
7. Barach, A. L., *Am. Rev. Tuberc.*, 1926, v13, 293.

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Uptake of Radioactive Sulfur by Chondrosarcomas in Man. (19615)

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The ground substance of cartilage contains sulfuric esters of polysaccharides. Dziewiatkowski has shown that the radioactive sulfur of labeled sulfate given to rats is fixed by cartilage(1) and incorporated into chrondoitin sulfuric acid(2). The other tissues of the rat retain S-35 in smaller amounts and for shorter periods(3). There is little exchange between inorganic sulfur and the sulfur of amino acids and proteins(4), so that the major portion of the labeled sulfate given to rats is excreted within 24 hours(3). In man the excretion is somewhat slower(5). It is known that some differentiated tumors have metabolic patterns similar to those of the tissue of origin. Metachromatic staining properties indicate specifically the presence of sulfuric esters of polysaccharides(6) in the ground substance of

chondrosarcomas, as well as in that of cartilage. On the basis of these facts it appeared likely that radioactive sulfur given as sulfate to man would be taken up selectively by neoplastic as well as by normal cartilage. Two cases of chondrosarcoma offered an opportunity to verify this assumption. Sulfur-35 has seldom been used in man, and only in small amounts(5,7), presumably on account of its relatively long physical half-life (87 days). Recently Walser gave 100 μc of S-35 sulfate to measure the extracellular fluid of normal human subjects(8).

The uptake of S-35 by tissue cultures of human cartilage and tumors was studied by Layton(9), who pointed out the feasibility of confirming the cartilaginous nature of the growths by this test *in vitro*. However, the histologic nature of the explants was not clearly ascertained. The cultures of the cartilaginous tumors studied (osteochondroma and osteochondrogenic tumor of osteoblasts and giant cells) appeared to fix about as much sulfur as the cultures of bone or cartilage invaded by other tumors.

Experimental. Radioactive sodium sulfate was prepared by neutralizing with sodium hydroxide the carrier free H_2 S-35 O_4 supplied by the Isotopes Division of the A.E.C., Oak Ridge, Tenn. It was dissolved to about 1 mc per ml in pyrogen free distilled water with 2.2 or 3.3 mg of sodium sulfate per mc as carrier. The solution was autoclaved and given in a single injection through the tubing of an intravenous drip of 5% glucose. The first patient (H., 26-year male) had a rapidly growing chondrosarcoma of the ilium partly resected by a previous hemipelvectomy. Local extension to the sacrum and to the soft tissues and metastasis to the lungs were known to be present. The patient was given 6.7 millicuries of S-35 sodium sulfate. Tissue samples were obtained by biopsy 65 hours later. The patient expired 11 days after the injection. Necropsy disclosed metastasis to the lungs, pleurae, liver, and lymph nodes, and neoplastic invasion of pulmonary arteries. The tumor consisted of round or elongated neoplastic cartilage cells within a variable amount of ground substance; in places the cells were closely grouped and resembled an undifferentiated sarcoma. The second patient (G., 4[illegible]-year male) had in the head of the tibia a slower growing tumor that had been repeatedly curetted. The leg was amputated 50 hours after injection of 2.9 millicuries of sodium sulfate. The chondrosarcoma had invaded the soft tissues. It was composed of neoplastic cartilage cells isolated within abundant ground substance. The patient was apparently well 3½ months after amputation. Numerous clinical tests in both cases disclosed no signs of toxicity referable to the radioactivity of the sulfur. Direct radiation counts were made with a 1.8 mg/sq cm mica end-window Geiger-Mueller tube adequately protected and applied against the cut surfaces of the tissues of both patients. Samples of tissues kept in the frozen state were analyzed in duplicate for their S-35 content. They were hydrolyzed with 1 ml of 10% sodium hydroxide. After addition of 5 ml of a 0.35% sodium sulfate solution as carrier, they were oxidized with 5 ml of Denis' reagent(10). The melt was dissolved with about 10 ml of 5% hydrochloric acid, and the sulfate was precipitated at pH 2 to 3 with 5 ml of 5% barium chloride. The activity of the barium sulfate was measured in metal cups with a G-M tube (1.8 mg/sq cm mica window) and with a Tracerlab autoscaler. Individual samples were counted for at least a total of 4096 counts to insure a standard counting error of $\pm 1.6\%$. The net counts were corrected for self-absorption to a standard weight of barium sulfate. In a typical experiment the recovery of S-35 added as sodium sulfate was 79 and 83%. The activity in each sample was referred to the wet weight of the tissue; it was compared to the activity of aliquots of the injected solution of S-35 simultaneously treated by the same methods.

Results. To permit a direct comparison of the concentrations of S-35 found in the tissues of different patients, they were referred to the injected doses of S-35 calculated on a body-weight basis. The "relative concentrations" of radioactive sulfur given in the charts and table are expressed as percents of the theoretical concentration that would have occurred if the injected isotope had been evenly distributed throughout the body and completely

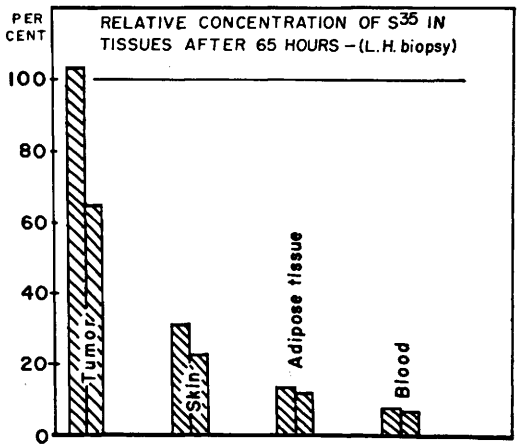

FIG. 1. The 100% relative concentration (initial avg body level) corresponds to .124 μc of S-35 per g wet wt of tissue on day of inj. decayed to .121 μc per g on day of biopsy.

retained ("initial average body level").

In patient H., 65 hours after the injection (Fig. 1), the concentration of S-35 in a subcutaneous nodule of chondrosarcoma was about 3 times as high as in the skin (epidermis and upper dermis), 6 times as high as in the subcutaneous adipose tissue, and 11 times as high as in the blood. At the death of this patient (Fig. 2), samples of both primary and metastatic chondrosarcomas contained large amounts of S-35. The concentration varied markedly between samples of tumors of different sites; even in adjacent samples of the same tumor the variation appeared greater than in adjacent samples of normal tissues. The average concentration of S-35 in normal cartilage was about two-thirds the average concentration in the neoplasm. Progressively lower levels were found in bone marrow, spleen, kidney, skin, liver, cortical bone, adipose tissue, and voluntary muscle. In the muscle the concentration of S-35 was about one two-hundredth that in the tumor. The ratio of concentration between tumor and soft tissues had increased with time, since the isotope level was about the same in the chondrosarcoma, and had rapidly fallen in the skin and adipose tissue. There was a similar fall of the level of S-35 in the blood (Table I).

The relative concentrations of radioactive sulfur in the tissues of the leg of patient G., amputated 50 hours after injection, are given in Fig. 3. In the skin and blood the relative concentrations were about the same as in the specimens of the first patient taken 65 hours

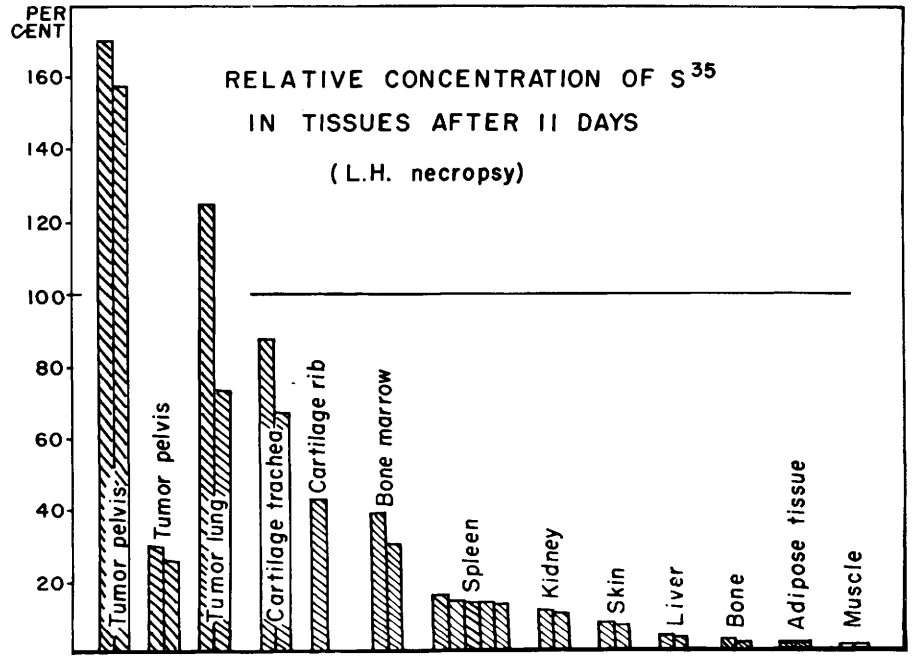

FIG. 2. The 100% relative concentration corresponds to .124 μc per g on day of inj. decayed to .114 μc per g at time of death.

TABLE I. Relative Concentration of S-35 in Blood of Patient H.

Hr after inj.	% conc.
12	61.7
65	7.4
180	1.4

after injection. The lower relative concentration in the adipose tissue of G. seems related to the better nutritional status and higher fat content. If the 2-day specimens of G. and the 11-day specimens of H. are compared, the expected fall of concentration is noted in the bone and muscle; on the other hand the similarity of level of S-35 in the specimens of cartilage suggest that in man, as in rats(2), sulfur is retained over long periods in cartilaginous tissues. In the chondrosarcoma of G., the relative concentration of S-35 was similar or higher than in the tumor of the first patient, reaching 2.5 times the theoretical initial average body level. The concentration in the chondrosarcoma averaged 4.4 times that present in the cartilage and 63 times that in voluntary muscle.

Discussion. The high but variable uptake of sulfate sulfur by the chondrosarcomas seems related to the amount of ground substance and to the proliferative activity of the cells. The concentration was maximal in the tumor of G. that had abundant ground substance.

Radioautographs of biopsy specimens of the patient H. (Fig. 4) disclosed larger amounts of isotope in the portions of the tumor where the neoplastic cells were interspersed in ground substance. However, all viable portions of the growth, even the undifferentiated sarcoma-like areas, contained more S-35 than the soft tissues. The concentration was generally higher at the growing edges of the tumor and minimal in necrotic portions. Small amounts of S-35 were taken up by the epidermis and by the fibrous tissue. Somewhat larger amounts were visualized in the probably newly formed dense connective tissue adjacent to the tumor or near the blood vessels supplying it. Labeled sulfate is probably incorporated, although to a lesser degree than in cartilage, in the intercellular substance of connective tissue, as indicated by the studies of Layton(11).

The observation that radioactive sulfate specifically localizes in chondrosarcomas suggests some possible diagnostic or therapeutic applications.

The counts on the exposed surfaces of the surgical or necropsy specimens of both patients grossly paralleled the counts on the purified sulfur fraction of the corresponding tissues. The Geiger-Mueller counter appeared more reliable than the gross appearance in differentiating between neoplastic nodules and scar tissue at the periphery of the curetted chondrosarcoma of G., as shown by subsequent microscopic examination. However, such a diagnostic application would be limited to the direct exploration of an operative field, possibly with a probe-shaped G-M tube, to

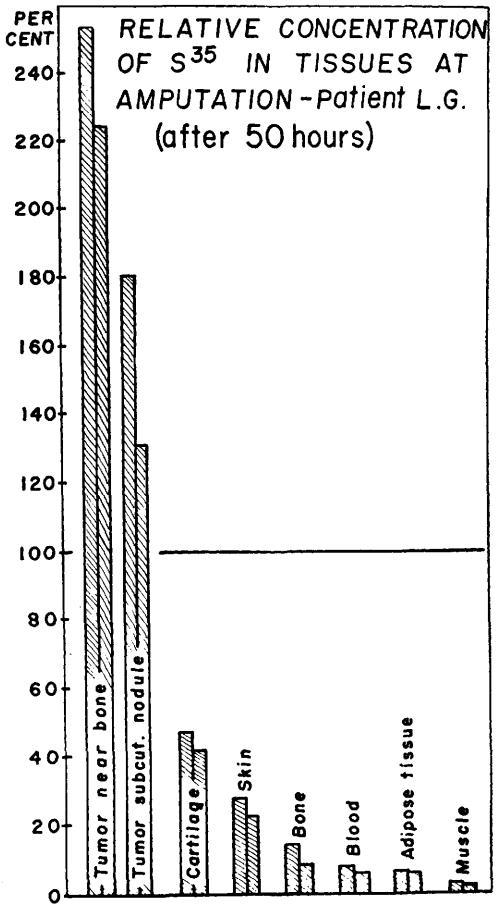

FIG. 3. The 100% relative concentration corresponds to .053 μc per g on day of inj. decayed to .052 μc per g at time of amputation.

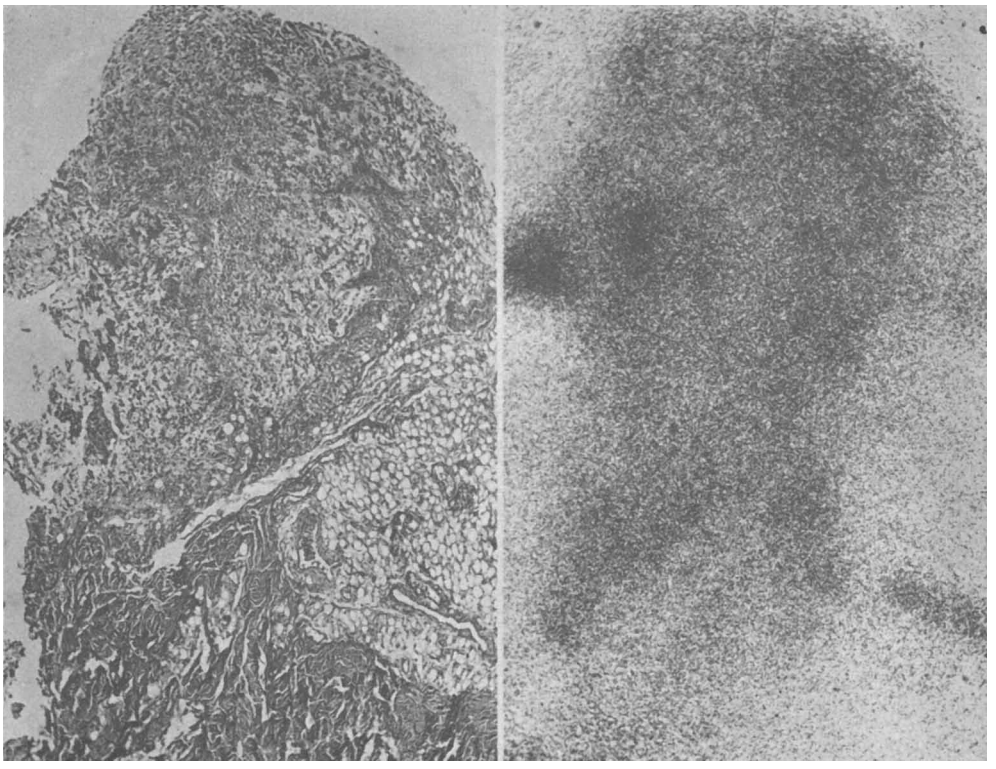

FIG. 4a (left). Subcutaneous nodule of chondrosarcoma obtained at biopsy from patient H. Neoplastic cells infiltrate the capsule (center). In lower and right fields fibrous and adipose tissues. Acid formalin fixation, paraffin embedding, 5 μ section, H. E. stain. $\times$ 42.
FIG. 4b (right). Radioautograph obtained from the section of Fig. 4a (Kodak Contrast Process Ortho film was held at about 20 μ from the surface of the unstained deparaffinized section, 97 days exposure). S-35 is demonstrated throughout the tumor, mainly in areas with abundant ground substance and at the growing edge. The dark areas in the right lower corner correspond to blood vessels. $\times$ 42.

determine the extent of a chondrosarcoma, since the soft beta rays of S-35 are absorbed by less than 1 mm of tissue.

Because of the low average energy of the radiation (0.055 MEV) and the rapid excretion of the sulfate, the doses of S-35 used in these studies can be considered to be tracer doses. Tissues with a 100% relative concentration of S-35 would have received initially 0.41 rep/day in the case of H., and 0.18 rep/day in the case of G. (calculated by formula 3 of Evans(12)).

The administration of very large doses of S-35 would be required to damage chondrosarcoma cells. Whether such doses could be tolerated by the human organism will depend not only upon the concentration in the different tissues, but also upon the time the radioisotope is retained in the respective tissue (effective half-life), and upon the radiosensitivity of the tissue. The ionization due to S-35 is limited to the tissue where it concentrates because of the very short range of the radiation. S-35 is retained at high levels in both primary and metastatic chondrosarcomas, mainly in the portions with abundant ground substance and in the areas actively growing. Large doses might, however, be harmful to normal cartilage, where they are retained for long periods, and to the bone marrow, which has a high radiation sensitivity. Recent results also indicate that very large amounts of radiation are delivered to the excretory organs immediately after administration of radioactive sulfur.

These toxic effects may outweigh the pos-

sible beneficial effects on the tumor. Additional studies on the radiation toxicity of S-35 are needed in order to evaluate whether any therapeutic benefit could be hoped for from the administration of radioactive sulfur in selected cases of chondrosarcoma.

Summary. Two patients with chondrosarcomas received respectively 6.7 and 2.9 millicuries of S-35 as sodium sulfate. Tissue samples were obtained at operations and at necropsy several days after the injection and were analyzed for S-35 content. The highest concentrations of isotope were found in the chondrosarcomas. Progressively smaller amounts were found in other tissues in the sequence: cartilage, bone marrow, spleen, kidney, skin, liver, bone, adipose tissue, and muscle. Radioautographs indicated maximum concentration in the growing portions of the chondrosarcoma and in those containing abundant ground substance. The findings are discussed in relation to the possibility of diagnostic or therapeutic applications.

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1. Dziewiatkowski, D. D., Benesch, R. E., and Benesch, R., *J. Biol. Chem.,* 1949, v178, 931.

2. Dziewiatkowski, D. D., *J. Biol. Chem.,* 1951, v189, 187.

3. ———, *J. Biol Chem.,* 1949, v178, 197.

4. Tarver, H., and Schmidt, C. L. A., *J. Biol. Chem.,* 1939, v130, 67.

5. Borsook, H., Keighley, G., Yost, D. M., and McMillan, E., *Science,* 1937, v86, 525.

6. Lison, L., *Histochimie Animale,* Paris, Gauthier-Villars, 1936.

7. Tarver, H., *Advances in Biol. and Med. Physics,* 1951, v2, 281.

8. Walser, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 372.

9. Layton, L. L., *Cancer,* 1949, v2, 1089.

10. Denis, W., *J. Biol. Chem.,* 1910, v8, 401.

11. Layton, L. L., PROC. SOC. EXP. BIOL. AND MED, 1950, v73, 570.

12. Evans, R. D., p. 428 in Glasser, O., *Medical Physics,* v2, Chicago, Year Book Publ., 1950.

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Serum Citric Acid in Hodgkin's Disease, Lymphoma and Carcinoma.* (19616)

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Thunberg(1) published an enzymatic method for quantitating serum citric acid. Benni, Schersten and Ostberg(2), employing this method, reported normal serum values of 1.5 to 3.75 mg per 100 ml of serum. Schersten (3) noted hypocitricemia in some patients with cancer. Ostberg(4) tested sera for citric acid in 8 patients with malignant disease and found values to be normal, below normal, or above normal.

Since we were unable to find any values on citric acid levels in persons with Hodgkin's disease, we undertook to make determinations of serum citric acid in 50 patients with this disease and, in addition, in 19 patients with cancer, 10 with lymphosarcoma, and in 7 with leukemia. The control group embraced 37 normal persons and 60 patients with various non-malignant diseases. In the 67 patients with Hodgkin's disease, lymphosarcoma, and leukemia 137 serum determinations were made. In 34 subjects single determinations were done, and in each of the remaining 33 persons, 2 to 5 determinations were made over a period of 4 months. The study covered a period of one year. In the Hodgkin's group data were obtained on patients during relapse and remission, in the early and terminal stages of the disease, and in mild and severe forms

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