Acta orthop. Scandinav. 39, 549-564, 1968

From the Department of Medicine,[1] Serafimerlasarettet, the Department of Orthopedic Surgery,[3] Norrbacka Institutet, and the Department of Anatomy and Histology,[2] Karolinska Institutet, Stockholm.

CASE OF CHONDROSARCOMA WITH PULMONARY AND SKELETAL METASTASES AFTER HEMIPELVECTOMY, SUCCESSFULLY TREATED WITH ^{35}S-SULFATE

By

HARRY BOSTRÖM,[1] BO EDGREN,[1] ULF FRIBERG,[2] K. SUNE LARSSON,[2] ULF NILSONNE,[3] BO WENGLE[1] & PER OLOF WESTER[1]

Received 9.ii.68

INTRODUCTION

In a recent paper in this journal (*Boström et al.* 1968) the need for new methods of treatment of chondrosarcoma was discussed. On the basis of present knowledge of the biochemistry of these tumors the following two biochemical principles for the retardation of tumor growth were suggested. 1. Retardation of tumor growth by interference with the formation of its matrix, *e.g.* by drug inhibition of the biosynthesis of sulfated glycosaminoglycans (*Boström et al.* 1964) or by enhancing the breakdown of preformed cartilaginous matrix (*Thomas* 1956). 2. Introduction of a precursor which, when built into the sulfated glycosaminoglycans, will cause damage to the malignant cells. Both principles are related to the fact that sulfated glycosaminoglycans are important constituents of the matrices of such tumors and, moreover, that there is indication that the tumors have an increased formation rate of these compounds.

The approach of *Gottschalk et al.* (1959 a, b), *Andrews et al.* (1960)

Supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council, King Gustav V 80th Birthday Fund, the Knut and Alice Wallenberg Foundation and the Association for the Aid of Crippled Children, New York.

A preliminary account of this work was given at the Annual Meeting of the Swedish Orthopedic Association, Dec. 1–4, 1966, Stockholm, Sweden.

and *Botstein & Marcus* (1963), who administered large doses of ^{35}S-sulfate to patients with advanced chondrosarcoma, exemplifies the utilization of the second of the two principles. The radioisotope was given in amounts of 0.6–1.2 C. With this dosage, temporary arrest of growth and relief of pain were noted in several instances, but in no case was a cure of the patient obtained. It was also established that the limiting factor in the therapeutic use of ^{35}S-sulfate is the radio-sensitivity of the hematopoietic system. Marked depression of bone marrow activity invariably followed the administration of the large doses of ^{35}S employed. However, the marrow function was completely restored in about one month. Consequently, the possibility of multiple courses of ^{35}S-treatment for patients who respond well was suggested by *Botstein & Marcus* (1963).

The purpose of the present paper is to report the effects of treatment with ^{35}S-sulfate in an operated case of chondrosarcoma, in which the principle of multiple courses referred to above was applied. In order to increase the ratio of exposure between tumor and bone marrow, arterial injection of the isotope was used on some occasions. In this way it was possible to administer a total dose of radioactivity, higher than any reported earlier, without protracted damage to the bone marrow. The result of the treatment was also more favorable. An apparent devitalization of a skeletal metastasis was obtained. There was also arrest of growth of pulmonary metastases.

CASE REPORT

Patient, male aged 74, previously healthy with no family history of malignancy. In 1959 he complained of pain in the left gluteal region. Roentgenological examination early in 1960 revealed skeletal destruction in the tuberosity of the left ischium; the diagnosis of chondrosarcoma was verified histologically from biopsy material. In February 1960, a resection of the left ischium was performed at the Department of Orthopedic Surgery, Norrbacka Institutet and the tumor was regarded as radically removed. At the end of 1963, however, local recurrence of the tumor was observed; and during the following year there was a slow growth of the tumor into the left proximal femoral region.

The patient was admitted to the Department of Surgery at the Serafimer hospital in September 1964. During the next six months he was treated with cytostatics (methotrexate) through an indwelling catheter in the left iliac artery. This treatment was ineffective. Tumor growth continued, and the patient was readmitted to the Orthopedic clinic in March 1965.—At that time the tumor occupied part of the pelvis and impaired the bladder function. Since no metastases could be detected in the lungs or elsewhere, a left hemipelvectomy was performed as a palliative and

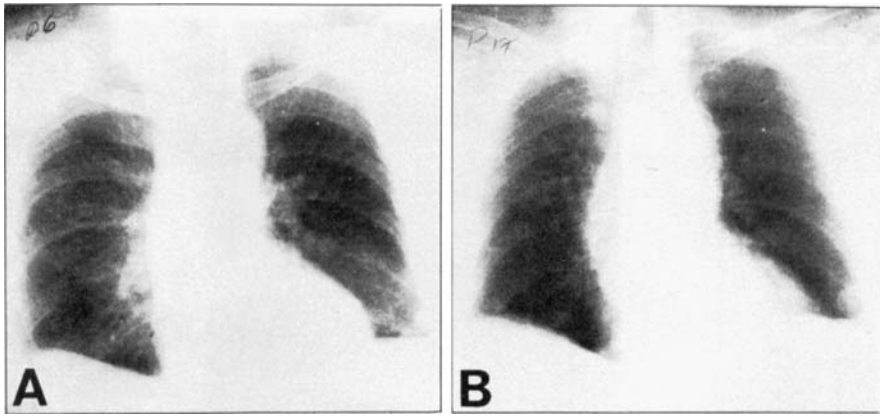

Figure 1. Roentgenologic survey of lungs before (A), and 2 years after (B), introduction of ^{35}S-sulfate treatment. Widespread metastases were observed on both occasions, but there is no evidence of progress in the disease.

hopefully curative measure, in April 1965. The operation was considered radical, as judged by macroscopic and microscopic examination of the tissues removed. The postoperative course was uncomplicated and training with a prosthesis was started.

In November 1965, pulmonary roentgenograms showed (Figure 1 A) the presence of a large number of small areas of increased density bilaterally, which were considered to be chondrosarcoma metastases. Attempts to obtain needle biopsies for histologic confirmation of this diagnosis were unsuccessful.

Soon thereafter treatment with high doses of radioactive sulfate was started at the metabolic ward of the Medical clinic at the Serafimer hospital. The total amount of radioactivity given was 1.735 C, in seven separate doses, some of which were given intravenously and others intra-arterially, as shown in Table 1.

After the first four injections the patient started to feel pain in the distal part of the right tibia. A roentgenogram of this region showed destruction of the bone; and the presence of a bone metastasis was suspected (Figure 2 A). Surgical exploration in January 1966 revealed a subcutaneous, bluish, gelatine-like, lobated tumor, destroying the cortical layer of the tibia and growing into the marrow cavity. Macroscopically, the tumor had the appearance of a chondrosarcoma; the diagnosis was confirmed by microscopic examination. In order to achieve the highest possible concentration of radioactive sulfate in the tibial tumor, the next dose was given intra-arterially into the right popliteal artery. After two additional intravenous doses of radioactivity the isotope treatment was concluded in July, 1966.

During the spring of 1966 the pains in the right tibia disappeared slowly, and the patient was again able to stand on his leg and to resume training with the prosthesis. At the end of November, *i.e.* about 10 months after the bone biopsy, roentgenograms of the chondrosarcomatous region in the tibia showed no increase in the size of the tumor. On the contrary, a marked demarcation and sclerosis of

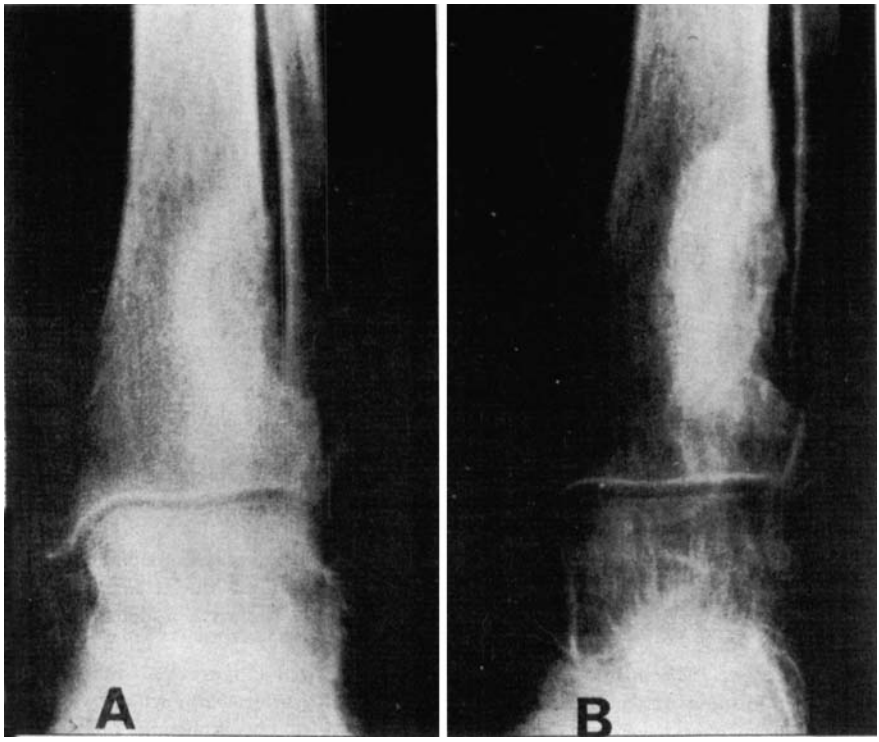

Figure 2. Tibial metastases of chondrosarcoma in January 1966 (A), and 10 months after introduction of ^{35}S-sulfate therapy (B). On the former occasion diffuse bone destruction and cortical fragmentation were observed, whereas after treatment there was marked sclerosis, with a sharp border between the lesion and the surrounding bone and soft tissue.

the metastasis was observed (Figure 2 B). A second surgical exploration of this region was made in November 1966. No extraskeletal tumor tissues were then seen macroscopically. A dense fibrotic scar tissue was found in the muscles; and the cortical layer of the tibia was hard and sclerotic. No tumor tissue was observed in the cortical or cancellous bone. The absence of viable tumor cells, in the extensive biopsy material, was confirmed by a detailed microscopic investigation. The last available roentgenogram of the tibia, taken in October 1967, showed no signs of recurrence of the malignant process. With regard to the pulmonary metastases, frequent roentgenologic surveys of the lungs, from the beginning of the isotopic treatment in November 1965 until October 1967, have failed to show any definite increase in size of the metastases, or any other sign of progress in the pulmonary involvment (Figure 1 B).

The patient's general condition, which was reasonably good throughout the isotopic treatment, has steadily improved during the two last years. Since his discharge from the metabolic ward in March 1966, he has been leading an essentially

Table 1.

Injection no.	Date	Dose mC	Administered into	Urine collection days
I	29 Nov 1965	250	Superior vena cava	7
II	6 Dec 1965	250	Right bronchial artery	14
III	20 Dec 1965	250	Right bronchial artery	7
IV	27 Dec 1965	250	Cephalic vein	14
V	17 Feb 1966	250	Right popliteal artery	–
VI	17 May 1966	235	Cephalic vein	–
VII	18 July 1966	250	Cephalic vein	–

Date, dose and route of administration of the seven injections of ^{35}S-sulfate. Duration of urine collection for radio-assay is also given; – indicates that urine was collected for safe disposal only.

normal but sedentary life in his home, except for four short periods in the hospital for treatment or checkups. However, the patient recently (Oct. 1967) developed a diabetes mellitus which is well controlled with a small dose of tolbutamide.

EXPERIMENTAL

Isotope Treatment

The isotope used in the treatment was obtained as a carrierfree, neutral, sterile solution of sodium sulfate-^{35}S, from the Radiochemical Centre Amersham, England. After carefully controlling the radioactivity of each batch of the isotope, the actual dose for administration was diluted to 10 ml with sterile normal saline.[1] The isotope was injected over a period of about five minutes through an indwelling catheter. Table 1 shows the amount of ^{35}S-sulfate given each time, the administration routes, and the injection days.

Strict precautions were taken in order to reduce the hazards of undue isotopic contamination. Routines normally employed in cases of highly infectious diseases were used in the general management of the patient during the first two months of intensive treatment, and subsequently for at least 10 days after each single dose of ^{35}S-sulfate. Moreover, during the first week after the injection the urine was quantitatively collected by means of an indwelling urethral catheter. The

[1] Mr. L. Johansson, M.A., Radiophysical Institute, Karolinska Institutet, performed the radioactivity controls and the preparation of the different doses to be injected.

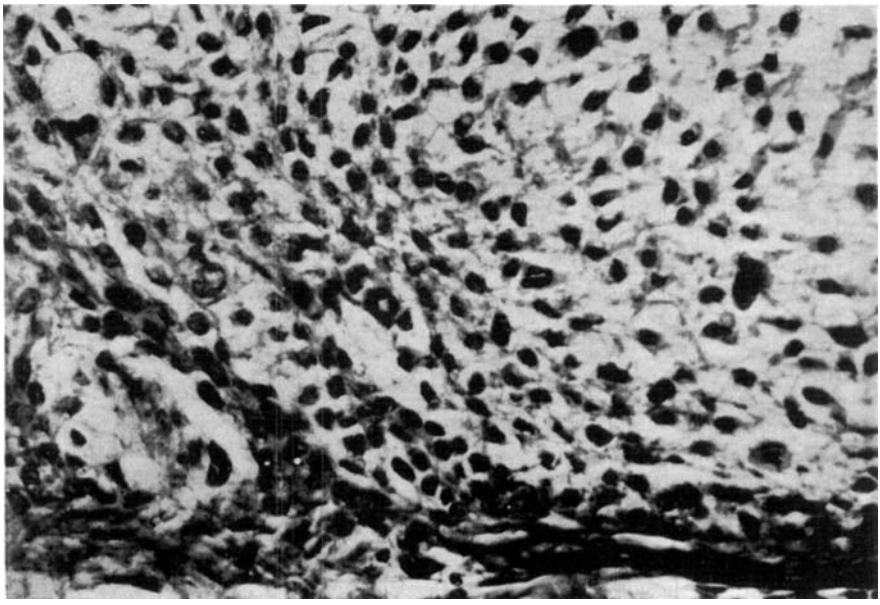

Figure 3. Microscopical section, from biopsy of tibial metastasis (cf. Figure 2 A) obtained in January 1966. Low-differentiated chondrosarcoma with typical structure containing immature chondroblastic tumor cells (× 400).

catheter was connected to plastic tubing draining into a plastic bottle, which was kept in ice in order to prevent bacterial growth and subsequent derangement of sulfate conjugates. The bottles were changed at scheduled times, sealed in the patient's room and transported to a laboratory where facilities for the handling, assay and disposal of high doses of radioactivity were available.

Histologic Studies

Material for histologic examination of the chondrosarcomatous lesion was obtained on several occasions. The primary tumor in the tuberosity of the ischium had the appearance of a typical chondrosarcoma. The recurrent pelvic tumor had a very similar appearance, with only very slight regressive changes in spite of the preceding treatment with cytostatics.

The characteristic, chondrosarcomatous appearance of the tibial metastasis before treatment with ^{35}S is shown in Figure 3. A section of the same metastasis ten months later was strikingly different (Figure 4). There was marked sclerosis and hyalinization of the tissue. The

Figure 4. Section of the same metastasis 10 months after instituting ^{35}S-sulfate treatment (cf. Figure 2 B). Marked hyalinization and sclerosis of the tumor tissue, containing but few tumor cells without signs of viability (× 400).

tumor seemed to be completely devitalized, and there were no signs of viability among the few remnant tumor cells.

Hematologic Studies

An estimate of the radiation received by the tumor tissue, as compared with that received by the bone marrow could not be worked out. Therefore, frequent hematologic studies were made during the entire course of treatment, to guide the timing of the isotope administration.

Studies on the peripheral blood included determination of the number of erythrocytes, leukocytes and thrombocytes, as well as hemoglobulin concentration. The results are summarized in Figure 5. In general, as illustrated by this figure, the hematologic complications were not very serious. On two occasions during the treatment, about one month and six months after its inception, the hemoglobin level dropped to values around 9 g/100 ml. The erythrocyte and thrombocyte counts remained at safe levels, with the lowest values one month and four months after initiation of ^{35}S-therapy. The leukocyte count

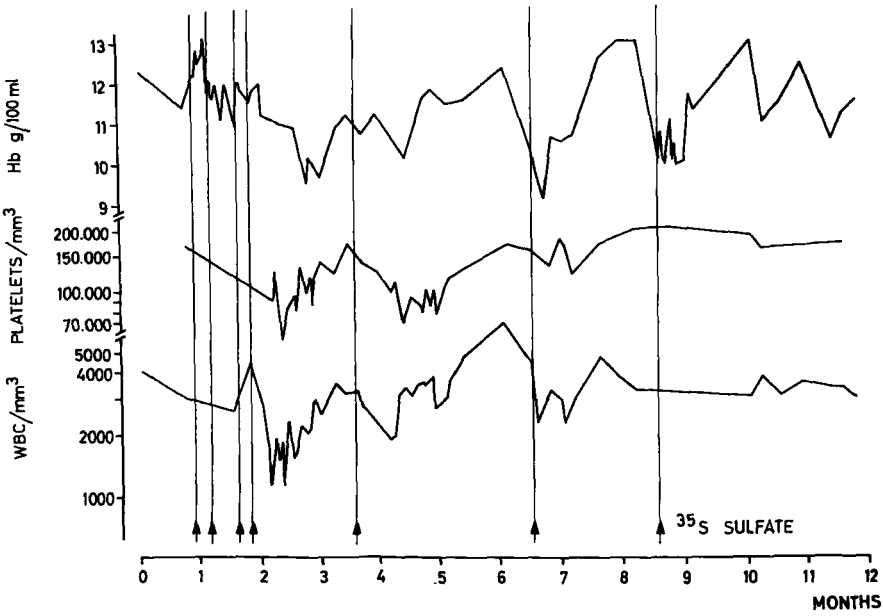

Figure 5. Level of hemoglobin, platelets and white blood cells during treatment of the patient. The arrows indicate the times when the isotope was injected.

was somewhat more strongly influenced by the ^{35}S-treatment. It fell to 1,200 cells/mm^3 after six weeks of treatment, at which time one curie of radioactivity had been given. Subsequent single doses of the isotope caused only minor reductions in the white cell count.

Frequent samples of bone marrow were taken by sternal puncture during the course of treatment, particularly during the first two months, when marrow smears were investigated several times weekly. No significant pathological changes were ever noted with respect to erythropoesis, thrombocytopoesis, or the appearance of reticular cells. Between the tenth and fortieth day after the beginning of treatment, myelopoesis was found to be hypoplastic, with the occurrence of various numbers of damaged cells. This was interpreted as indicating a mild to moderate toxic effect on myelopoesis. Two months after the beginning of the treatment the bone marrow had normalized. Subsequently no significant pathological changes in the bone marrow were observed. At the last examination (Oct. 1967) all the above mentioned hematologic parameters were normal.

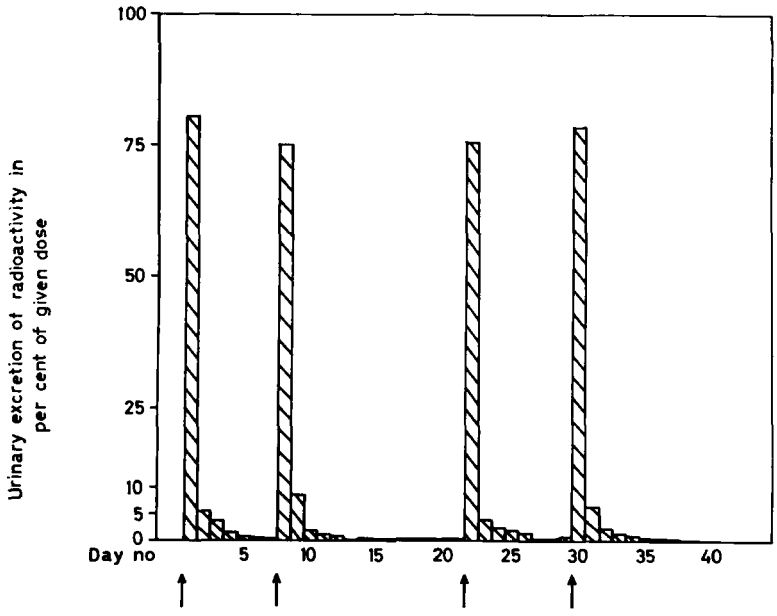

Figure 6. Schematic representation of daily urinary excretion of radioactivity following injections I–IV, expressed as per cent of given dose. The arrows indicate the times of isotope injection.

Urinary Radio-Assays

The excretion of the radioisotope into the urine was examined after injections I–IV. The radio-assays included determination of total radioactivity and of ester sulfate radioactivity; the ^{35}S-activity in the inorganic sulfate fraction was calculated as the difference between total and ester sulfate activity. No attempt was made to examine the occurrence of ^{35}S as neutral sulfur, since previous studies have shown the excretion in this fraction to be negligible (see page 12). Total ^{35}S-activity was measured at "infinite thinness" with a Geiger-Müller counter equipped with a thin endwindow tube. Aliquots of 100 μl of properly diluted (up to 10,000 times) samples of urine were applied to frosted aluminum plates, and dried at 80–90° C. As a reference, 100 μl aliquots of a dilution of the isotope solution given to the patient, were always used.

The following method was applied for determination of ester sulfate-^{35}S. The ester sulfate fraction was separated from inorganic sulfate by precipitation of the latter as $BaSO_4$. After centrifugation, the supernatant was filtered through Munktell filter paper no. OOH. The excess

Table 2.

Collection period	Fraction	Injection no. I	II	III	IV
Whole period	Inorganic sulfate	79.2	71.2	67.1	81.6
	Ester sulfate	13.2	18.6	19.5	10.3
	Total	92.4	89.8	86.6	91.9
First 24 hours	Inorganic sulfate	69.6	58.9	57.7	70.0
	Ester sulfate	10.7	16.0	17.8	8.5
	Total	80.3	74.9	75.5	78.5
Second 24 hours	Inorganic sulfate	4.6	7.3	3.2	5.8
	Ester sulfate	0.8	1.4	0.7	0.7
	Total	5.4	8.7	3.9	6.5
Third 24 hours	Inorganic sulfate	2.8	1.5	2.1	2.2
	Ester sulfate	0.9	0.4	0.4	0.2
	Total	3.7	1.9	2.5	2.4

Urinary recovery of ^{35}S-activity in per cent of administered dose after the first four injections.

of barium ions was precipitated as $BaCO_3$ by bubbling CO_2 through the solution. Centrifugation and filtration rendered a clear solution, aliquots of which were plated for radio-assay, as described above.

As shown in Figure 6 and Table 2, approximately 90 per cent of the administered radioactivity was recovered in the patient's urine. Most of

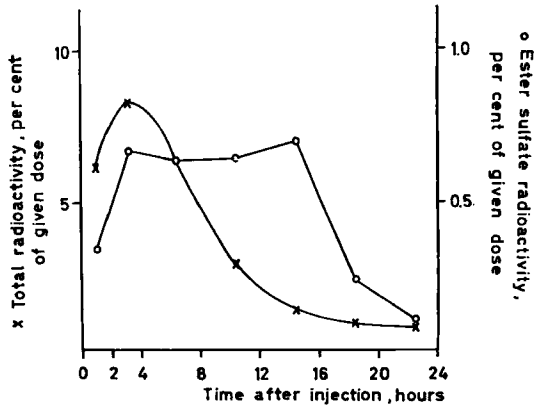

Figure 7. Excretion rate of total, and of ester sulfate, radioactivity during the first 24 hours after injection I. The excretion rates are expressed in per cent of the given dose excreted per hour.

this radioactivity was recovered during the first 24 hours and occurred in the inorganic sulfate fraction. However, as shown in Table 2, 10 to 20 per cent of the dose was excreted in the form of ester sulfates. This fraction was somewhat higher after doses II and III, injected into the bronchial artery, than after doses I and IV, which were administered intravenously. An analysis of urinary radioactivity on an hourly basis was performed during the first 24 hours of collection following injection I. The excretion of total radioactivity, mainly representing inorganic sulfate, was highest during the first few hours with a peak at about 4 hours after injection (Figure 7). Radioactivity in the ester sulfate fraction, on the other hand, showed a more delayed excretion pattern, presumably reflecting the appearance in the urine of different ester sulfates, formed and excreted at different rates.

DISCUSSION

The diagnosis of pelvic chondrosarcoma in the present case is well founded. It is based on the typical symptoms, the course of the disease, and the characteristic roentgenographic and morphologic findings. The same applies to the tibial metastasis; the nature of which was also confirmed by microscopy. With regard to the pulmonary lesions, microscopical characterization of the suggested chondrosarcomatous structure is still lacking, due to the obvious difficulty in obtaining representative biopsy material.

The clinical course was strikingly changed in a favorable direction after initiation of ^{35}S-therapy. The patient's general condition improved considerably. The tibial metastasis apparently was completely devitalized, there was no further progress of the pulmonary involvement, and there have been no signs of the appearance of new metastases. To our knowledge such a therapeutic effect has not been demonstrated previously with ^{35}S-treatment or cytostatic drugs. The question then arises to what extent the advantageous course of the disease during the last two years can be credited to the ^{35}S-therapy.

It must be pointed out that some types of chondrosarcomas grow very slowly, and that generalized metastases usually appear late in the course of the disease. Neither can the possibility of a spontaneous regression of the metastases during the period of observation be completely excluded. However, in view of the recurrence and rapid growth of the primary tumor, the failure of cytostatic treatment, and the rapid

appearance of pulmonary metastases after hemipelvectomy, it seems unlikely that the change in course was spontaneous. We assume therefore that the cessation of growth of the pulmonary metastases and the devitalization of the tibial metastasis was in fact a result of the ^{35}S-treatment. The more favorable effect of this therapy than in previously reported cases may in part be due to a greater radiosensitivity of the tumor or to an earlier institution of treatment. However, in our opinion the most important factor was probably that the patient received the highest total dose of ^{35}S-sulfate thus far reported in the literature.

In spite of the higher dose of ^{35}S-sulfate in the present case the bone marrow was only slightly affected in comparison to previous cases (*Gottschalk et al.* 1959a, and b, *Andrews et al.* 1960, *Botstein & Marcus* 1963). Nor were there any signs of damage to other tissues, or any symptoms of a generalized radiation sickness. On the contrary, the patient's general condition has been good ever since the treatment was completed. Whether his recently discovered diabetes has been precipitated by radiation injury of the beta cells of the pancreas or is only coincidental can not be established. However, it seems unlikely that such a radiation injury would not become manifest until two years after the institution of ^{35}S-therapy. Nevertheless, animal experiments have shown that ^{35}S-sulfate is taken up by the pancreatic islets. This uptake occurs mainly in the vascular connective tissue strands and only to a limited extent in the specific cells (*Odeblad & Boström* 1952, *Norhagen & Odeblad* 1955, *Diderholm & Hellman* 1957).

The urinary radio-assays performed in the present case gave results that are in agreement with those of previous studies in animals (*Laidlaw & Young* 1948, *Dziewiatkowski* 1949a and b, *Boström et al.* 1963), and in human (*Gottschalk et al.* 1959a and b, *Andrews et al.* 1960, *Botstein & Marcus* 1963): the bulk of an administered dose of ^{35}S-sulfate is swiftly excreted into the urine, mainly as inorganic sulfate, but to some extent also as ester sulfate. It should be of interest, in further therapeutic trials with ^{35}S-sulfate to subject the urinary excretion of ^{35}S-labeled ester sulfates to a closer analysis, *e.g.* in order to elucidate the possible relationship between the mode of administration of the isotope and excretion of ^{35}S-labeled ester sulfates.

The experience gained from this case indicates that it is possible to administer high doses of ^{35}S-sulfate without untoward effects through the use of multiple doses. Conceivably, a considerably higher total dose of ^{35}S-sulfate could have been tolerated by the patient, and might be utilized in future cases. A proposed therapeutic scheme could be week-

ly single doses of about 250 mC four times bi-monthly, until from two to three curies or possibly more had been administered. In the execution of such a scheme attention should be given to the hematological response. Whenever possible, arterial injection or perfusion should be employed rather than intravenous administration. Furthermore, since the greater portion of a therapeutic dose is eliminated in the urine measures should be taken to reduce undesirable irradiation of the urinary passages, particularly the bladder, and to ensure complete recovery and safe disposal of the isotope. A high urinary output volume should be maintained. The use of an indwelling urethral catheter, preferably with continuous lavage, is also advisable.

The possibility of combining ^{35}S-treatment with other methods known to depress the synthesis of mesenchymal tissue constituents, either by drugs (*Boström et al.* 1964) or roentgenologic treatment (*Andrews & Holland* 1965), must be further explored. It is our opinion that particular attention should be paid to the appropriate timing of such auxilliary measures, in relation to the course of ^{35}S-sulfate treatment. The value of ^{35}S-sulfate in the treatment of chondrosarcoma cannot as yet be considered as fully established. In our opinion, however, the present promising observations are a stimulus to further attempts to utilize this radioisotope in the search for an effective, non-surgical treatment of this malignancy.

SUMMARY

A case of chondrosarcoma, with pulmonary and skeletal metastases occurring after hemipelvectomy, is described in which treatment with ^{35}S-sulfate was successfully applied.

The total dose was 1.735 C and is the highest thus far reported. The isotope was given in seven separate injections by various intra-arterial and intravenous routes during a period of seven months.

The patient's general condition improved markedly with the institution of ^{35}S-therapy. For the last two years he has been leading an essentially normal but sedentary life. There has been no increase in the size of the pulmonary metastases. Moreover, devitalization of the tibial metastasis was obtained as verified histologically.

The treatment did not cause any serious damage to the bone marrow. Nor were there any signs of damage to other tissues, or any symptoms of a generalized radiation sickness. Hence, a therapeutic scheme involv-

ing even higher doses is suggested for future attempts to utilize ^{35}S-sulfate in the treatment of chondrosarcoma.

RESUME

On décrit un cas de chondrosarcome avec métastases pulmonaires et osseuses après hemipelvectomie pour lequel un traitement par sulfate de ^{35}S a été appliqué avec succès.

La dose totale a été de 1,735 C et est la plus haute rapportée jusqu' à présent. L'isotope a été administré en 7 injections séparées par diverses voies intraartérielles et intraveineuses pendant une période de 7 mois.

L'état général du patient s'est amélioré de façon notable avec ce traitement.

Au cours des deux dernières années, il a mené une vie essentiellement normale, mais sédentaire.

Il n'y pas eu d'accroissement dans la dimension des métastases pulmonaires.

De plus, il a été vérifié histologiquement qu'une dévitalisation des métastases du tibia a été obtenue. Le traitement n'a causé aucun dommage sérieux à moelle osseuse.

Il n'y a pas eu non plus de signes de dommages à d'autres tissus ainsi que de symptomes de maladie consécutive à une irradiation généralisée.

Ainsi, une thérapie incluant des doses encore plus fortes est suggérée dans les tentatives futures d'utilisation du sulfate dans le traitement de la chondrosarcome.

ZUSAMMENFASSUNG

Eine erfolgreiche Behandlung von pulmonalen und Skelett-Metastasen von Chondrosarcom mit ^{35}S-Sulfat nach Hemipelvectomi wird mitgeteilt.

Die totale Dosis war 1.735 C und stellt die höchste bisher publizierte Dosis dar. Die Isotope wurde in 7 separaten Injektionen, teils intravenöse, teils intraarterielle, im Laufe von 7 Monaten verabreicht.

Der Allgemeinzustand des Patienten verbesserte sich während der Behandlung mit ^{35}S-Sulfat deutlich. In den letzten beiden Jahren lebte er nahezu normal, jedoch mit begrenzter Aktivität. Es wurde keine Vergrösserung der Lungen metastasen beobachtet. Ausserdem konnte eine Devitalisierung einer Tibiametastase histologisch nachgewiesen werden.

Die Behandlung hatte keine Schäden des Knochenmarkes zur Folge.

Auch traten keine Zeichen von Gewebsschädigung oder generelle Strahlenschäden in Erscheinung.

Auf Grund dessen beabsichtigt man die Dosierung von ^{35}S-Sulfat bei zukünftiger Behandlung von Chondrosarcom zu erhöhen.

REFERENCES

Andrews, J. R., Swarm, R. L., Schlachter, L., Brace, K. C., Rubin, Ph., Pergenstal, D. M., Gump, H., Siegel, S. & Swain, R. W. (1960) The effects of one curie of sulfur 35 administered intravenously as sulfate to a man with advanced chondrosarcoma. *Amer. J. Roentgenol.* **83**, 123–134.

Andrews, J. R. & Holland, P. (1965) Sulfur 35 studies in human chondrosarcoma. *Am. J. Roentgenol.* **94**, 798–806.

Boström, H. (1952) On the metabolism of the sulfate group of chondroitinsulfuric acid. *J. biol. Chem.* **196**, 477–481.

Boström, H. & Vestermark, A. (1959) Studies on ester sulphates. 4. Ester sulphate patterns in the urine of different species. *Acta Soc. Med. Upsalien.* **64**, 194–203.

Boström, H., Gustavsson, B. E. & Wengle, B. (1963) Studies on ester sulphates 18. Ester sulphate formation in the germfree rat. *Proc. Soc. Exp. Biol. Med.* **114**, 742–747.

Boström, H., Berntsen, K. & Whitehouse, M. W. (1964) Studies on ester sulphates. 20. Biochemical properties of antiinflammatory drugs. II. Some effects on sulphate-^{35}S-metabolism *in vivo*. *Biochem. Pharmacol.* **13**, 413–420.

Boström, H. & Rodén, L. (1965) Metabolism of glycosaminoglycans, in "The Amino Sugars", Vol. IIB. E.A. Balazs and R. W. Jeanloz, eds. Academic Press, New York, p. 45–80.

Boström, H., Friberg, U., Larsson, K. S. & Nilsonne, U. (1968) On the *in vitro* incorporation of ^{35}S-sulfate in chondrosarcomatous tissues. *Acta orthop. scand.* (in print).

Botstein, C. & Marcus, N. (1963) A case of recurrent chondrosarcoma of the maxilla treated unsuccessfully with sulphur 35. *Amer. J. Roentgenol.* **89**, 555–558.

Diderholm, H. & Hellman, B. (1957) An autoradiographic study of the uptake of labelled sulphate in the pancreatic tissue of the mouse. *Acta Soc. Med. Upsal.* **62**, 23–28.

Dziewiatkowski, D. D. (1949 a) Rate of excretion of radioactive sulphur and its concentration in some tissues of the rat after intraperitoneal administration of labelled sodium sulfate. *J. biol. Chem.* **178**, 197–202.

Dziewiatkowski, D. D. (1949 b) On the utilization of exogenous sulfate sulfur by the rat in the formation of ethereal sulfates as indicated by the use of sodium sulfate labeled with radioactive sulfur. *J. biol. Chem.* **178**, 389–393.

Gottschalk, R. G., Alpert, L. K. & Alpert, R. E. (1959 a) The use of large amounts of radioactive sulfur in patients with advanced chondrosarcomas. I. Clinical and hematologic observations. *Cancer Res.* **19**, 1070–1077.

Gottschalk, R. G., Alpert, L. K. & Miller, P. O. (1959 b) The use of large amounts of radioactive sulfur in patients with advanced chondrosarcomas. II. Distribution and tissue irradiation. *Cancer Res.* **19**, 1078–1085.

Laidlaw, J. C. & Young, L. (1948) Studies on the synthesis of ethereal sulfates *in vivo*. *Biochem. J.* **42**, (Proc.).

Norhagen, A. & Odeblad, E. (1955) Uptake of radiosulfate in the islets of Langerhans of mice. *Arch. Biochem. Biophys.* **54**, 562–563.

Odeblad, E. & Boström, H. (1952) An autoradiographic study of the incorporation of S^{35}-labeled sodium sulfate in different organs of adult rats and rabbits. *Acta path. et microbiol. scandinav.* **31**, 339–344.

Thomas, L. (1956) Reversible collapse of rabbit ears after intravenous papain, and prevention of recovery by cortisone. *J. exp. Med.* **104**, 339–344.