A Study of the Factors Responsible for the Accumulation of Radioactive Iodinated Human Serum Albumin (RIHSA) by Intracranial Tumours and Other Lesions

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Radioactive iodinated (I\textsuperscript{131}) human serum albumin (RIHSA) was first used in external brain scanning by Chou et al.\textsuperscript{10} in 1951. Its popularity persists because of its effectiveness, its ready availability and the convenient rate of decay of its radioactivity (I\textsuperscript{131} half-life is 8 days). A knowledge of the mechanisms involved in the uptake of radioactive materials by tumours and other lesions should lead to improvements in diagnostic techniques and perhaps eventually even to the therapeutic use of isotopes in patients with tumours. This article presents data obtained from the study of the fate of RIHSA in intracranial tumours and certain other lesions. The decision was taken to limit the investigation to the fate of RIHSA alone because this material was the one currently in diagnostic use at this centre. It is, of course, recognised that the observations on the behaviour of RIHSA in the body and the conclusions drawn from them are not all applicable to other radioactive substances.

Material and Methods

Surgical or postmortem specimens were taken from patients who had received RIHSA for brain scanning. Radioactivity was determined in a scintillation well-counter, gross and microscopic autoradiographs were prepared, and from some tumour-skin cultures were grown from which autoradiographs were also made. Material from 50 cases was studied.

Received for publication March 6, 1964.

* This article is based on the winning entry by C.H.T. for the Eighth Annual Award of the American Academy of Neurological Surgery. The work was generously supported by the Ontario Cancer Treatment and Research Foundation, Project Nos. 64 and 147.
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Administration of RIHSA. Most patients received 1 intravenous dose of 350 µc of RIHSA-I\textsuperscript{131} 24 hours after the oral administration of Lugol's iodine. Two patients received less than 350 µc. and in these cases the counting data have been standardised to a dose of 350 µc. Two patients (Cases 15 and 20) received a second dose of 350 µc. of RIHSA-I\textsuperscript{131}, but have not been included in the uptake curves. Three patients received RIHSA-I\textsuperscript{125} for purposes of autoradiography.* Scanning took place 24 hours and sometimes again 48 hours after the administration of RIHSA.

Well-Counting Procedures. Specimens containing RIHSA-I\textsuperscript{131} were counted in a well-scintillation counter containing a 2 in. crystal. Specimens containing RIHSA-I\textsuperscript{125} were counted in a similar well, but a scaler with a discriminator was used. The system for counting RIHSA-I\textsuperscript{125} had an efficiency of about 20 per cent yielding approximately $4.5 \times 10^5$ counts per min. per µc. A sample error of 2 per cent was usually obtained easily and was determined by the method of Loewinger and Berman.\textsuperscript{27} However, as a result of a combination of the relatively low efficiency of the counting apparatus and the low radioactivity of some specimens, an error of 5–10 per cent occasionally had to be accepted.

Standard solutions of the isotope were counted periodically, and it was found that the efficiency of the counting apparatus did not vary significantly during the course of this study. A sample of blood from each patient was counted so that the uptake in tumours could be expressed as a percentage of the uptake in blood.

Surgical specimens were placed in 10 per cent formalin as soon as possible after removal, and the formalin was changed 3 or 4 times in order to remove any surface blood. It was found that up to 20 per cent of the radioactivity might be leached from a specimen if it were placed in saline or water before fixation. This was probably because albumin is water-soluble and mainly exp-

* According to the suppliers both iodinated albumin solutions—I\textsuperscript{131} and I\textsuperscript{125}—contain less than 2 per cent unbound radioactivity.
tracellular, and so may be removed by washing with water or saline. When necessary, surface blood was also removed by blotting the specimens with paper towelling, and areas of obvious operative haemorrhage were excised under the dissecting microscope. The removal of the surface blood was necessary to avoid spuriously high counts, as radioactivity in blood was almost invariably higher than radioactivity in tissue. Brains obtained at autopsy from patients who had received RIHSA were perfused through the basal vessels with 10–20 per cent formalin in order to hasten fixation. After perfusion, the autopsy specimens were treated in the same manner as the surgical specimens. The meninges were removed from the samples of brain before they were counted in the well-counter.

After fixation, excessive fluid was removed from the specimens with blotting paper, and 300–500 mg. samples were weighed and counted in the well. The specimens that had been counted were then embedded for histological examination and, in some cases, autoradiography. The concentration of radioactivity in the specimens was calculated in terms of counts per min. per gm. (c./min./gm.) and corrected for decay to the time of the injection of RIHSA.

Autoradiographic Techniques. (a) Macroscopic autoradiography. With specimens of low radiation flux, thick sections (up to 1 cm.) were covered with a thin polyethylene membrane, placed in contact with Ilford or Gevaert X-ray film, and exposed for periods up to 60 days at 4°C. When the radiation flux was higher, 5 to 25 μm. sections were placed in contact with Kodak Type No Screen Autoradiographic Plates and exposed for similar periods. Kodak D-19 Developer and Kodak Fixer were used for processing these autoradiographs. The processing solutions were maintained at 17° to 18°C. Times for development varied from 4 to 12 min., depending on the density of the image observed.

(b) Microscopic autoradiography. Microscopic autoradiographs were prepared by modification of the coating technique of Kopriwa and Leblond. Specimens were cut at 5 to 25 μm. and mounted on microscopic slides with 0.5 per cent gelatin. Kodak NTB3 emulsion was used in most instances. Unstained sections were dipped in emulsion at 40°C. and allowed to dry at room temperature and humidity for about 30 min. After drying, the slides were placed in black plastic slide boxes in the presence of anhydrous calcium sulphate and exposed at 4°C. The time of exposure varied from 11–63 days depending on the isotope used and the radiation flux of the specimens. The autoradiographs were developed with Kodak D-72 for 2 min., placed in a water stop-bath for 30 sec., and fixed in Kodak Fixer for 4 min. The slides were then washed in running water for 30 min. All solutions were maintained at 17°–18°C. When dry, the autoradiographs were stained with Harris' haematoxylin and eosin. Then they were placed in a 1:1 mixture of cedar oil and absolute alcohol for 1 hour, followed by a 1:1 mixture of Malinol and xylol for 1 hour. The slides were mounted with Malinol.

(c) Autoradiography of tissue cultures. Intracranial neoplasms and neoplasms from the spinal canal were grown in tissue culture according to the technique of Morley in dilute homologous human serum. The cultures were grown on coverslips in Carrel flasks and Leighton tubes. At varying times after growth had begun, the medium was removed and 2 ml. of fresh medium containing RIHSA-131 or RIHSA-125 in a concentration of 2.5 μc./ml. were added to each culture. The incubation time in the presence of radioisotope was generally 48 hours. The cultures were fixed in formalin-saline and washed 5 times with saline to remove excessive tracer. The coverslips were removed, mounted on microscopic slides with Krylon, and microscopic autoradiographs were then made according to the technique described above. Cultures to which tracers had not been added were coated with photographic emulsion to serve as controls.

Results

a) Sample Counting Data. There was often considerable variation in the concentration of radioactivity in different parts of the same specimen of tumour. Usually the variation could be accounted for by histological differences, but occasionally different parts of a tumour which appeared homogeneous contained varying amounts of radioactivity. Gurdjian et al. found that the radioactivity of the surface areas of many tumours was higher than that found beneath the surface. In our specimens of tumours the variation in radioactivity was not related to position. When several samples of each specimen were available, mean values were recorded.

The concentration of radioactivity in small specimens of neoplastic or brain tissue taken by needle biopsy was always less than that of larger pieces of adjacent tissue of similar composition. The reason for this is not clear but it may be that proportionately more fluid is lost from a small fragment of tissue during fixation because of its relatively larger surface area. In view of this finding, specimens taken by needle biopsy were not used when