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\(^{131}\)) human serum albumin (RIHSA) was first used in external brain scanning by Chou et al.\(^{10}\) in 1951. Its popularity persisted because of its effectiveness, its ready availability and the convenient rate of decay of its radioactivity (I\(^{131}\) 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 tumours tissue cultures were grown from which autoradiographs were also made. Material from 50 cases was studied.

Administration of RIHSA. Most patients received 1 intravenous dose of 350 μc of RIHSA-I\(^{131}\) 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\(^{131}\), but have not been included in the uptake curves. Three patients received RIHSA-I\(^{125}\) 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\(^{131}\) were counted in a well-scintillation counter containing a 2 in. crystal. Specimens containing RIHSA-I\(^{125}\) were counted in a similar well, but a scaler with a discriminator was used. The system for counting RIHSA-I\(^{131}\) had an efficiency of about 20 per cent yielding approximately 4.5 × 10\(^{3}\) 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.\(^{27}\) 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 ex-

* According to the suppliers both iodinated albumin solutions—I\(^{131}\) and I\(^{125}\)—contain less than 2 per cent unbound radioactivity.
Accumulation of RIHSA by Intracranial Tumours 61

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 µ 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 µ, 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 xylo1 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 mc/µl. 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
TABLE 1
Radioactivity of glioblastomas and cerebral cortex from patients injected with RIHSA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Interval Between Injection* and Death (Days)</th>
<th>Glioblastoma† (c./min./gm.)</th>
<th>Cerebral Cortex‡ (c./min./gm.)</th>
<th>Tumour-Brain Ratio</th>
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</thead>
<tbody>
<tr>
<td>20</td>
<td>1.5 and 10</td>
<td>10,545</td>
<td>534</td>
<td>19.7</td>
</tr>
<tr>
<td>15</td>
<td>4 and 7</td>
<td>5,254</td>
<td>234</td>
<td>22.5</td>
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<td>76</td>
<td>6</td>
<td>5,055</td>
<td>263</td>
<td>19.2</td>
</tr>
<tr>
<td>52</td>
<td>7</td>
<td>3,733</td>
<td>136</td>
<td>27.4</td>
</tr>
<tr>
<td>95</td>
<td>8</td>
<td>2,227</td>
<td>122</td>
<td>18.3</td>
</tr>
</tbody>
</table>

* Cases 20 and 15 received 2 injections of 350 µc. of RIHSA. The other cases each received 1 injection of 350 µc. of RIHSA.
† The meninges were removed.

quantitative observations and comparisons were being made.

b) Ratios of Tumour-Brain Uptake. Tables 1 and 2 compare the concentration of RIHSA in brain with the concentration of RIHSA in glioblastomas and acoustic neuromas. The glioblastomas were postmortem specimens, and the concentration of RIHSA in cerebral cortex remote from the tumour was also determined. The acoustic neuromas were surgical specimens; the concentration of RIHSA in portions of cerebellum excised at the same operation was also determined. The ratios of tumour-brain uptake range from 18.3 to 27.4 in the glioblastomas (Table 1), and from 3.0 to 8.0 in the acoustic neuromas (Table 2). The ratio is lower in the acoustic neuromas because, in general, these tumours accumu-

TABLE 2
Radioactivity of acoustic neuromas and cerebellum from patients injected with RIHSA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Interval Between Injection and Operation (Days)</th>
<th>Acoustic Neuroma* (c./min./gm.)</th>
<th>Cerebellum† (c./min./gm.)</th>
<th>Tumour-Brain Ratio</th>
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</thead>
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<tr>
<td>20</td>
<td>1.5 and 10</td>
<td>3,622</td>
<td>453</td>
<td>8.0</td>
</tr>
<tr>
<td>33</td>
<td>3</td>
<td>7,201</td>
<td>2,410</td>
<td>3.0</td>
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<td>113</td>
<td>4</td>
<td>2,535</td>
<td>444</td>
<td>5.7</td>
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<tr>
<td>87</td>
<td>16</td>
<td>1,231</td>
<td>215</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* The meninges were removed.

lated less RIHSA than the glioblastomas, and cerebellar tissue was more radioactive than cerebral cortex presumably because of operative trauma; for in the autopsy cases when nontraumatized tissues could be selected, the radioactivity of cerebral cortex and cerebellum was approximately equal.

The ratio of tumour-brain uptake depends on two factors: the inhibition of passage of albumin from the capillaries into nervous tissue (the blood-brain barrier), and the accumulation and retention of albumin by neoplastic tissue. The relative importance of these two mechanisms may be assessed by referring to Table 3 which lists the radioactivity of glioblastomas, cerebral cortex and other organs from 3 patients who came to autopsy after receiving RIHSA. The radioactivity of the specimens of Case 15 is higher than the others because this patient received 2 injections of 350 µc. of RIHSA, 4 days and 7 days before death. Case 52 and Case 95 each received only 1 injection of 350 µc. of RIHSA 7 days and 8 days before death respectively. At autopsy formalin was injected into the basal vessels in an attempt to perfuse the brain. However, the autopsies were performed several hours after death, and clotted blood prevented the passage of formalin through most vessels. Gross and microscopic examination revealed that most cerebral vessels contained blood.

Cerebral cortex was the least radioactive

TABLE 3
Radioactivity* of glioblastomas and normal tissues from patients injected with RIHSA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Case 15</th>
<th>Case 52</th>
<th>Case 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma†</td>
<td>5,254</td>
<td>3,733</td>
<td>2,227</td>
</tr>
<tr>
<td>Cerebral cortex†</td>
<td>284</td>
<td>186</td>
<td>122</td>
</tr>
<tr>
<td>Muscle</td>
<td>4,387</td>
<td>555</td>
<td>747</td>
</tr>
<tr>
<td>Liver</td>
<td>10,203</td>
<td>1,753</td>
<td>2,651</td>
</tr>
<tr>
<td>Kidney</td>
<td>4,796</td>
<td>2,169</td>
<td>2,486</td>
</tr>
<tr>
<td>Thyroid</td>
<td>297,424</td>
<td>198,464</td>
<td>137,488</td>
</tr>
<tr>
<td>Lung</td>
<td>3,976</td>
<td>3,976</td>
<td>3,976</td>
</tr>
<tr>
<td>Spleen</td>
<td>2,718</td>
<td>2,718</td>
<td>2,718</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1,110</td>
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<td>1,110</td>
</tr>
</tbody>
</table>

* The values correspond to counts/min./gm. of tissue.
† Brains were partially perfused with formalin at autopsy and the meninges were removed.
Accumulation of RIHSA by Intracranial Tumours

Fig. 1. Concentration of RIHSA in glioblastomas (11 cases), meningiomas (12 cases) and normal brain (6 cases) from 5 to 520 hours after injection. The broken line represents the estimated concentration.

of all tissues examined. This demonstrates the relative impermeability of the normal brain to intravenously injected RIHSA. Table 3 also demonstrates that the glioblastomas accumulated more RIHSA than many other tissues.

c) Comparison of the Uptake of RIHSA in Different Classes of Neoplasms and Other Lesions of the Nervous System. The analysis of the radioactivity of 50 lesions of the nervous system is shown in Figs. 1–4. The data on counting and the period from 5 to 520 hours after the injection of RIHSA have been plotted logarithmically. Only cases which had received 1 injection of RIHSA-131 are plotted, and in all cases the counting data have been standardised to a dose of 350 μc. All the lesions were removed at operation except 1 intracerebral haematoma (at 360 hours, Fig. 4), which was an autopsy specimen. Three of the neoplasms, 1 meningioma (at 16 hours, Fig. 1), 1 metastatic tumour from breast (at

Fig. 2. Concentration of RIHSA in metastatic tumours (7 cases) and astrocytomas (3 cases) from 5 to 520 hours after injection.

Fig. 3. Concentration of RIHSA in whole blood (7 cases), acoustic neuromas (4 cases) and pituitary tumours (3 chromophobe adenomas, 1 mixed adenoma and 1 primary carcinoma) from 5 to 520 hours after injection. The broken lines represent the estimated concentrations.

Fig. 4. Concentration of RIHSA in various neoplastic and non-neoplastic lesions of the nervous system from 5 to 520 hours after injection.
8 hours, Fig. 2) and 1 ependymoma (Fig. 4), were spinal, and 1, the malignant neurofibroma (Fig. 4), was removed from the thigh. The concentration of RIHSA in whole blood is seen in Fig. 3, and the concentration of RIHSA in normal brain is seen in Fig. 1.

There were not enough acoustic neuromas and pituitary tumours studied to draw uptake curves over the entire 5- to 520-hour interval. Therefore, portions of these two curves (indicated by broken lines) were inferred from the general shape of the other uptake curves. Since only 3 astrocytomas were studied, no uptake curve for them could be drawn. The paucity of points plotted for normal brain (Fig. 1) indicates how seldom normal brain was found in tissue removed surgically. The presence of haemorrhage, oedema or softening altered the radioactivity of specimens of brain, and most operative specimens of cerebral tissue obtained during the course of this study contained such areas.

For comparison with the neoplasms, several other lesions are shown in Fig. 4.

In general, glioblastomas and metastatic tumours accumulated most RIHSA, followed by meningiomas and acoustic neuromas. Next came the pituitary tumours and last the astrocytomas. The astrocytoma removed 36 hours after injection was a malignant astrocytoma, but the concentration of RIHSA in this tumour was related more closely to that of the 2 benign astrocytomas, removed at 45 and 192 hours (Fig. 2), than to the glioblastomas (Fig. 1). The ependymoma of the spinal cord (Fig. 4) took up about as much RIHSA as the astrocytomas. The wall of an epidermoid cyst and the wall of a chronic brain abscess (Fig. 4) concentrated as much RIHSA as many of the neoplasms.

The highest count obtained in any tumour after 1 injection of 330 μc. of RIHSA was in a metastatic papillary carcinoma of unknown origin removed 64 hours after injection (Fig. 2). The astrocytoma removed 192 hours after injection (Fig. 2) contained the lowest amount of RIHSA of any tumour studied.

d) **Interpretation of the Uptake Curves.** The initial upward phase of the curves drawn for the neoplasms lasts for at least 24 hours after injection. This is indicative of a gradual transfer of RIHSA across the capillary walls of the tumours. Only a few patients were operated upon in the first 24 hours after injection, and therefore it is not known for certain which tumours accumulated RIHSA most rapidly. A provisional calculation obtained by measuring the slope of the initial upward phase of the curve suggests that, in the 8- to 24-hour interval, the glioblastomas took up RIHSA at the fastest rate, followed by the metastatic tumours and then the meningiomas.

The curves reach their maxima between 24 and 80 hours: the meningiomas at about 24 hours and the glioblastomas at about 80 hours (Fig. 1). The curve of metastatic tumours (Fig. 2) reaches its maximum at a time midway between those of the meningiomas and the glioblastomas.

After reaching the maxima, the radioactivity of the tumours declines parallel to the decreasing radioactivity of blood (Fig. 3). No tumour became more radioactive than blood at any time.

The relationship between the radioactivity in blood and tumour is seen in Fig. 5. The concentrations of RIHSA in 7 metastatic tumours (the same tumours that are shown in Fig. 2) were calculated as a percentage of the concentrations of RIHSA in the blood and plasma at the time the tumours were removed. The percentage of uptake of each tumour is plotted against the elapsed time between the injection of RIHSA and operation.

e) **Radioactivity and the Detection of Lesions**
by External Scanning. Table 4 shows that a relationship exists between the radioactivity of a tumour and its detectability by scanning. The numerous factors that influence the correlation are beyond the scope of this paper. They will be considered in a future clinical report.

f) Uptake of RIHSA by Necrotic Neoplastic Tissue. Large areas of necrotic tissue were found in 13 tumours. These necrotic areas were examined for radioactivity by counting and by autoradiography and compared with adjacent viable neoplastic tissue. Areas of recent necrosis were almost invariably more radioactive than living tumour (Fig. 6) whatever the pathological type, but necrotic areas which had become organized were in most instances less radioactive than viable tumour.

g) Uptake of RIHSA by Stroma and Parenchyma of Tumours. Autoradiography showed that the stroma usually became more radioactive than the parenchyma. This could be demonstrated more easily in secondary than in primary tumours because of greater abundance of stroma in secondary tumours (Figs. 7 and 8).

h) Uptake of RIHSA by Cysts. The cystic fluid of most tumours—and in particular the fluid of the cystic gliomas examined later than 1 hour after injection—was more radioactive than the neoplastic tissue itself (Fig. 9). It was noted in gliomas that the clear pale yellow cystic fluid which solidified on standing was highly radioactive, while in a metastatic carcinoma the turbid fluid from a cyst was less radioactive than the neoplastic tissue (Table 5).

i) Uptake of RIHSA in Cerebral Oedema. Oedematous brain was often more radioactive than the adjacent tumour (Fig. 10). It was also found (Fig. 6) that oedematous white matter was more radioactive than cortex; this presumably was because the oedema in white matter was more intense than in cortex, for in numerous autoradiographs the abrupt change from high to low radioactivity coincided with the junction of oedematous white matter with less oedematous cortex.

j) Uptake of RIHSA in Intracerebral

<table>
<thead>
<tr>
<th>Neoplasms in Decreasing Order of Radioactivity</th>
<th>Result of Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastomas</td>
<td>Positive</td>
</tr>
<tr>
<td>Metastatic tumours</td>
<td>9</td>
</tr>
<tr>
<td>Meningiomas</td>
<td>4</td>
</tr>
<tr>
<td>Acoustic neuromas</td>
<td>8</td>
</tr>
<tr>
<td>Pituitary tumours</td>
<td>1</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>0</td>
</tr>
<tr>
<td>* The astrocytomas which were detected contained large amounts of highly radioactive cystic fluid (see Table 5, Cases 59 and 28).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neoplasms in Decreasing Order of Radioactivity</th>
<th>Result of Scan</th>
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</thead>
<tbody>
<tr>
<td>Glioblastomas</td>
<td>Positive</td>
</tr>
<tr>
<td>Metastatic tumours</td>
<td>9</td>
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<tr>
<td>Meningiomas</td>
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<td>Acoustic neuromas</td>
<td>8</td>
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<tr>
<td>Pituitary tumours</td>
<td>1</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>0</td>
</tr>
<tr>
<td>* The astrocytomas which were detected contained large amounts of highly radioactive cystic fluid (see Table 5, Cases 59 and 28).</td>
<td></td>
</tr>
</tbody>
</table>

Haemorrhage. In the 6 cases of non-neoplastic intracerebral haemorrhage studied the radioactivity of the haematoma has been variable, some showing as much activity as tumours, others showing very little (Fig. 4). The oedema surrounding a haematoma would often show more activity than the haemorrhage itself (Fig. 11).

k) Uptake of RIHSA by Tissue Cultures of Tumours. The cultures of glioblastomas and acoustic neuromas showed the greatest uptake of RIHSA, and an ependymoma showed the least of all (Fig. 12). There are some uncertainties in the technique of tissue-culture autoradiography, and therefore, the results must be interpreted with caution. For example, a sparsely dense accumulation of silver grains covering cells and coverslip alike may be obtained unless the excessive albumin is washed away before the fixative is added. The increased background of silver grains in Fig. 12 is explained in this way. Also, even with repeated washing before fixation, the location of the tracer cannot be identified. It may be adsorbed on to the surface of the cells or incorporated into their substance.

Discussion

Most of the radioactivity in the tissues (except thyroid) is probably attributable to the accumulation of RIHSA. Some may be the result of free radioiodine or radioiodinated degradation products of RIHSA.
(chiefly radioiodotyrosine) which either were present in the injected solution or were produced in vivo from the metabolism of RIHSA. We tried to ensure that the RIHSA was free from impurities and was not likely to be degraded quickly in vivo. It is known that RIHSA of high specific activity and low concentration in albumin may be degraded rapidly, releasing much free radioiodine in vitro and in vivo. The same may occur with older preparations of RIHSA and in those not refrigerated. Therefore, we used

Fig. 6. (a) Coronal section of brain from a patient who died 5 days after the partial excision of a glioblastoma multiforme. The patient received 2 injections of RIHSA-111 10 days and 1.5 days before death. Thickness of section, 1 cm.

<table>
<thead>
<tr>
<th>Area</th>
<th>Tissue</th>
<th>Radioactivity in c./min./gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Tumour—necrotic and viable</td>
<td>7,896</td>
</tr>
<tr>
<td>B</td>
<td>Tumour—necrotic and viable</td>
<td>11,475</td>
</tr>
<tr>
<td>C</td>
<td>Tumour—mostly viable</td>
<td>2,490</td>
</tr>
<tr>
<td>D</td>
<td>Tumour—invading corpus callosum</td>
<td>1,607</td>
</tr>
<tr>
<td>E</td>
<td>White matter—occasional tumour cells around petechial haemorrhages. Early infarction and oedema of white matter dorsal and ventral to the haemorrhages</td>
<td>10,673</td>
</tr>
<tr>
<td>F</td>
<td>White matter—slight oedema, no tumour</td>
<td>1,945</td>
</tr>
<tr>
<td>G</td>
<td>Cerebral cortex—no tumour</td>
<td>470</td>
</tr>
<tr>
<td>H</td>
<td>Haematoma</td>
<td>2,299</td>
</tr>
</tbody>
</table>

(b) Corresponding contact autoradiograph. Note radioactivity of necrotic tumour compared with non-necrotic tumour. Note radioactivity of area E; in the superior frontal convolution dorsal to the petechial haemorrhages there were oedema and early infarction. There is an abrupt change in radioactivity at the junction of cortex and white matter. The nonradioactive “island” in the superior frontal convolution is cerebral cortex which cannot be recognized in (a). The external surface of the section is outlined on the autoradiograph because of the radioactivity of the blood in the vessels of the subarachnoid space.
Accumulation of RIHSA by Intracranial Tumours

67

FIG. 7. (a) Metastatic papillary carcinoma of unknown primary site removed from cerebellar hemisphere 64 hours after the injection of RIHSA-1131. A, B and C are areas of vascular, oedematous connective-tissue stroma bordered by neoplastic cells. D is an area of recent necrosis which contains numerous polymorphonuclear cells. E and F are recent haemorrhages probably caused by operative trauma. Thickness of section, 20 μ. Haematoxylin and eosin, ×12.

(b) Corresponding contact autoradiograph. The high uptake of RIHSA in stroma of tumour is seen at A, B and C. The uptake in necrotic tissue at D is as high as in viable stroma. The haemorrhage at E may contribute to the radioactivity of this area of stroma, although the haemorrhage at F appears to be no more radioactive than the surrounding tissue.

preparations of low specific activity (less than 500 μc./ml.), and with a concentration in albumin greater than 5 mg./ml. Fresh shipments were obtained weekly and were kept at 5°C. Even with these precautions, however, we cannot be certain that the radioactivity in the specimens was ascribable only to RIHSA. We have not performed chemical

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Hours After Injection of RIHSA</th>
<th>Comparative Radioactivity of Cystic Contents to Solid Tumor Expressed as Cystic Activity in c./min./gm.×100</th>
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<tbody>
<tr>
<td>108</td>
<td>Glioblastoma</td>
<td>1</td>
<td>56.0</td>
</tr>
<tr>
<td>108</td>
<td>Glioblastoma</td>
<td>120</td>
<td>189.5</td>
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<td>31</td>
<td>Glioblastoma</td>
<td>17</td>
<td>190.2</td>
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<tr>
<td>52</td>
<td>Glioblastoma</td>
<td>168</td>
<td>388.2</td>
</tr>
<tr>
<td>59</td>
<td>Astrocytoma</td>
<td>36</td>
<td>319.9</td>
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<td>28</td>
<td>Astrocytoma</td>
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<tr>
<td>107</td>
<td>Metastatic carcinoma</td>
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<td>95.2</td>
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<tr>
<td>113</td>
<td>Acoustic neuroma</td>
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<td>226.7</td>
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<td>82</td>
<td>Haemangioendothelioma</td>
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<td>79</td>
<td>Epidermoid cyst</td>
<td>72</td>
<td>2.1</td>
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Fig. 8. Microscopic autoradiograph of section adjacent to that in Fig. 7a. The uptake of RIHSA by the stroma (left) is higher than the uptake by the neoplastic cells. The number of grains over the neoplastic cells is not greater than the number in the background (right). Thickness of section, 10 μ. Haematoxylin and eosin, ×730.

Fig. 9. Microscopic autoradiograph of a cystic glioblastoma multiforme removed 45 hours after injection of RIHSA-IP. There are numerous grains over the cystic fluid and relatively few over the neoplastic tissue. Thickness of section, 10 μ. Haematoxylin and eosin, ×775.
Fig. 10. Microscopic autoradiograph of metastatic epidermoid carcinoma of cervix and adjacent cerebral tissue removed from the parietal lobe 42 hours after injection of RIHSA-I\(^\text{132}\). Compression by the tumour (left) caused severe oedema and gliosis of surrounding brain (right). There is a distinct boundary between tumour and white matter and the latter was not infiltrated by tumour cells. The concentration of grains over the white matter is about twice that over the neoplastic tissue. Thickness of section, 5 \(\mu\). Haematoxylin and eosin, \(\times 775\).

Accumulation of RIHSA by Intracranial Tumours

This assumption may not be wholly justified\(^9,11,22,31\). Bell\(^4\) measured the radioactivity of 'normal brain' and cerebral tumours after the injection of RIHSA. One can calculate from his data that 24 hours after injection Grade IV astrocytomas took up 1.75 to 12.5 times more RIHSA than did normal brain. Our ratios of uptake were higher (Table 1), perhaps because our patients were all examined at least 44 hours after the administration of RIHSA. Bell found the radioactivity of brain as high as 11 per cent that of plasma; in our studies the radioactivity of normal brain was never more than 1 to 2 per cent that of plasma. Rothschild et al.\(^39\) found a similarly low figure. The discrepancy between Bell's findings and ours may be explained by the presence of cerebral oedema in his samples of 'normal brain' especially if they were taken from white matter close to the tumours.

Table 3 shows the radioactivity of various separations on the specimens and we do not know how much radioactivity was caused by free radioiodine or radioiodotyrosine. Other evidence suggests that each of the latter is probably responsible for only a small amount of the total radioactivity of the tumours. Bell\(^4\) found that almost all the radioactivity of tumours from patients injected with RIHSA was precipitated with 5 per cent trichloroacetic acid, which indicated that very little free radioiodine was present. Similarly, there is evidence that radioiodotyrosine cannot be utilized for protein biosynthesis either by normal cells\(^21,47\) or by neoplastic cells.\(^40\) The amount of radioiodotyrosine accumulated by the stroma of tumours is unknown.

In order to understand some of the findings with RIHSA we have assumed that its behaviour in the body, including its uptake in tumours, is similar to that of naturally occurring human serum albumin.
autopsy specimens. It is seen from the low concentration of radioactivity in brain that an effective barrier exists to exclude RIHSA from normal brain. This is the most important factor in the detection of brain tumours with RIHSA, for if a tumour accumulates even a modest amount of RIHSA it will stand out sharply against the background of the relatively nonradioactive normal brain. Other organs are not suitable for scanning with RIHSA because, unlike the brain, they do not discriminate against RIHSA; in them, the tumour lies concealed in a bed as radioactive as itself.

Table 3 and Figs. 1–4 show the range in radioactivity of human tissue after the injection of RIHSA. The accumulation of RIHSA in brain tumours depends, as in any other tissue, on the metabolic activity of the cells, the permeability of the vessels, and the spatial arrangement of the components of the tissue. The detection of a tumour by scanning depends, in addition, on its lying in the relatively low radioactive field of the brain.

The high radioactivity of the thyroid (Table 3) indicates that the blocking dose of Lugol’s solution was inadequate. More recently we have increased the dose of Lugol’s iodine to 10 minims 3 times a day for 24 hours before the administration of the tracer, and continuing for 1 week, as recommended by Beierwaltes et al.³

The curves (Figs. 1–4) show that there are differences in the uptake of RIHSA, both in rate and amount, between the various classes of intracranial neoplasms. However, there is considerable overlap, especially among the glioblastomas, meningiomas, and metastatic tumours. Schlesinger et al.⁴ thought that by repeated scanning after the injection of RIHSA it was possible “to predict the exact nature of the pathologic lesion by its time of appearance” on the scan. Our findings suggest that the patterns of uptake in the different classes of intracranial neoplasms are not distinctive enough to permit the diagnosis of the pathological nature of a lesion on the basis of the RIHSA scan. Indeed, RIHSA is such a nonspecific tracer that a focus of radioactivity on the scan may not even be caused by a neoplasm. The chronic abscess (Fig. 4) accumulated as much RIHSA as many of the neoplasms and gave a positive scan indistinguishable from them. A scan which becomes positive soon after the administration of RIHSA is likely to be caused by a very vascular lesion such as an arteriovenous malformation, but 1 glioblastoma was detected by the scan after only 2 hours.

The high concentration of RIHSA in most of the cystic fluids that we encountered did not confirm the experience of most au-
thors. They reported that cystic lesions are frequently not detected by RIHSA scanning and, further, that a large cyst may be so inactive that brain in the corresponding area on the opposite side may by comparison be more active. This may be interpreted wrongly, and the lesion diagnosed on the normal side. With lesions such as the epidermoid cyst (Table 5 and Fig. 4) it is understandable how this could occur. The cyst occupied most of one frontal lobe, but was not detected by brain scanning. The wall accumulated an appreciable amount of RIHSA but the inert cystic contents had taken up very little. Di Chiro reported findings similar to ours, i.e. that cystic astrocytomas were easier to detect than solid astrocytomas. Schlesinger et al. found that cystic lesions did not show up well on the scan until 48 hours after injection. The time of scanning may be important, because there is a tendency for the fluid to accumulate RIHSA at a slower rate than does the neoplasm (Table 5). This is seen most clearly in Case 108 which was sampled twice. With the exception of the epidermoid cyst, the only cystic fluid sampled after 24 hours which was less radioactive than the neoplasm was from the haemangioendothelioma. Shy et al. also found less radioactivity in the cystic fluid of a haemangioblastoma than in the solid portion of the tumour, but they did not record the interval between the injection of RIHSA and sampling.

The uptake of RIHSA in necrotic portions of tumours tended to be higher when the necrosis was recent. Lee and Olszewski found a high concentration of RIHSA in necrotic brain, especially in the first 24 hours after experimental lesions had been made. Rothschild et al. found that the concentration of RIHSA in recently infarcted myocardium was about 50 per cent higher than in noninfarcted myocardium. Other reports conflict. It is possible that the differences are the result of the varying ages of the lesions. We have found that areas which have undergone necrosis only lately accumulate RIHSA more readily than older necrotic lesions.

A high uptake of radioiodinated albumin and fluorescein-labelled albumin has been reported in oedematous white matter following experimental lesions. Shy et al. found that the radioactive focus seen on the RIHSA scan in a case of glioblastoma was larger than the size of the tumour seen at autopsy and thought that “some of the activity is probably dependent upon oedema.” On the other hand McAfee and Taxdal did not find a high uptake in oedematous areas surrounding neoplasms. Our data on counting and autoradiographs have shown a high uptake of RIHSA in oedematous brain (especially white matter) associated with brain tumours. In some cases the radioactivity of oedematous brain exceeded that of the adjacent tumour. The implications of these findings are twofold. The added radioactivity from an area of cerebral oedema may allow detection of a tumour which accumulates only a small amount of radioactivity or which is small in size; and the adjacent cerebral oedema may interfere with the accurate localization of a tumour for biopsy or excision by enlarging the area of radioactivity.

The considerable variation in the uptake of RIHSA found in intracerebral haemorrhages is probably caused by many factors, such as the age of the lesion, the interval between injection and sampling, and the presence or absence of active bleeding while RIHSA is in the blood stream. We have found that oedematous or necrotic brain surrounding intracerebral haematomas may contain higher concentrations of RIHSA than the haematoma itself. This may contribute to the detection of the lesion by scanning. Feindel et al. measured the uptake of RIHSA in 1 intracerebral haematoma and found it to be low, and in another they found that the RIHSA scan showed a much smaller lesion than was found at operation. They concluded that RIHSA “does not penetrate, to any great extent, into an inert blood clot within the brain.” Our findings support this view.

The resolution obtained with autoradiography was not precise enough to distinguish intracellular from interstitial tracer. It has
Accumulation of RIHSA by Intracranial Tumours

FIG. 12. Microscopic autoradiographs of neoplastic cells in tissue culture. After growth was established each culture was incubated for 48 hours in a medium containing 2.5 μc. per ml. of RIHSA-I\(^{125}\).

(a) Culture of glioblastoma multiforme. A dense accumulation of grains is seen over the cell bodies. Haematoxylin and eosin, ×1400.

(b) Culture of acoustic neuroma. There are fewer grains over these cells than over those in (a). Haematoxylin and eosin, ×1100.

(c) Culture of spinal ependymoma. There is almost no accumulation of grains over these cells as compared with those in (a) or (b). Haematoxylin and eosin, ×1100.

been shown\(^2\) that the low-energy electrons of I\(^{125}\) give better resolution for autoradiography than the beta particles of I\(^{131}\), and it was thought that the use of this isotope would allow better localization of RIHSA. However, in our study thick sections had to be used because of the low radiation flux in the tissues; and precise autoradiographic localization of RIHSA demands thin sections. Some of the difficulties in interpretation of tissue-culture autoradiographs have already been mentioned. We could not distinguish between RIHSA fixed to the surface of the cell by the process of fixation, and RIHSA incorporated into the cell or biologically absorbed on to its surface. The adsorption and incorporation of RIHSA has been demonstrated in suspensions of Ehrlich ascites tumour cells,\(^4\) and it is possible that the same phenomenon takes place in brain-tumour cultures. However, even if we could be sure of the mechanisms of the uptake of RIHSA by the cultured cells, we should hardly be justified in assuming that the same occurred in vivo. Furthermore, while we believe that the cells observed in our cultures are derived from the neoplastic cell type, we cannot always be certain of this, and we do not feel as confident as some that cells in cultures of tumours can always be identified accurately.\(^{35}\)

Other studies have shown that albumin can be taken up intracellularly by neoplastic and non-neoplastic cells. Busch et al.\(^7,8\) found that C-14 labelled albumin was incorporated intracellularly in the Walker tumour of rats. Klatzo et al.\(^23,24\) demonstrated the presence of intracellular fluorescein-labelled albumin
in glial cells and neurons in areas adjacent to experimental cold lesions. It is, therefore, probable that at least some of the RIHSA taken up, in our studies, by tumours and other cerebral lesions was incorporated into the cells.

Conclusions

A number of factors must be considered in any attempt to account for the uptake of RIHSA in variable amounts by different tissues and lesions.

a) Increased vascularity. The high concentration of RIHSA in blood ensures that any lesion or tissue with a high content of blood will also have a high content of RIHSA and that the concentration of RIHSA will reach a peak soon after the administration of RIHSA. The converse is not necessarily true. Those meningiomas that showed on microscopic examination a relative paucity of vessels had a high content of RIHSA.

b) Increased capillary permeability. It has long been recognised that there may be increased permeability of capillaries in tumours. This property of capillaries in tumours is, in numerous reports, held to account for the recovery from tumours of diverse substances injected into the blood stream.

c) Active metabolic uptake of RIHSA. It is improbable that RIHSA takes part in metabolism of cells as actively as other tracers in ionic form have been shown to do. However, the concentration of RIHSA in cystic fluid in 1 glioblastoma (No. 52, Table 5) exceeded that of plasma, and the radioactivity of the solid part of the tumour exceeded that of all other tissues examined except thyroid. This suggests that the RIHSA has been actively drawn into the metabolism of the tumour, although simple retention of RIHSA in cyst and tumour after blood levels had fallen may account for this finding.

d) The blood-brain barrier. The role of increased permeability of capillaries has already been discussed. The importance to gamma encephalography of the integrity of the barrier in normal brain has also been stressed. Perhaps a breakdown of the barrier can be postulated to account for the increased radioactivity of cerebral oedema, recent infarction and the earlier stages of cerebral abscess.

e) The interstitial space. Of the total plasma albumin in the body about 50 per cent is intravascular, 50 per cent interstitial and only a small amount intracellular. Our findings suggest that the distribution of RIHSA in brain lesions follows a similar pattern. The exact volume of the interstitial space in human brain tumours is not known. Matthews and Molinaro have shown that the extracellular space (bromide space) in fibrosarcomas of rats is 44 per cent. Electron-microscopic studies on brain tumours from humans have shown that some tumours have an appreciable interstitial space. We suspect that the uptake of RIHSA in different classes of brain tumours is related to the volume of their interstitial spaces. However, there is insufficient evidence available to be sure of this.

This theory of the distribution of RIHSA may explain why the specimens of oedematous brain contained so much RIHSA. If the fluid of oedema were located interstitially it could be expected that it would accumulate large amounts of RIHSA. Electron-microscopic studies of oedematous grey matter have shown that fluid of oedema is held within glial cells. On the other hand, electron-microscopic studies of oedematous white matter have shown a significant enlargement of the interstitial space. Klatzo et al. found in oedematous white matter large amounts of fluorescein-labelled albumin which they thought was partly extracellular. Cumings found a large amount of protein which migrated like serum albumin in starch-gel electrophoresis in oedematous brain surrounding tumours. This was found more often in oedematous white matter than in grey matter. Cumings thought that this protein was located extracellularly. From their studies on cerebral oedema Aleu et al. concluded that the "ultrastructural aspect of cerebral fluid accumulation varies according to the species, the region of the brain, and the
cause of the edema.” It may also be that the composition and physiological properties of the fluid of oedema depend upon species, site and cause. In our studies of oedematous human brain adjacent to brain tumours, the high uptake of RIHSA by white matter suggests that the fluid from oedema here behaves like interstitial fluid and is probably located mainly interstitially.

Summary
1. The fate of RIHSA has been studied in cerebral tumours and other intracranial lesions by measuring the radioactivity of samples of tissue in a well-counter and by autoradiography. Similar techniques were applied to tissue cultures of tumours.
2. It is suggested that the distribution of RIHSA in brain tumours and other lesions follows the same pattern as the distribution of total plasma albumin in the body.
3. The mechanisms considered to be important in the uptake of RIHSA by neoplasms are vascularity, abnormally permeable vessels and a large interstitial space. There may be active incorporation of RIHSA into the metabolism of some tumours.
4. Areas of adjacent cerebral oedema, necrosis of tumour or cystic fluid may accumulate more RIHSA than solid viable neoplastic tissue.

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References