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COLLOIDAL RADIOALBUMIN AGGREGATES FOR ORGAN SCANNING

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by

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ABSTRACT

The exhibit shows that colloidal aggregates (10-20 mμ) of human serum albumin I^{131} may be used safely by intravenous injection to perform photoscans of the heart, liver, spleen, stomach and salivary glands in man. Large particle size suspensions (10-50 μ) of the same material are being investigated experimentally in animals for scanning the lungs after intravenous injection and the brain following injection into an internal carotid artery. The advantages of this test material are the relatively low radiation exposure to the target organs and the number of organs that may be examined. Radiation exposure is low because of the rapid turnover in the target organs and removal from the body mainly by urinary excretion within 72 hours.

The mechanism of liver-spleen localization with this organic colloid is the same as for inorganic colloidal radiogold^{198}, namely, rapid removal from the blood by the phagocytic cells of the liver and spleen. However, in contrast to the inorganic colloid, which remains in the phagocytic cells permanently, albumin is digested by proteolytic enzymes and the I^{131} label is set free to re-enter the general circulation. With the thyroid blocked, the I^{131} is excreted mainly in the urine as free iodide together with other labeled albumin degradation products, such as tyrosine and peptides. The calculated radiation dose to the liver is at least 100 times less from colloidal albumin I^{131} than from an equal dose of Au^{198}.

PREPARATION OF HUMAN SERUM ALBUMIN SUSPENSIONS BY HEAT TREATMENT AND pH ADJUSTMENT

(a) Small size colloids (10-20 mμ).

1. Prepare from commercially available human serum albumin I^{131} a 1% solution in physiological saline in a sterile multiple injection type rubber stoppered bottle.
2. Adjust pH to 10 ± 0.2 using sterile 0.2 normal NaOH (check with pH meter).
3. Immerse bottle in water bath at 70° C. for 20 minutes and agitate continuously.
4. Repeat step #3 with water bath at 79° C. for 15 minutes.
5. Cool to room temperature and add sterile 0.2 normal HCl sufficient to reduce the pH to 7.5 to 8.0.
6. Test for sterility and store in refrigerator at 5° C.

Note: The preparations usually contain < 0.2% free I^{131}. Use pyrogen free saline containing 0.9% decyl alcohol as a preservative.

(b) Large size suspensions (10-50 μ).

1. Same as for (a).
2. Adjust pH to 5.7 ± 0.1 using 0.2 normal HCl.
3. Immerse in water bath at 75° C. for 20 minutes and agitate continuously.
4. Cool to room temperature immediately and store in refrigerator at 5° C.
5. Remove supernatant and replace with 1/2 volume of sterile normal saline.
6. Test for sterility.
7. Shake well before using to resuspend large particles.

PARTICLE SIZE AND ORGAN DISTRIBUTION

The work of Dobson and Jones indicate that particle size of colloidal materials is a determining factor in organ distribution. Large size suspensions ($> 1\ \mu$) accumulate in high percentage of injected dose in the lungs. Intermediate size colloids ($0.1 - 1\ \mu$) localize primarily in the liver and spleen. Small size colloids ($< 0.1\ \mu$) remain in the circulation for much longer times and finally accumulate in relatively high concentration in the bone marrow.

Recent studies in this laboratory verify Dobson's observations. Specifically, large size albumin aggregates ($5-50\ \mu$) disappear rapidly from the blood of dogs and rabbits with a half-time of approximately 30 seconds. After 30 minutes 80-90% of the intravenously injected dose is found in the lungs. With the small size colloids of human serum albumin (10-20 mμ) 80-90% of the injected dose is found in the liver and spleen within 15-30 minutes. The turnover rate of the small size aggregates in the liver is rapid. The half-time in the liver following intravenous injection of trace quantities in man is approximately 20 minutes. This rapid rate of proteolytic digestion and free iodine release from the liver can be slowed by a factor of 2 or more by the simultaneous administration of unlabeled aggregates of the same material in doses of 3-5 mg/kg. With simultaneous administration of unlabeled albumin aggregates the levels of liver radioactivity remain relatively constant from 15-60 minutes, thus permitting organ scanning. The turnover rate in the spleen as determined by repeated photoscanning indicates a slower turnover than in the liver. There is some evidence that particles in the $5-10\ \mu$ range are selectively removed from the blood by the spleen. This aspect is now under investigation.

Preliminary data on turnover of large particle size aggregates of albumin in the lungs indicate that the label is released at a considerably slower rate. The half-time disappearance of free iodine from the lungs is ~120 minutes. Likewise, when the large size particles are injected into the internal carotid artery of the dog, a high percentage (60-90%) is retained initially in the ipsilateral side of the brain and the half-time of I^{131} release is several hours.

EXTRA THYROIDAL SITES OF IODIDE METABOLISM

Free I^{131} is largely released from the liver after 3-5 hours. For the next few hours it is possible to scan the stomach and salivary glands, because iodide recycles continuously through these organs. These organs concentrate iodide to about the same degree (about 30:1) as thyroid tissue. In addition, iodide is not reabsorbed by the stomach but is rapidly absorbed from the small bowel. Excess iodide ingestion which blocks thyroid uptake does not interfere with the iodide concentrating capacity of either the stomach or the salivary glands. Therefore, these organs are scanned after the thyroid is blocked to avoid its exposure to radiation unnecessarily.

The concentration of I^{131} in the epithelial lining of the stomach provides the primary mechanism for stomach visualization by photoscanning. I^{131} in the gastric juice is of secondary importance because the organ is visualized equally well during continuous gastric suction. Another point of practical importance is the complete disassociation between iodide and chloride secretion by the stomach. For example in pernicious anemia with achlorhydria, iodide is still secreted and the stomach may be scanned following injection of either sodium I^{131} or colloidal albumin I^{131}.

Certain gastric malignancies are reported by Baptista of Lisbon, Portugal, to selectively concentrate I^{131}. So far no instances of this kind have been found. In our experience, gastric carcinomas concentrate less I^{131} than do surrounding gastric tissues. However, if some tumors selectively concentrate I^{131} then small deep-seated metastatic lesions to the liver would be easily detectable by standard photoscanning techniques. Also the primary lesion could be distinguished from the usual gastric carcinoma and/or from peptic ulcer by scanning methods.

The salivary glands may be visualized by photoscanning immediately following intravenous injection of sodium I^{131} (100-300 μc) and for several hours thereafter because of similar selective concentration in these tissues and the continuous iodide recycling process. They can be visualized 2-4 hours after intravenous injection of similar doses of human serum albumin I^{131} on the same principle just described for gastric scanning with this agent. In addition, the salivary glands are readily visualized for several days following therapy doses (10-100 mc) of I^{131}. They must and can be distinguished from thyroid metastases by their location, shape and size. It should be noted also that the liver may be visualized following therapy doses of I^{131} by its content of endogenous thyroxine I^{131}.

MULTIPLE ORGAN SCANNING WITH ALBUMIN I^{131} AGGREGATES

The exhibit presents the equipment and test agents used for scanning the heart, liver, spleen, stomach, salivary glands, lungs and brain. Under basic studies, the advantage of photo vs. dot scanning of large organs is demonstrated with the international phantom and the Alderson human abdominal phantom. Deep-seated tumors of small size (< 2 cm. diameter) may be visualized easily provided tumor radioactivity exceeds that of the surrounding tissue by factors of 5-10. On the other hand, radionegative tumors, which are far the most common, are much more difficult to localize unless they exceed 2 cms. in diameter and are located close to the liver surface.

Electronmicrographs of the small particle size albumin aggregates are demonstrated. They show that the individual particles are quite uniform in size varying from 10-20 mμ. The largest clumps of such particles seldom exceed 0.2 μ. Photomicrographs of the large particle size albumin suspensions demonstrate that the individual particles produced by heating at low pH measure 1.0 to 1.5 μ or about 100 times larger than those produced by heat at pH 10.0. Aggregates of these micron size particles range in size from 1.0 - 50 μ with a mean of $\sim$ 20 μ.

SCANNING TECHNIQUE WITH SMALL PARTICLE SIZE COLLOIDS

To visualize the heart, scanning is begun immediately following an intravenous injection of 250 μc of tracer which is administered together with 4 mg/kg loading dose of unlabeled particles of the same size. The liver and spleen may be scanned between 15 and 75 minutes after injection. During this time a large fraction of the injected dose accumulates in these organs.

Typical photoscans are exhibited of the normal liver, the liver and spleen in compensated and uncompensated Laennec's cirrhosis and in Hodgkin's disease and the liver with abscesses and with metastatic malignancy. The exhibit also shows other organs which may be visualized by photoscanning with human serum albumin I^{131} aggregates as test agents. The stomach becomes visible by photoscanning 3-5 hours after injection of 200-300 μc. The parotids, submaxillary and sublingual glands may also be scanned after the same interval. The mechanisms are described in a previous section of this reprint.

The stomach and salivary glands are nearly equally as well visualized following similar small doses of sodium iodide intravenously (200-300 μc). In all patients, the thyroid gland is blocked by prior administration of a few drops of a saturated solution of potassium iodide, orally.

EXPERIMENTAL APPLICATION OF LARGE PARTICLE SIZE RADIOALBUMIN AGGREGATES

The lungs may be scanned immediately after the intravenous injection of 50-100 μc of this test material. An example of a lung scan in the dog is exhibited. Similar scans were made in rabbits with the same test material. Serial lung scans and external monitoring indicate that I^{131} leaves the lung slowly with a half-time of approximately two hours. Thus the lungs may be scanned immediately and/or at any time during the first few hours post injection. Application of this test agent in man is being deferred pending complete investigation of the safety of such injections. Preliminary toxicity and histopathological studies in rabbits and dogs indicate that the LD_{50} values exceed the amounts required for human lung scans by at least 50-fold. Dogs and rabbits both tolerate repeated intravenous injections of large particle size material in doses 30 times in excess of those required for human scanning.

The brain may be scanned immediately following carotid injection of the large particle size material. The ipsilateral hemisphere is readily visualized by photoscanning for several hours post injection. The potential advantage of this type of test agent for brain scanning is its immediate localization in high concentration in one hemisphere of the brain. Therefore small quantities of radioisotope are required and radiation exposure to other organs is minimal.

ANTIGENICITY OF ALBUMIN AGGREGATES?

In man there is now considerable evidence that human serum albumin is not made antigenic by the amounts of heat used in its conversion to particulate aggregates. No anaphylactoid reactions have occurred during or after more than 1,000 intravenous injections, including 20 individuals who had multiple tests over periods varying from 3 weeks to 30 months. There were no positive skin reactions to the material in any of 70 patients tested three weeks to several months following potential sensitization. No albumin antibody was detectable in more than 50 patients following multiple injections of the test materials. The possibility of serious sensitivity reactions now appears to be remote especially when the material is used once in any one individual for scanning purposes. However, further clinical experience is needed to prove the complete lack of antigenicity of these agents when used repeatedly for months or years. Other evidence regarding the antigenicity of human serum albumin aggregates is their relatively low antigenicity and absence of anaphylactoid reactions when administered repeatedly to rabbits, mice and dogs. In these animal species, human serum albumin is many times less antigenic than egg albumin. Antibodies to the human serum albumin are produced more slowly and in far lower titer.

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