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ARGONNE CANCER RESEARCH HOSPITAL
950 EAST FIFTY-NINTH STREET • CHICAGO 37 • ILLINOIS

Semiannual Report to
THE ATOMIC ENERGY COMMISSION

MARCH 1955

LEON O. JACOBSON, M.D.
Editor

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THE DESTRUCTION OF THE PITUITARY GLAND IN METASTATIC
CANCER WITH YTTRIUM-90 PELLETS*

By

T. B. Rasmussen,[†] P. V. Harper, E. Yuhl,[‡] and D. M. Bergenstal^{**}

Recent encouraging results in attempts to cause the regression of metastatic tumors in man by altering the hormonal milieu of the body by the administration of hormonally active compounds or by removing hormone-producing glands has stimulated interest in the possible effect of total hypophysectomy in producing similar or more profound regression of metastatic tumors in man.

The role of the ovary and the adrenal in controlling the growth of some types of tumors of the breast and prostate gland has been described.⁽¹⁻³⁾ The pituitary gland produces trophic hormones that significantly affect the function of the ovary and adrenal. In addition, the pituitary is capable of producing other protein hormones that act directly upon other tissues. It is therefore possible that the hypophysis may be a significant controlling factor in the continued growth of certain neoplasms in man. Two of the protein hormones that may act directly upon neoplastic cells are growth hormone and prolactin. Thus, if the pituitary can be removed completely without significant hazard, such a procedure might be more effective in the long-term control of metastatic neoplasms in man than previous types of endocrine therapy. The fact that the time of tumor regression following oophorectomy and adrenalectomy may vary from a few months to several years may be explained by the increased function of accessory adrenal tissue, which is found frequently in such cases at autopsy. If ACTH and gonadotrophins can be removed by total hypophysectomy, the accessory endocrine tissues might not renew function.

In 1952, Perrault⁽⁴⁾ described a case of carcinoma of the breast with pulmonary metastasis in which hypophysectomy had been performed 2 months earlier. A general improvement of the patient's condition and an increase in weight resulted. In 1952, Shimkin⁽⁵⁾ and his collaborators reported the result of hypophysectomy in a patient with a malignant melanoma. The patient died from progression of the malignant tumor 2 months after the operation. At autopsy it was found that the hypophysis had been incompletely removed.

* Paper presented at the meetings of the Society of Neurological Surgeons, Chicago, May, 1955.

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Luft and Olivecrona,⁽⁶⁾ in 1953, reported that surgical hypophysectomy produced objective regression in patients with mammary cancer and prostatic cancer after simpler methods of treatment had no longer been effective and that the operation was well tolerated and comparatively free from danger. In some of the cases, small remnants of pituitary tissue were left in the sella turcica.

During the past 3 years, surgical removal of the hypophysis has been carried out at this clinic on nine patients who had advanced metastatic cancer. The clinical response has been uniformly poor as far as the malignancy was concerned. There was no significant beneficial effect on the course of the tumors of these patients following hypophysectomy. At autopsy, all of them had demonstrable significant amounts of residual pituitary tissue. In view of this experience, it was believed that if hypophysectomy was to be useful for the control of certain types of metastatic carcinoma, some procedure had to be developed that could ensure total hypophysectomy.

Development of a new technic for destruction of pituitary gland: Surgical hypophysectomy is performed through a right frontal craniotomy flap. The chiasm and pituitary are exposed by elevating and retracting the right frontal lobe. The pituitary stalk is clipped and divided, the diaphragma sellae incised, and the pituitary gland removed piecemeal by suction and curettage. The removal cavity is then swabbed thoroughly with Zenker's solution in an attempt to destroy any remaining tissue that has not been removed surgically. This method has some disadvantages. Retraction of the frontal lobe may be prolonged and increases the risk of postoperative central edema. Brisk bleeding from entry into the cavernous sinus may occur not infrequently. The use of corrosive solution so close to the optic nerves may be hazardous, and most important of all, in our hands, the hypophysectomy was never complete (Figure 1).

Figure 1. Photomicrograph (H & E) of the pituitary gland following surgical hypophysectomy showing a large amount of histologically normal residual tissue.

These considerations led us to seek a different approach for destroying the hypophysis. The injection of radioactive colloid material, such as CrPO_4 or colloidal gold (Au^{198}), into the pituitary gland, suggested itself as a possible alternative. This, however, was abandoned because we felt that there was too great a hazard of reflux of colloid along the needle tract and infundibular stalk producing damage to such adjacent structures as the optic nerves.

For this reason it was decided to use discrete radioactive sources such as those described by Kisielecki.⁽⁷⁾ With these, it was believed the location of the radiation fields could be better controlled. Yttrium-90 was chosen as the most suitable agent because of its strong β radiation (2.3 mev), short half-life (62 hr), and adequate activation cross section for thermal neutrons (1.24 barns). It was found that compressed pellets of Y_2O_3 powder could be sintered at 1650°C for an hour to produce solid ceramic pellets. These were formed as small cylinders, 1 to 3 mm in length, to fit into a 17-gauge needle (Figure 2). Activation of these pellets in a thermal neutron flux of $1 \times 10^{13} \text{ n/cm}^2/\text{sec}$ for 24

Figure 2. Seventeen-gauge shielded needle for implantation of Y^{90} pellets. (A pellet is shown just beyond the curved needle tip.)

hrs resulted in a specific activity of about $0.4 \text{ mc/mg/Y}_2\text{O}_3$ or 1 to 2 mc per pellet. Some difficulty was experienced with long-lived γ activity from rare earth contamination in the yttrium oxide. Terbium-160 was the principal offender. However, this contamination in the Y_2O_3 that we used* was not of significant magnitude. Handling the activated pellets

* Obtained through the kindness of Dr. Spedding, University of Iowa.

presented no problem because a centimeter of water offers complete shielding against the β radiation. It was found that the pellets had a tendency to chip slightly when handled, but this was prevented almost completely by coating them with a thin layer of lucite prior to activation.

A 2-mm lead shield, several cm long, was placed around the proximal end of a 4-inch 17-gauge spinal puncture needle (Figure 3). Under water, the pellet was placed in the needle behind the lead shield. This reduced the radiation level to less than 20 mr per hr at the operator's finger tips, and permitted the loaded needle to be handled without haste at the time of operation.

Figure 3. The curved needle with the lead jacket (lower) and the nylon stylet (upper) used to eject the pellets.

Considerable effort was devoted to measurement of the radiation dosage in the tissue surrounding the pellet.^(8,9) This was accomplished with reasonable precision, and isodose curves surrounding a representative pellet are shown in Figure 4.

A series of experiments was carried out in monkeys in which the hypophysis was reached through an anterior midline approach.⁽⁹⁾ Yttrium pellets of various sizes were inserted, and after various intervals, the animals were sacrificed and the resulting areas of destruction examined. These were uniformly found to be spherical volumes of complete necrosis, 4 to 8 mm in diameter, depending on the activity of the pellet, surrounded by apparently normal tissue. The zone of transition between normal and necrotic tissue was extremely narrow (Figure 5) (see also Figure 11). These findings are precisely what was anticipated from the isodose curves. The gradient of the radiation field is so steep that the transition from a cauterizing radiation dose to an insignificant dose must necessarily be sharp. The apparent dose level at which transition occurred varied somewhat due probably to variations in the tissue swelling, fixation artifact, etc. The point at which necrosis

Figure 4. Isodose curves (total dose in rep) around a 1.5-mc Y^{90} pellet in a lucite phantom.

Figure 5. Photomicrograph (H & E) through the hypophysis and hypothalamus of a monkey (X indicates location of 1.3 mc Y^{90} pellet) sacrificed 31 days after operation.

occurred in the hypophysis and various parasellar structures is shown below in Table 1.

On the basis of measurements taken on typical human pituitary glands, it was felt that the whole gland could be destroyed by 4 properly placed, 1-mc pellets without risk of ra-

Table 1

RADIATION DOSAGE REQUIRED TO PRODUCE NECROSIS

Anterior lobe of hypophysis	110,000 to 190,000 rep
Hypothalamus	60,000 to 120,000 rep
Optic chiasm or tract	60,000 to 140,000 rep
Oculomotor nerve	30,000 to 60,000 rep

diation damage to the neighboring structures.

Clinical application of yttrium-90 for the destruction of the human pituitary: After completion of the preliminary experiments on monkeys, the clinical application of this procedure was first undertaken in February, 1954. The patient was a 52-year-old woman who had been suffering from carcinoma of the cervix that had metastasized and failed to respond to radiation therapy. Four 0.88-mc yttrium beads were inserted into the sella. On the following day, a 67-year-old man, who had had widespread disseminated cancer of the prostate that had responded previously but only temporarily to castration and estrogen therapy, was subjected to the same procedure. Four 0.63-mc pellets were inserted into the sella.

The first patient had a smooth course after operation until the fifth day at which time she began to show evidence of diabetes insipidus. On the seventh postoperative day, an increasing paresis of the left third and fourth cranial nerves was encountered, and the lesion progressed to complete paralysis of these nerves. Roentgenographs of the skull in the lateral and stereo-axial projection were obtained and revealed that all 4 beads were located in the posterior half of the sella turcica. One bead had been placed well out to the left of the clinoid process. These roentgenographic findings explained the diabetes insipidus as resulting from incomplete destruction of the hypophysis, and the paresis of the left third and fourth cranial nerves from the misplaced pellet.

The second patient failed to respond after operation, remained comatose, exhibited episodes of hypotension and hypothermia, and expired on the fourteenth postoperative day.

The first patient was reoperated, and 2 additional pellets of 0.75-mc activity were inserted into the sella. Within 4 days after surgery, the diabetes insipidus, which had necessitated daily injections of 1 cc of pitressin, began to decrease. From that time until the time of her death 3 months later, no further evidence of diabetes insipidus was noted. Roentgenographs on the tenth postoperative day after the second operation revealed that both of the additional pellets lay in the anterior sella.

Roentgenographs that were taken on the second patient postmortem revealed that all 4 pellets were situated in the posterior half of the sella (Figure 6). It became apparent that, in the standard craniotomy position for exposure of the optic chiasm and sella turcica, the angle of approach almost precluded placement of the beads in the anterior half of the sella if a straight needle was used. The needles were then modified by curving the ends

(Figures 2 and 3).

Four patients were subsequently subjected to the procedure. In each instance, 4 pellets were placed within the bony sella. Attempts were made to insert 1 pellet in the midline in the anterior portion of the sella, 2 on either side of the midline in the midportion of the gland, and 1 in the midline in the posterior aspect of the gland. Of these 4 additional patients, one died on the first postoperative day; one survived 4 months and developed moderately severe diabetes insipidus on the eighth postoperative day, which persisted until death; and another survived 8 months, developing diabetes insipidus of moderate degree on the fourteenth day, which decreased somewhat but was present to a slight degree until the time of death. The last patient survived 2 months without evidence of diabetes insipidus. No cranial nerve damage was noted in these patients.

Figure 6. Photographic enlargement of a lateral roentgenograph of the sella turcica in one of the early patients. Note the silver clip on the pituitary stalk above the sella and the four Y^{90} pellets in posterior half of the pituitary gland near the posterior clinoid process.

At this time, it became apparent on the basis of autopsy findings that 4 pellets were not sufficient to yield anatomically complete hypophysectomy. It also became obvious that the proper placement of the pellets in the pituitary was not as easily accomplished as had been thought. Review of the histologic sections revealed that, because of the sharp border of the region of radiation necrosis, a pellet that deviated as little as 1 or 2 mm from its

proper position left histologically undamaged pituitary remnants.

It was then decided to increase the number of pellets. Three additional patients were subjected to the procedure, and in each patient 6 pellets were inserted into the hypophysis. The intent at this time was to place 4 beads in the anterior half of the sella and 2 beads in the posterior half. Two of these patients died during the first postoperative week. The third survived 5 months and did not develop diabetes insipidus. However, on the fourth postoperative day, semi-coma ensued which deepened for three days and then rapidly disappeared. There was an associated transient paresis of the right sixth cranial nerve. It seems probable that the altered consciousness and cranial nerve palsy were direct results of reversible radiation damage.

In the subsequent 8 patients, 7 to 8 radioactive pellets, averaging a total dose of 6.0 mc (Figure 7), were inserted into the sella. Five of the seven operations were carried out

Figure 7. Posterior-anterior roentgenograph after operation. Note the right frontal craniotomy opening. Seven Y⁹⁰ pellets are in the sella turcica; four anteriorly and three posteriorly.

using local anesthesia. It was believed that because many of these patients had advanced metastatic lesions, particularly to the lung and to the liver, the use of a general anesthetic would increase the hazard. The uneventfulness of the course after operation may be due materially to the choice of the anesthetic. We consider local anesthesia indicated in patients who are in poor preoperative condition.

One of the eight patients died suddenly one week after operation of a cause unrelated

to the procedure. One of the eight patients exhibited symptoms of cerebral edema for four days postoperatively. After this period of time he improved rapidly, and was asymptomatic by the eighth postoperative day. No other complications were observed. As pointed out previously, the choice of anesthetic depends upon the condition of the patient. The procedure can be carried out quite easily under local anesthesia and this should be the choice for patients who have widespread hepatic or pulmonary metastases; or in those patients whose preoperative condition is poor and in whom general anesthesia would increase the surgical risk. For those patients who present a fairly stable general condition and in whom no specific contraindication exists, the choice is endotracheal anesthesia.

The surgical technique as used at present is essentially as follows. A standard right frontal craniotomy is performed. The infundibular stalk is always clipped and divided. The stalk should be clipped as close to the hypothalamus as possible. Eight to 10 pellets of Y^{90} are inserted into the hypophysis (Figures 7 and 8); 4 into the anterior-most portion,

Figure 8. Lateral roentgenograph after operation in the same patient as in Figure 7. Note the distribution of the Y^{90} pellets in the sella turcica.

approximately 4 mm apart; 3 in the midportion; and 3 in the posterior region of the gland 4 mm apart.

CLINICAL COMPLICATIONS

The patients discussed in this paper are divided into two groups: an initial series of 9 patients and a subsequent one of 7. In the initial group, 4 patients died without regaining consciousness in the immediate postoperative period. Of the remaining 5 patients, 1 developed paresis of the third and fourth cranial nerves; and 1 had transient paresis of the sixth cranial nerve on one side. In each instance, postoperative roentgenographs revealed

that a radioactive pellet had been misplaced lateral to the bony sella. This accounted for cranial nerve palsy.

Of the 5 patients who survived the operative procedure, only 1 did not develop diabetes insipidus. The onset of the diabetes insipidus varied from as early as the fifth postoperative day to as late as the fourteenth. In most instances, the diabetes varied in severity from day to day, and in all cases was well controlled by intermittent doses of 1 cc of pitressin given intramuscularly. No other clinical complications were incurred in this series.

In the second group of 7 patients, no operative fatalities were encountered. One patient suddenly developed massive intrathoracic hemorrhage on the eighth postoperative day after a previously uneventful course. This patient died 1 day thereafter. Necropsy revealed erosion of a pulmonary artery radical by a large pulmonary metastasis.

Two of the 7 patients exhibited partial paralyses of extra-ocular movements. One patient developed a left sixth cranial nerve palsy that began 2 days after operation and was permanent throughout the three-month survival time. Another patient developed a partial paresis of the left third cranial nerve, which began 4 days after surgery, and still exhibited some evidence of ocular motor paresis 6 months after operation. No other clinical complications were encountered.

RESULTS OF ANATOMICAL STUDIES

Twelve of the total of 16 patients have died, and autopsies have been obtained on each of these. The bony sella was removed in toto, and the entire content of the sella was embedded. Each of the preparations was sectioned serially. Ten-micron sections were obtained, and every 40th section was mounted and studied. By this means, an average of 20 to 25 sections of each pituitary gland was studied to determine the completeness of the hypophysectomy. The hypothalamus was likewise imbedded and studied.

Observations of the specimens have revealed histologically normal-appearing remnants of the pituitary gland in each of the twelve cases that came to autopsy. (Typical findings are shown in Figures 9, 10, and 11.) The amount of this portion varied from patient to patient, depending upon the number of pellets that had been inserted and their implantation site. It became clear that, in the majority of instances, these remnants of functioning pituitary tissue are present usually in the lateral extremities of the gland, close to the dural covering and usually in either the anterior- or posterior-most portion, on the right side. These areas escaped radiation-induced destruction because one or the other of the beads was placed as little as 1 mm from its proper locus. The sharp demarcation between completely necrosed pituitary tissue and normal-appearing tissue is extremely clear cut so that precise positioning of the pellets is of paramount importance to obtain complete destruction of all pituitary tissue.

A detailed presentation of the studies on the hypophysis and hypothalamus will be made subsequently.

Figure 9. Photomicrograph (H & E) of the pituitary gland of a patient who died 1 month after operation. Note extensive necrosis, cellular debris, and phagocytosis.

Figure 10. Photomicrograph (H & E) of the pituitary gland of the same patient as in Figure 9. Note the nearly total histologic destruction of the gland. One small area of normally stained pituicytes is seen (dark stained area, upper right).

Figure 11. Photomicrograph of same patient as in Figure 10 showing sharp transition zone between area of necrosis and area of histologically undamaged pituitary tissue.

CLINICAL RESULTS

All of the first group of 9 patients have died. The longest survival was 8 months, and the shortest, 1 day. Of those patients who survived the operative procedure (5), the average length of life was 4.6 months.

Of the second group of 7 patients, the survival times ranged from 1 week to 6 months, the time of writing when 4 were still surviving. Three of the seven patients have died. The longest survival time of these 3 was 3 months.

The 4 patients who are still alive have the following types of metastatic cancer; hypernephroma, melanoma, prostatic cancer, and breast cancer. It is difficult to evaluate the rate of growth of the tumor following hypophysectomy unless the growth ceases entirely or there is objective regression.

In the patient with carcinoma of the prostate there has been both subjective and objective evidence of beneficial effect of the procedure. Preoperatively, the patient had bone pain of such severity that he required 5 to 6 grains of codeine a day. This medication has been stopped with cessation of pain. The rather severe anemia observed preoperatively has disappeared, and the hemoglobin has returned to a normal value. The acid phosphatase, which was 13 units preoperatively, has dropped to 7 units in the postoperative period. This patient developed a transient diabetes insipidus, which rapidly disappeared. His BMR, which was plus 10 preoperatively, has dropped to a level of minus 23 approximately 2 months after surgery, and there is clinical evidence of mild hypothyroidism.

The patient with metastatic carcinoma of the breast had severe pelvic bone pain before operation which disappeared entirely thereafter. Her appetite has returned, and she has a sense of well-being. The hemoglobin values are at normal levels. She still has roentgenographic evidence of lung lesions. This patient developed a mild diabetes insipidus for a few weeks but this has gradually disappeared.

The 4 survivors are being maintained on from 37.5 to 50 mg of cortisone a day, and at the present time, no thyroid medication is given.

There does not appear to be any single or group of endocrine tests that will yield unequivocal proof of the completeness of hypophysectomy. We have observed the development of pituitary myxedema in 6 patients who had had hypophysectomy, and at autopsy, were found to have small amounts of anterior pituitary tissue remaining in the pituitary fossa. The patients who have had intact adrenals at the time of hypophysectomy have, on occasion, been maintained without any adrenal hormone replacement. During this period, they did not have a sense of well-being nor a good appetite. However, their electrolytic balance remained essentially normal and their blood pressure, although somewhat lowered, did not reach shock levels. In at least 6 patients who were maintained on a very small maintenance dose of DOCA and/or cortisone, there were rather stressful reactions secondary to complications from the metastatic carcinoma. During these periods, they developed symptoms of what appeared to be adrenal insufficiency with low serum sodiums and hypotension.

The onset of diabetes insipidus is difficult to evaluate, but it does seem clear that all of the patients who had significant amounts of anterior pituitary tissue remaining in the pituitary fossa following hypophysectomy developed moderately and prolonged diabetes insipidus. There have been a few patients in whom the diabetes insipidus gradually disap-

peared. However, these patients still did not have normal water or solute balance. It is of interest that in the last 4 cases in which we have used larger number of pellets with resultant higher doses of radiation, there has been only a very mild transitory diabetes insipidus or none at all.

The preoperative and postoperative hormonal support for the patient undergoing hypophysectomy is accomplished quite easily. The day before surgery the patient is given intramuscularly 100 mg of cortisone acetate and 2 mg of DOCA. On the day of operation, the patient is given intramuscular injections of 150 mg of cortisone acetate and 2 mg DOCA before operation. Following operation, cortisone acetate is continued at 50 mg every 6 hours for 24 hours beginning the first day. On the subsequent days, the doses of cortisone acetate are reduced to about 150 mg on the 2nd day, 100 mg on the 3rd, 75 mg on the 4th, and then to 50 mg a day as maintenance. Lower maintenance doses of 25 to 37.5 mg have been used successfully. Intramuscular injections of pitressin (0.5 to 1.0 ml) are used to control diabetes insipidus should it develop.

SUMMARY OF CLINICAL OBSERVATIONS

The insertion of radioactive point sources into the hypophysis for destruction of the pituitary gland by the method that is described is a relatively simple and safe procedure. With recent refinements, fatalities resulting from the technique have been negligible. In this regard, we believe that a right frontal craniotomy and the insertion of radioactive pellets into the hypophysis represent less surgical hazard than attempts at surgical removal of the pituitary gland or adrenalectomy in association with gonadectomy. We have performed 9 surgical hypophysectomies. Autopsies and serial sections of the pituitary glands from these 9 patients have revealed that in all instances the surgical removal of the gland had been incomplete. In all 9 patients, relatively large fragments of normal viable pituitary tissue remained in situ. Whereas, although we have been unable to obtain anatomically complete hypophysectomy with the insertion of the radioactive pellets, a comparison of the amount of viable tissue remaining after the insertion of beads with that left after surgical procedure has revealed that the pellets are more effective. In addition, with surgical hypophysectomy there is the attendant risk of bleeding from the cavernous sinus, and mechanical trauma to the parasellar structures is a greater danger to the patient than the complications that follow insertion of the radioactive sources.

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EFFECTS OF RADIATION IN TISSUE SURROUNDING Y^{90} -IMPREGNATED GEL-FOAM*

By

T. T. Kennedy,[†] P. V. Harper, K. A. Lathrop, and T. B. Rasmussen[‡]

Local applications of radioactive materials are being used widely to effect tumor destruction. The authors are exploring the feasibility of using locally implanted isotopes for radiation cautery of the tumor bed after removal of tumors of the brain. Dosimetry and tissue effects, as well as the clinical applicability of such a technique, were investigated in cats.

Several important factors are involved in the development of this method of localized irradiation. The unpredictable degree of postoperative edema necessitates the selection of a vehicle for the radioactive isotope that will adhere to the cut surface of the brain. An isotope with short-range radiation is necessary so that changes in the geometry of the isotope distribution, caused by postoperative swelling, will not affect the radiation dose that is delivered to the selected tissue. In addition, the introduction of a foreign body into the brain for an extended period of time should be avoided.

These considerations led us to investigate the use of gelatine sponge (Gel-foam)⁽¹⁾ impregnated with an insoluble radioactive beta emitter. The isotope that was chosen was yttrium-90, which has a half-life of 62 hours and emits a strong beta ray with mean beta energy of 0.90 mev and a maximum energy of 2.3 mev. This isotope may be produced in sufficient quantity by irradiation of natural Y^{89} in the pile for one week at a flux of 3×10^{12} neutrons per cm^2 per sec. Under these conditions it reaches the specific activity of 0.5 mc per mg of yttrium. Considered as an infinite sheet with an isotope density of 1 mc per cm^2 , this material will give a surface dose rate of approximately 5000 rep per hour.⁽²⁾ Photographic film measurements⁽⁴⁾ of surface dosages were made in the central zones of several Gel-foam pledgets (see below) in which U_3O_8 was used as a standard.⁽³⁾ The data confirmed the calculations to within 20 per cent.

It is necessary to know the rate at which the isotope leaves the Gel-foam and also the penetration and absorption of isotopic radiation in the surrounding tissue in order to determine the depth dose in tissue surrounding the Gel-foam applicator. In this study these factors were measured directly.

* Paper presented before the Radiation Effects Section, The Inter-society Session of the Federation of American Societies for Experimental Biology, San Francisco, April 11-15, 1955.

† Department of Surgery (Neurosurgery), The University of Chicago.

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Gel-foam pledgets, 1.0 cm in diameter and 0.5 mm thick, were prepared. These were impregnated with insoluble yttrium hydroxide, which was precipitated directly on the Gel-foam. Yttrium nitrate, which had been activated in the pile, was dissolved in 50 per cent ethanol, containing a few drops of phenolphthalein. One-tenth cc of this solution, containing approximately 30 mg of yttrium nitrate, was pipetted onto the pledget, which had been flattened to remove air spaces. The solution soaked uniformly through the pledget in 5 to 10 seconds. The pledget was then placed in ammonia vapor for about 5 minutes until the phenolphthalein became uniformly red. Then the pledgets were placed in a Buchner funnel, washed with 0.8 per cent saline to remove ammonium nitrate, and made almost dry by suction. During this process, usually no more than 5 to 10 per cent of the activity was lost. Pledgets were prepared this way containing between 40 μ c and 4 mc.

A trephine hole was made in the skulls of adult cats, and the pledgets were introduced either intra- or extradurally.

In one group of cats, a lucite well was inserted into the trephine hole and wired into place (Figure 1) in such a way that the lucite window (0.02 in. thick) at the bottom of the well was in contact with the pledget. Between measurements, the well was closed with a

Figure 1. Lucite well in place in trephine hole in skull with skin sutured around it. The lucite plug is left inserted between readings (loosely wired to well). The ionization chamber fits in place of plug for readings.

lucite plug, which served as a radiation shield and simulated back scatter from the tissue. Measurements of relative activity were made at intervals by inserting an ionization chamber into the well (Figure 2). Readings were taken with a Beckmann micro-microammeter. Simultaneous measurements were made on a pledget that was embedded in a lucite phantom.

An alternative method of measurement was carried out using photographic film⁽⁴⁾ for pledgets with activities too low to permit accurate measurements with the ionization cham-

Figure 2. A view of the ionization chamber assembly. The lucite well and its plug are shown at the bottom of the picture. The end window of the well is 0.02 in. thick and is in contact with the Gel-foam pledget. The flange is used to wire the well to the skull.

The ionization chamber is 13 mm in diameter. Its accelerating electrode is an aluminum foil disc, 0.03 mm thick, cemented on the end of the chamber and connected to the high voltage line of the micro-microammeter by a polyethylene insulated wire. A brass plate, 6 mm in diameter, forms the collecting electrode, while the outer brass guard ring is attached to the ground of the ammeter. The capacity of the sealed-in air space is 0.5 cc.

The cables from the ammeter are shown above. The heavy cable served to reduce the dielectric strain caused by movements of the animals.

ber. Such low dose pledgets were placed in contact with the dura, and a sheet of sterile plastic was placed over the wound to prevent isotopic and bacterial contamination. A small square of photographic film (sealed in black paper) was fixed on the end of a lucite rod and placed upon the pledget for varying periods of time, after which the bone button was reinserted and the wound closed. The wound was reopened and further film exposures were made on succeeding days. Exposure to a comparable pledget in a lucite phantom was carried out as a control measure.

The data from the two groups are plotted in Figure 3. The measurements obtained by means of the ionization chamber show a decrease in activity that is accounted for entirely by the natural decay of the isotope. These indicate that negligible quantities of isotope left the Gel-foam during the 2-week period of observation. The results obtained with the photographic film give evidence of disappearance of the isotope from the pledget. The data probably reflect both mechanical loss of the isotope during measurements and the fact that the isotope was in contact to a much greater extent with viable tissue at the lower radiation level.

The depth dose in tissue was determined by film measurements⁽⁴⁾ that were made by placing a pledget in a lucite phantom that permitted insertion of X-ray film at right angles to the plane of the pledget (Figure 4). Figure 5 shows the result of such an exposure. Isodose curves were plotted (Figure 6), using the methods described previously.⁽⁵⁾ Absolute surface dose measurements on this and the other standard pledgets were made with film, using a U_3O_8 standard (see above).

The pathological changes resulting from the irradiation were demonstrated in sections of the brain taken for histologic study. These sections were prepared by the usual techniques, using hematoxylin and eosin stain. Figure 7 shows a representative section of the area beneath the pledget, with the isodose curves superimposed. There is a fairly sharp borderline of radiation-induced necrosis, which corresponds approximately to the 7 per cent of the central surface dose or, in this case 75,000 rep. It appears that the grey matter is slightly more resistant to the necrotizing effects of irradiation.

SUMMARY AND CONCLUSIONS

Y^{90} , in the form of yttrium hydroxide, impregnated into Gel-foam and placed extra- or intradurally in normal cats produces necrosis. A sharp border zone appears at about 75,000 rep. The yttrium, when prepared in this fashion, remains in place for at least two weeks, by which time the activity has decayed to 1 per cent of the original level and the Gel-foam is fairly well absorbed.

The technique, however, has certain disadvantages. The preparation of large quantities of the radioisotope that would be needed to line tumor beds would involve prohibitive exposure and contamination hazards. The intracranial insertion of inactive absorbant material, sealed in plastic envelopes, followed by the introduction of the radioactive material through plastic tubing at any desired time postoperatively circumvents these difficulties,

while preserving many of the advantages of the Gel-foam technique, and work in this direction is in progress at the present time.

Figure 3. The results of in situ activity.

The open triangles are readings taken with the ionization chamber on cats with high level Gel-foam pledgets. The initial surface dose rates ranged from 90 to 360 rep per minute. The black triangles are readings on 9 cats in which the Gel-foam was placed extradurally. The white triangles are readings on 3 cats in which the Gel-foam was inserted intradurally.

The circles are readings taken from photographic film in cats with low level Gel-foam pledgets. The initial surface dose rates ranged from 3 to 14 rep per minute. The pledgets were placed extradurally in all 6 cats of this group.

The straight line is the decay curve for Y^{90} , with half-life of approximately 2.5 days.

Figure 4. Diagram of location of film in lucite phantom for depth dose measurements.

Figure 5. An 8-second exposure in phantom of a Gel-foam pledget containing 1.1 mc of Y^{90} . A millimeter scale is reproduced.

Figure 6. A diagram of isodose curves derived from density readings of film exposed for varying periods of time to a Gel-foam pledget.

Figure 7. A photomicrograph of the area beneath a pledget, with the isodose curves superimposed. Radiation-induced necrosis appears to approximately the 7 per cent isodose curve, which, in this animal, corresponds to about 75,000 rep.

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RADIATION DOSAGE IN TISSUE SURROUNDING SMALL BETA-RAY SOURCES*

By

P. V. Harper, K. A. Lathrop, and T. T. Kennedy†

INTRODUCTION

The use of small beta-ray sources for the destruction of the hypophysis⁽¹⁾ and for the production of localized lesions in the central nervous system⁽²⁾ has made desirable a study of the radiation dosage in tissue surrounding such sources. Methods have been proposed for calculating these dosages.^(3,4) However, since we were using sources of various shapes and sizes and since each source had a complex self absorption that altered the energy spectrum of the beta radiation to an unknown degree, we believed that direct measurements would probably give a more realistic estimate of the radiation field than would calculation.

Tochilin and Golden have shown⁽⁵⁾ that X-ray film determinations of beta-ray depth dosage reproduce very exactly those made by means of the extrapolation chamber. Moreover, above a maximum beta energy of 1.7 mev, the film shows no energy sensitivity. This approach to beta-ray depth dose measurement has been reviewed favorably by Marinelli.⁽⁶⁾ Bass et al.⁽⁷⁾ measured the beta-ray surface dose rate of U_3O_8 and found it to be 0.207 rep per hour. This provides an excellent, reproducible absolute standard for high energy (2.2 mev) beta radiation.

For energy below 1.7 mev, the film determinations may be corrected according to the data of Tochilin and Golden.

PROCEDURE

The radioactive source to be measured was embedded in the surface of a lucite block (Figure 1). In the darkroom, this was placed in direct contact with Eastman Blue Brand Medical X-ray film backed by lucite. For the purpose of these experiments, the lucite was considered to be the same density as tissue. When the radiation caused visible fluorescence in the lucite, the film was protected by black paper. A series of exposures was made. The range was spread between 1 and 256 seconds, depending on the strengths of the source. Radiation from the U_3O_8 standard was measured, and a film sensitivity curve was made with each experiment to control variations in the developing process.

A photograph of a representative X-ray film is shown in Figure 2. The transmission

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Figure 1. Method of exposing source to film in lucite phantom.

Figure 2. Film blackening resulting from a 16-second exposure to a P^{32} source. (Millimeter scale)

gradient across this exposure field was measured by mounting the film on a microscope slide and moving it across the stage of a microscope (Figure 3) equipped with a Photovolt light meter and a mechanical stage. The position of the film is determined by the vernier scales on the mechanical stage, and the optical transmission is measured by means of the light meter, assuming transmission to be 100 per cent in the unexposed film. Using an oil immersion objective (1.25 mm), the diameter of the field viewed through the lens, which is focused on the film, is 0.2 mm.

Film density measurements made with this apparatus were found to be practically identical with those made by the Ansco MacBeth densitometer. The gradient across the exposure in Figure 2 is shown in Figure 4. This is compared with the U_3O_8 standard ex-

Figure 3. Microscope showing light meter in place of eye-piece for densitometry.

Figure 4. Transmission gradient across the exposure area shown in Figure 2.

posure by means of the sensitivity curve, and the rep per hour delivered at each point by the source can thus be calculated. By using a wide range of exposures, the entire dose rate curve may be constructed. Figure 5 shows the complete curve for the source that is shown in Figures 3 and 4. This curve is a composite of five exposures taken at different

time intervals, and it demonstrates the usual scatter of points. A number of beta-ray sources (Table 1) have been prepared and studied in this fashion.

Figure 5. Composite dosage curve calculated from exposures at five different time intervals.

Table 1
BETA-RAY SOURCES

Isotope	Half-life	$E_{\max}$ (mev)	Chemical nature	Mass	Shape	Dimension	Plane of measurements
Pd^{109}	13 h	.95	Pd	.78 mg	cylinder	Length 1 mm Diam. 0.3 mm	Tangent to side of cylinder
Ag^{111}	7.5 d	1.05	Ag in Pd				
P^{32}	14.3 d	1.7	$\text{Mg}_2\text{P}_2\text{O}_7$	.67 mg	sphere	Diam. 3 mm	Tangent
Y^{90}	62 h	2.3	Y_2O_3	2.3 mg	cylinder	Length 1.5 mm Diam. 0.9 mm	Tangent to end of cylinder

The rep per hour delivered by these sources at various distances is shown in Figure 6 for sources corrected to 1-mc activity. When the ordinate scale for each curve is multiplied by its average life in hours, the curves shown in Figure 7 result. These give the total dose to complete decay at any distance from the source. The activities for all these sources were calculated from data on neutron bombardment. The flux was determined by measuring induced P^{32} activity in $\text{Mg}_2\text{P}_2\text{O}_7$, using a Tracerlab simulated P^{32} standard.

The total energy released outside the source may be calculated roughly since $dE = 93 D \times 4 \pi r dr$, where D is the total dose in rep at distance r from the source, assuming

Figure 6. Dosage rates in tissue surrounding various beta-ray sources.

Figure 7. Total dose in tissue surrounding various beta-ray sources.

a point source. For the P^{32} source, about 80 per cent of the energy expected from the decay of the isotope may be accounted for in this way, indicating a self absorption of about 20 per cent. This agrees well with the self absorption of the source that is estimated by counting it directly in a planchet and then dissolving it and distributing it evenly over the surface of the planchet. The self absorption in the Y^{90} and Pd^{109} sources that was estimated in a similar way was about 80 per cent.

Isodose curves may be plotted surrounding the source rather than the gradient, as done here by using similar techniques for measuring film density.

DISCUSSION

In general, the gradient curves conform rather closely to expected magnitudes, estimating self absorption by counting measurements and assuming point source geometry with inverse square attenuation superimposed upon an appropriate exponential absorption. The principal cause of deviation far from the source is the finite range of the beta particles, and close to the source is the departure from point source geometry. The correction for self absorption is sometimes quite large as mentioned above.

SUMMARY AND CONCLUSION

A method for using X-ray film to determine the beta-ray dosage in tissue surrounding small point sources is presented together with measurements on a number of beta-ray sources.

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SUMMARY OF THE THERAPEUTIC APPLICATION OF THE
VAN DE GRAAFF GENERATOR AND
THE COBALT-60 UNIT

By

J. W. J. Carpender

Therapeutic application of the Van de Graaff two-million volt X-ray machine was initiated on May 11, 1953. Since that time, 82 patients have been treated with its radiation. In general, the results have been what was expected in the light of other work done with similar high energy sources. The great advantage with this particular machine, however, is the increased depth dose that it provides. This permits adequate treatment with little or no skin reaction.

The patients have been selected carefully from several standpoints. In all cases, there has been absolute proof of the site of primary lesions and adequate classification of type of malignancy. Very few patients have been treated where only palliation was the aim. Most patients, even those in which the disease was quite extensive, have been exposed to dose levels that might potentially be curative.

So far, the most promising results have been obtained in patients with carcinoma of the larynx. Seven of eight of such individuals have shown an excellent, immediate response. As yet, the interval between the time that therapy was initiated and this writing is much too short and the series too small to give more than the breakdown shown in Table 1.

The Vande Graaff generator has proven to be a fairly reliable device, and only two major cessations have occurred in the two years that it has been in operation. One of these was caused by circumstances that were beyond our control which involved failure of the air conditioning system. The other stoppage was a month that was devoted to preventative maintenance and necessary alterations.

Results in the 34 patients that were treated by means of the radioactive cobalt unit, which has been in operation for therapy since March 8, 1954, have been disappointing (Table 2). Only seven individuals have been treated to a possible cure. Evidence points to the fact that a much higher dose is necessary with this quality of radiation than with the conventional 250-kv X-ray machine. This has not only been borne out in clinical studies but also in experiments on mice.

The cobalt source has been as completely reliable as could be expected. As the first source (500 curies) decayed, the length of time required to administer the prescribed daily dose increased to such an extent that the irradiation sessions became rather trying for the patients, some of which could not tolerate remaining motionless for a half-hour or more. Recently, a new very active source (1225 curies) was installed without interrupting

Table 1

SUMMARY OF THERAPEUTIC APPLICATION OF THE VAN DE GRAAFF
GENERATOR (82 patients)

Pathology	Total dosage delivered (r) over		Clinical response
8 lesions of larynx			
Stage 1	5200	41 days	Good result 2 yrs.
Stage 2	5640	49 days	Failure in 6 mo.
Stage 1	6000	51 days	Good result 1 yr.
Stage 2	6000	46 days	Good result 1 yr.
Recurrent larynx or new epiglottis (Hemilaryng. 1950 June)	6000	46 days	Good result 1 yr.
Stage 1	6000	35 days	Good result 7 mo.
Stage 1	6000	42 days	Good result 5 mo.
Stage 1	6000	34 days	Good result 3 mo.
11 miscellaneous lesions of mouth and airways			
Epiglottis	5600	7 wks	Failure in 3 mo.
Floor of mouth	4000	4 wks	Therapy stopped - mental deterioration - patient died in 3 mo.
Gingival margin	6100	51 days	Good result 1.5 yrs.
Ethmoid sinuses	6300	49 days	Good result 1.5 yrs.
Reticulum cell nasopharynx	5000	42 days	Metastasized under treatment
Lymphosarcomatous tonsil	4000	28 days	Good result 1.25 yrs.
Transitional cell nasopharynx	5000		Prev. treated - failure good palliation
Transitional cell nasopharynx			Died during treatment - pneumonia
Transitional cell nasopharynx	6100	36 days	Primary lesion healed - has metastases 6 mo. after treatment
Max. antrum	7000	45 days	Doing well at 6 mo.
Squamous cell left maxilla and antrum	7500	55 days	Recurrence at 2 mo.
16 Bronchogenic tumors	6 showed what can be called good palliation either from symptoms or improvement observed by means of roentgenographs		
6 Brain tumors	4 survive. Ependymoma - large amount of tumor left by surgeon subjected to 5000 r in 17 days; doing very well at 1.5 yrs. Astrocytoma - 5000 r in 30 days; doing well at 1 yr.		

The remaining 41 miscellaneous lesions were treated for a variety of reasons, e.g., size of patient, previous irradiation, and curiosity regarding the response (Hürthle cell).

Table 2

SUMMARY OF THERAPEUTIC APPLICATION OF COBALT-60 UNIT

Pathology	Total dosage delivered (r) over		Clinical response
7 Carcinomas of the tongue			
Recurrence in area previously treated with radium	4000		Immediate failure experienced
Posterior rt. side - no notes	6000	7 wks	Apparent cure at 10 mo.
Base of tongue	6000	7 wks	Ulcer persists - bleeding
Base of tongue left	6000	8 wks	Proven recurrence tonsillar fossa
Tongue mid third	6000	6 wks	Proven recurrence
Posterior tongue with cervical metastases	7500	7 wks	No recurrence
Tongue with large neck nodes	8400	9 wks	No recurrence
4 Carcinomas of the mouth			
Alveolar ridge right	6900	9 wks	Recurred - died post-operatively aplastic anemia
Floor of mouth	1400		Patient discontinued
Floor of mouth right	7000	7 wks	Recurrence in 4 mo. and osteonecrosis
Floor of mouth, jaw and buccal post 3 surg.	6000	2 mo	Too early to evaluate
3 Carcinomas of the tonsil and other areas			
Tonsil	5000	30 days	Cure at 10 mo.
Epiglottis	7500	39 days	No evidence of recurrence at 4 mo.
Tonsil	6500	6 wks	No recurrence at 2 mo.
2 Carcinomas of the esophagus			
Perhaps metastatic	7000	5 wks	Good palliation
Squamous - small lesion	7500	6 wks	Appears cured at 3 mo.

4 Miscellaneous lesions in lungs - all failures but 1 improved under therapy

1 lesion in bladder appears cured at 8 mo.; 12 miscellaneous cases, some with palliation - either previously treated or palliation problems are involved.

the treatment schedule. This has reduced the treatment time to less than half of what it was previously. As a consequence, the irradiation period is more tolerable and a greater number of patients can be treated.

2-MEV ISODOSE CURVES FROM A VAN DE GRAAFF X-RAY GENERATOR

By

M. L. Rozenfeld

INTRODUCTION

The dosage distribution inside an absorbing medium, resulting from an external source of 2-mev radiation, has been described in terms of the variation of dose along the central ray.⁽¹⁻⁴⁾ The variation of dose in an entire plane containing the central ray has also been given.⁽²⁻⁵⁾ These data, in the form of isodose curves, were obtained either by point by point measurements with an ionization chamber or by interpretation of density measurements on exposed films. Because of limitations of space, however, only reduced size samples of these data have been published.

A set of 17 isodose curves, obtained with the aid of a servo system from a Van de Graaff X-ray generator under the same conditions that are used for patient treatment, has been plotted and is presented as an appendix to this text. The X-ray machine was operated at 2 million volts with no added filter. The half-value layer (HVL) of the radiation was 12.4 mm Cu. The distance from the focal spot on the X-ray tube to the surface of the water phantom (FSD) was 100 cm. The field sizes, ranging from 5 x 5 to 20 x 20 cm, were determined from measurements on the light beam from the light localizer.

EXPERIMENTAL PROCEDURE

The dosage was measured with a cylindrical air-filled ionization chamber with an internal diameter of 3 mm and a length of 11 mm. The polystyrene wall of the chamber was 1 mm thick. An aluminum coating on the inside surface of the cap and a central aluminum wire, 0.5 mm thick, served as the electrodes of the ion chamber. A water tight chamber was provided by cementing the cap onto the stem.

The isodose curves were constructed with the aid of a servo system that automatically moved the ion chamber along lines of constant dosage. The servo system compared the output of the hunting chamber with the output of a monitor chamber, thereby eliminating any effects caused by variation in intensity of the X-ray beam. The system was operated slightly underdamped, so a small amplitude oscillation was superimposed on the curves. These oscillations were easily smoothed out.

The following procedure was followed in the construction of the isodose curves for a given field size. After the physical setup of the equipment, the servo system was used to find the maximum dose in the field, and the output of the monitor chamber was set equal to this maximum. Then 90 per cent of the new output was selected with a precision ten-turn potentiometer, and the servo system was allowed to trace the 90 per cent curve. Se-

lection of the various percentages in turn resulted in the complete data for that field size.

DISCUSSION

The size of the field depends on the size of the lesion to be treated. Thus, a large number of curves for various field sizes is required. Some means for estimating the dose distribution for odd sizes therefore must be devised to circumvent the large accumulation of data that would otherwise be necessary.

For central axis depth dose data, a correction is usually applied to the dose from the square field that has the same area as the required rectangular field. From the set of isodose curves, however, it can be seen that a rectangular field, such as 5×10 cm, is similar to the square 5×5 cm field. Thus, a correction might be applied to the depth for a given dose from the appropriate square field. It has, in fact, been found that for any given elongation, the increase in depth and lateral spread of the isodose curves is independent of the size of the field. Thus, the isodose curves for any rectangular field can be drawn from the curves of the square field equal to the shorter side of the rectangle. This is demonstrated in Table 1 for the construction of the curves for a 5×10 cm field from those for a 5×5 cm field. Column 2 of the table gives the depth of the central axis doses shown in column 1 for the 5×5 cm field.

Table 1
COMPARISON OF CALCULATED AND MEASURED DEPTH DOSE
FOR 5×10 cm FIELD

Depth dose (%)	Measured depth for 5×5 cm field (cm)	Calculated depth for 5×10 cm field (cm)	Measured depth for 5×10 cm field (cm)
90	2.75	2.9	2.8
80	4.4	4.64	4.65
70	6.2	6.5	6.5
60	8.2	8.6	8.6
50	10.35	10.9	11.0
40	13.1	13.8	13.85
30	16.65	17.6	17.6

Figure 1 shows that for an elongation of 2, i.e., a 5×10 cm field, the correction factor is 1.052. The product of this number with the depths of column 2 is shown in column 3 of the table. Comparison with the measured depths as shown in column 4 indicates the accuracy of the method. The maximum error to be expected is about 3 mm.

Since no full size isodose curves have been made available for this energy, comparison with previously published data must be limited to the central axis. This task is compli-

Figure 1. Correction factors to be applied to isodose curves from square fields to construct curves for rectangular fields.

cated by the fact that the various workers made their measurements under slightly different conditions and presented their results in different ways. Fortunately, all of these results have been correlated in the British Journal of Radiology, Supplement No. 5. The average of the best work normalized to the conditions of 12-mm Cu, HVL; 100 cm, FSD are presented. The work reported in this paper gives central axis depth doses slightly greater than those in the Supplement. Part of this variation might be explained by the large HVL of the radiation from the X-ray machine used in the Argonne Cancer Research Hospital.

SUMMARY

A set of isodose curves has been obtained from a Van de Graaff X-ray generator operated at an energy of 2 mev. These curves show a relationship that enables one to estimate the isodose curves for odd field sizes.

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APPENDIX

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5 CM

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90%

80

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HVL 12.4 MM CU

100 CM FSD

30

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CHOLESTEROL—A PRECURSOR OF URINARY ADRENAL STEROID METABOLITES IN MAN*

By

H. Werbin and G. V. LeRoy

The role of cholesterol as a precursor of steroid hormones in man has been the subject of relatively few reports in the literature. In 1945, Bloch⁽¹⁾ demonstrated the conversion of deuteriocholesterol to deuteropregnane-3- α -20- α -diol in a pregnant woman. Ungar and Dorfman recovered labeled androsterone and etiocholanolone from the urine of a woman with an adrenal cortical carcinoma who had received dietary 3-C¹⁴-cholesterol.⁽²⁾ Hellman et al.⁽³⁾ have indicated that dietary 4-C¹⁴-cholesterol is converted to urinary steroid metabolites.

The following experiment was devised to learn whether the perhydro-1,2-cyclopentenophenanthrene rings of cholesterol were utilized intact in the formation of adrenal steroid metabolites in man. A male arthritic patient was given intravenously 30 ml of sterile human plasma containing 4.24 μ C of 4-C¹⁴-cholesterol and 33.8 μ C of cholesterol-t in 2.3 g of propylene glycol. Blood and urine samples were collected for 8 days. The tritium and carbon-14 specific activities of the free and ester blood cholesterol and five urinary steroids (tetrahydrocortisone, tetrahydrohydrocortisone, 11-ketoetiocholanolone, and rosterone, and etiocholanolone) isolated in a crystalline state were determined.

The distribution of tritium in the side chain and in the perhydro-1,2-cyclopentenophenanthrene rings of cholesterol was established by two methods; that of Bloch and Rittenberg⁽⁴⁾ and that of Fukushima and Gallagher.⁽⁵⁾

Having determined the ratio of T/C¹⁴ specific activities of the blood free cholesterol and knowing the distribution of tritium in the blood free cholesterol, it was possible to calculate the ratio of T/C¹⁴ to be expected in the steroids that were isolated. The ratios that were found agreed within 6 per cent of the calculated values. The excellent agreement is strong evidence for the direct conversion of cholesterol to the adrenal steroids.

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ABNORMAL HUMAN SERUM GLOBULINS^{*†}

By

F. W. Putnam

Many diseases induce an elevation in serum proteins but only multiple myeloma and certain related obscure disorders result in the formation of abnormal serum globulins in the human being. Globulins from individual sera from many patients have already been isolated and characterized by their physical constants⁽¹⁾ and by their N-terminal amino acid content,[‡] i.e., by the number and nature of the terminal residues having a free α amino group.⁽²⁾ The biosynthesis of these globulins has been studied by means of isotopic amino acids.^(3,4)

Electrophoretic analyses of sera from multiple myeloma patients reveal that the increase in plasma protein is confined to a major component that is remarkably homogeneous when compared with globulins from normal human subjects.⁽⁵⁾ However, each patient may elaborate individual globulins that are electrophoretically distinguishable from those of other patients. The mobility of most of the myeloma globulins is within the wide range of the normal gamma globulins, and the molecular size and shape of both normal and abnormal proteins are similar.⁽¹⁾ The greater homogeneity of the abnormal globulins results in more precisely defined mobilities, varying individually from -0.7 to -1.7×10^5 $\text{cm}^2 \text{sec}^{-1} \text{volt}^{-1}$ at pH 8.6. There are some myeloma globulins, e.g., the M or β types, that have a greater mobility at this pH.

Although the multiple myeloma globulins fall into two immunological groups of proteins that are interrelated and cross react, each protein appears to have individual specificity.⁽⁶⁾ The chemical characteristics of these proteins have been identified by means of N-terminal amino acid analysis, which has revealed that structurally abnormal serum globulins are synthesized in response to multiple myeloma.⁽²⁾

Cold-insoluble globulins, the cryoglobulins, occur only in rare diseases, e.g., multiple myeloma and cryoglobulinemia. These proteins may be purified readily as a result of their insolubility at lowered temperatures. Their relationship to normal plasma components is difficult to assess because relatively little is known about the abnormal proteins. It thus was our purpose to determine the sedimentation constant, isoelectric point, solu-

* Based on a paper that has been accepted for publication in Science.

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‡ The N-terminal amino acid analyses were initiated in the Department of Biochemistry, University of Cambridge, with the aid and encouragement of Dr. Fred Sanger.

bility, and other physical properties of a few cryoglobulins and to clarify their relationships to each other and to globulins that occur in normal human beings.

Eight highly purified cryoglobulins have been investigated by physicochemical and N-terminal amino acid analysis.[†] All were electrophoretically homogeneous at pH 4.7, 6.7, or 8.6, depending upon which was suitable for the particular cryoglobulin. Two of those studied crystallized spontaneously in the cold, a phenomenon that has not been observed for normal globulins. The chemical and physical properties of the 8 cryoglobulins and of normal human gamma globulin are summarized in Table 1.

Table 1
PHYSICAL PROPERTIES AND N-TERMINAL GROUPS OF
HUMAN PLASMA CRYOGLOBULINS

Protein	Crystal form	pI	Mobility at 1°C		s _{20,w} S	N-terminal groups	
			pH 8.6 10 ⁻⁵ cm ² sec ⁻¹ v ⁻¹	pH 4.7 10 ⁻⁵ cm ² sec ⁻¹ v ⁻¹		Aspartic acid moles per 160,000 g	Glutamic acid moles per 160,000 g
Normal γ_2		7.2	-1.3	3.0	6.6	1.12	1.75
Th		7.5	-1.1	3.0	6.6	1.80	0.16
Ag		6.4	-1.1		6.6	2.04	absent
Mi	needles		-1.3		6.75	2.06	0.16
R	hexagons		insoluble	3.0	7.6,*11	2.00	2.64
Se			-0.8		6.4	absent	2.65
Wk		5.5			7.18,*28	0.12	3.14
I			insoluble	3.3	7.6,*11	0.15	2.77
Gu			insoluble	3.3	6.0	2.29	2.21

* Mobilities and sedimentation constants were determined in 0.1 ionic strength Veronal buffer pH 8.6 or in 0.1 ionic strength acetate buffer pH 4.7. All the cryoglobulins migrated with a single sharp boundary upon electrophoresis. s_{20,w} is extrapolated to infinite dilution except for ultracentrifugally heterogeneous globulins, in which case the major component is indicated by an asterisk. S signifies the Svedberg unit and pI, the isoelectric point.

[†] We are indebted to the following persons for specimens of purified cryoglobulins and for information on their properties: Dr. David Barr and Miss Ella Russ, Cornell University Medical College, New York, N.Y. (Proteins R and I), and Dr. A. S. McFarlane, National Institute for Medical Research, Mill Hill, London, England (Protein WK). Sera containing cryoglobulins were kindly supplied by Dr. Charles B. Huggins, University of Chicago, Chicago, Ill., Dr. Steven A. Schwartz, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill., Dr. Elliott F. Osserman, College of Physicians and Surgeons, Columbia University, New York, N.Y., Dr. F. W. Gunz, Pathology Department, Christchurch Hospital, Christchurch, New Zealand, and Dr. Jan Waldenström, University of Lund, Lund, Sweden.

All of the cryoglobulins could be distinguished from the normal globulin by differences in two or more of the following properties: crystal form, solubility, isoelectric point, electrophoretic mobility, sedimentation constant, ultracentrifugal or electrophoretic homogeneity, and N-terminal amino acid residues. The cryoglobulins differed from each other in two or more of these properties. A significant finding was that the N-terminal residues of these cryoglobulins are not identical. The proteins fell into three groups each having different molecular structure. Their physical variability from patient to patient was confirmed.

It has been possible to classify the cryoglobulins, which were studied, as chemically different types by the fluorodinitrobenzene method.⁽⁷⁾ Three, designated as N-aspartyl globulins, have about 2 moles of N-terminal aspartic acid and are either without or have only traces of N-terminal glutamic acid. At pH 8.6 their mobilities are the same, and they have similar sedimentation constants. Two of the globulins, which are non-crystalline, are related immunologically to normal gamma globulin.

Three other proteins, designated as N-glutamyl globulins, have approximately 3 moles of glutamic acid per 160,000 g and little or no N-terminal aspartic acid. These differ from the N-aspartyl globulins in electrophoretic properties and in ultracentrifugal homogeneity. The N-glutamic globulins can be distinguished from each other by the distribution of their sedimenting components. In the N-terminal position, all of the cryoglobulins differ structurally from the gamma globulin obtained from pooled normal serum.

In addition to the cryoglobulins, eight myeloma globulins were subjected to N-terminal amino acid and physicochemical analyses. The distribution of N-terminal aspartic and glutamic acids in each of these was unlike that found in normal human gamma globulin. N-aspartyl and N-glutamic globulins were present in all of these globulins although only traces could be found in two. These, however, contained amino acid end-groups that are not present in normal globulins.* Both had a molecular weight of 160,000 but migrated with a mobility of only $-0.7 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$ at pH 8.6. One had 2 moles of N-terminal leucine per 160,000 g; the other had 1 mole of N-terminal leucine and approximately 1 mole of an unidentified N-terminal amino acid (or peptide). This component appeared to have some of the characteristics of phenylalanine but it moved faster than any known amino acid in the tertiary amyl alcohol system.

It might be postulated that patients with multiple myeloma or cryoglobulinemia synthesize N-aspartyl or N-glutamyl globulins or other normal components excessively and preferentially, but the data indicate a greater variety of amino acid end-groups and of physical properties than can be explained in this way. All of the cryoglobulins that we have studied are formed only in human beings with pathologic conditions. Although it is possible that these unnatural proteins are present in trace amounts in normal serum, it is very unlikely that 7 different cryoglobulins would be contained in normal serum. Globulins that

* Preliminary analyses indicate that N-terminal glutamic acid, aspartic acid, and serine are also present in human serum globulin, Fraction III. However, valine appears to be the major N-terminal group of the β_1 iron-binding globulin.

have N-terminal leucine seem to fit into the classification of abnormal proteins, but whether all myeloma globulins are specific abnormal proteins is difficult to ascertain. It may be that each myeloma globulin is the result of excessive and selective synthesis of one of many normal globulin components. In this regard, we have not been able to correlate the morphological characteristics of the myeloma plasma cell with pathological serum globulins or with proteinuria.

The multiple myeloma syndrome is characterized by a great increase in the synthesis of abnormal plasma globulins and/or profuse excretion of Bence-Jones proteins. Some patients produce twice as much abnormal globulin as healthy persons do total serum proteins; others excrete as much as one-third of their daily nitrogen intake by way of a protein that is specific for this disease. It may be that the clue to both the mechanism of normal growth and protein synthesis may be found in investigations of the deranged metabolism of nitrogen in this disease.

The Bence-Jones proteins have a molecular weight that is about one-fourth that of serum globulins and unusual heat coagulation properties. They are most often excreted by patients in which abnormal globulins are not synthesized. For our purposes, we have studied several patients in which both anomalies occurred.

The use of radioisotopes has not revealed any precursor relationship between the abnormal globulins and the urinary proteins. In point of fact, it has been shown that the Bence-Jones proteins are rapidly synthesized *de novo* from the free amino acid pool and are thereafter excreted at a rapid rate. Radioactive Bence-Jones protein was found to be present in the bladder of one patient 35 minutes after he had been given an injection of C^{14} -glutamic acid. The maximum specific activity of the urinary protein was reached at 6 hours, after which the radioactivity decreased at a logarithmic rate, with a half-time of 8 hours. Similar findings were obtained from another patient who was injected with C^{14} -labeled lysine.

In conclusion it may be stated that the mechanism of protein synthesis is perverted in patients with multiple myeloma. Such patients may synthesize individually specific abnormal serum globulins or excrete Bence-Jones proteins that are also individually specific or they may exhibit both aberrations. No function is known for either of these abnormal proteins.

The urinary proteins are essentially incomplete proteins and are excreted as waste products, possibly as the unused or unfinished precursors of normal globulin or as abortive products of the deranged mechanism of serum protein synthesis. The rapid synthesis and excretion of Bence-Jones proteins indicate their direct synthesis from metabolites that are diverted from normal pathways.

Further study of the nature and biosynthesis of the proteins that are characteristic of multiple myeloma should enable us to learn more of the mechanism whereby normal serum proteins are synthesized.

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METABOLIC FATE OF RADIOACTIVE DIGITOXIN
IN HUMAN SUBJECTS*†‡

By

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SUMMARY

Although digitoxin has been used for over 160 years to treat congestive heart failure, little is known of its metabolic fate in human beings. The difficulties involved in measuring minute amounts of the drug in body tissues were the main barrier to the study of this problem. The development of the isotope dilution technique and the use of C^{14} -labeled digitoxin permit the detection of as little as $0.02 \mu g$ of radioactive digitoxin in tissue samples. Using biosynthetically labeled digitoxin, it is also possible to follow metabolic products of digitoxin because of the "uniform" labeling of the parent compound. By means of this method, the metabolite-digitoxin ratio may be determined for various tissues, which in turn provides clues as to the possible site of detoxification of the cardiac glycoside.

The purposes of this study were to determine the distribution of unchanged digitoxin and its metabolic products in the various tissues of human beings and to determine the pathway by which the drug is removed from the body.

METHOD

Three terminal patients were selected for this investigation (Table 1). The radioactive digitoxin was isolated from digitalis plants that had been grown in an atmosphere of $C^{14}O_2$.⁽¹⁾ The specific activity of the labeled drug ranged from 0.48 to $0.65 \mu c$ per mg. Multiple doses were administered intravenously to the patients to maintain adequate concentration in the tissues. Tissue samples, which were taken approximately 2 hours after the death of each patient, were frozen and stored at $-17^\circ C$ to prevent degradation of the compound.

The unchanged digitoxin was extracted, and the metabolic products were fractionated

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‡ From the Departments of Pharmacology and Medicine and the Argonne Cancer Research Hospital, The University of Chicago, and the Department of Medicine, The Stritch School of Medicine, Loyola University, Chicago, Illinois.

Table 1

CLINICAL DATA

Patient	Sex	Age	Weight (kg)	Diagnosis and cause of death	Intravenous C ¹⁴ -digitoxin		Interval between last dose and death
					Mg	Days before death	
E.W.	F	74	40	Severe generalized atherosclerosis. Death due to cerebral vascular thrombosis	0.2	5	16 hrs
					0.1	2	
M.S.	M	66	87	Acute myocardial infarction, auricular flutter, uremia. Death due to uremia	0.1	11	36 hrs
					0.1	10	
					0.1	6	
					0.1	5	
					0.1	4	
					0.1	2	
T.K.	F	60	47	Hepatic carcinoma, metastatic. Death due to carcinomatosis	0.5	6	35 hrs
					0.1	5	
					0.1	4	
					0.1	3	
					0.1	2	
					0.1	1	

from tissue samples by slight modifications of a method that has been described previously.⁽²⁾

RESULTS

The concentrations on a tissue-weight basis of unchanged digitoxin and its metabolic products in the tissues of the three patients are given in Table 2. The tissue concentrations expressed as per cent of the total dose administered are given in Table 3.

Although it has long been postulated that the heart has a selective affinity for digitoxin, it is interesting to note that the myocardium in all three patients had no greater affinity for the glycoside than any of the other tissues. The kidneys, contents of the gall bladder, jejunum, ileum, and colon, on a tissue-weight basis, had higher concentrations of the unchanged drug than the cardiac tissue. However, a comparison of the concentration in the myocardium with that in the skeletal muscle indicates a greater uptake by heart muscle.

When the concentration of the unchanged drug was expressed in terms of the per cent of the total administered dose per organ, the liver had the greatest amount followed in decreasing order by the jejunum, jejunal contents, ileum, kidney, whole blood, and colonic contents. Lower concentrations were found in the ventricles, spleen, tissue of the colon, ileal contents, gall bladder and contents, and the auricles. It is believed that some of the glycoside is stored in skeletal muscle, but since the specific activity of the administered

Table 2

TISSUE DISTRIBUTION OF RADIOACTIVE DIGITOXIN IN SAMPLES FROM
HUMAN SUBJECTS. CONCENTRATION EXPRESSED IN μg OR μg
EQUIVALENT PER 100 g TISSUE

Sample	Subject E. W.			Subject M. S.			Subject T. K.		
	Digitoxin	Metabolites	Total C^{14}	Digitoxin	Metabolites	Total C^{14}	Digitoxin	Metabolites	Total C^{14}
Liver	0.67	5.40	6.07	0.53	6.74	7.27	0.94	5.75	6.69
Gall bladder	0.92	5.04	5.96	1.40	4.26	5.66	1.46	8.11	9.57
G.B. contents	1.20	9.53	10.73	2.12	19.00	21.12	3.66	21.09	24.75
Jejunum	0.80	6.00	6.80	0.60	5.26	5.86	1.77	12.22	13.99
Jej. contents	1.87	10.27	12.14	1.98	16.00	17.98	1.81	10.17	11.98
Ileum	0.44	2.73	3.17	0.75	6.59	7.34	0.33	2.34	2.67
Ileum contents	2.00	2.92	4.92	0.65	5.53	6.18	1.63	10.25	11.88
Colon	0.33	0.80	1.13	0.60	2.62	3.22	1.56	3.68	5.24
Colon contents	4.57	3.20	7.77	6.67	10.72	17.39	12.96	8.22	21.12
Kidney	2.33	4.67	7.00	1.22	5.83	7.05	2.45	6.10	8.55
Ventricle	0.83	1.40	2.23	0.75	1.87	2.62	2.04	4.73	6.77
Auricle	0.53	1.00	1.53	0.41	0.97	1.38	1.02	1.57	2.59
Spleen	0.94	16.20	17.14	0.67	7.49	8.16	9.56	10.63	11.19
Lung	1.00	1.78	2.78	0.87	1.93	2.80	1.56	2.51	4.07
Adrenal	0.67	2.00	2.67	0.75	1.95	2.70	2.16	3.87	6.03
Skel. muscle	ND*	ND	ND	ND	trace	trace	trace	0.32	0.32
Blood	0.01	0.50	0.60	0.10	0.35	0.45	0.25	0.70	0.95

* Not detectable.

Table 3

DISTRIBUTION OF RADIOACTIVE DIGITOXIN IN SAMPLES FROM HUMAN
SUBJECTS. CONCENTRATION EXPRESSED AS PER CENT OF
ADMINISTERED DOSE PER ORGAN

Sample	Subject E. W.			Subject M. S.			Subject T. K.		
	Digitoxin	Metabolites	Total C ¹⁴	Digitoxin	Metabolites	Total C ¹⁴	Digitoxin	Metabolites	Total C ¹⁴
Liver	2.81	22.67	25.48	1.52	19.10	20.62	2.51	15.29	17.80
Gall bladder	0.05	0.27	0.32	0.04	0.11	0.15	0.01	0.04	0.05
G.B. contents	0.05	0.39	0.44	0.26	2.28	2.54	0.06	0.36	0.42
Jejunum	0.85	6.40	7.25	0.49	4.34	4.83	0.53	3.63	4.16
Jej. contents	0.25	1.37	1.62	0.59	4.81	5.40	0.16	0.90	1.06
Ileum	0.61	3.80	4.41	0.56	4.95	5.51	0.13	0.92	1.05
Ileum contents	0.64	0.93	1.57	0.14	1.20	1.34	0.06	0.40	0.46
Colon	0.45	1.09	1.54	0.21	0.92	1.13	0.29	0.68	0.97
Colon contents	1.24	0.82	2.06	1.44	2.32	3.76	1.03	0.66	1.69
Kidney	1.79	3.57	5.36	0.91	4.33	5.24	0.49	1.22	1.71
Ventricle	0.88	1.49	2.37	0.55	1.37	1.92	0.51	0.84	1.35
Auricle	0.09	0.18	0.27	0.08	0.19	0.27	0.08	0.21	0.29
Spleen	0.20	3.46	3.66	0.19	2.15	2.34	0.05	0.85	0.90
Lung	2.00	3.56	5.56	1.41	3.13	4.54	0.47	0.75	1.22
Adrenal	0.03	0.10	0.13	0.03	0.09	0.12	0.03	0.05	0.08
Skel. muscle	?	?	?	?	?	?	?	?	?
Blood	0.95	4.76	5.71	1.08	3.64	4.72	0.72	2.00	2.72

drug was relatively low, quantitative determinations of skeletal muscle concentration were not made.

Considering that less than one per cent of the administered dose can be found in the ventricle as unchanged digitoxin, one is impressed by the minute amount of drug (approximately 2 μ g or less per 100 g of tissue) required for the cardiotonic action of digitoxin on the myocardium.

Information on the metabolic conversion of digitoxin was obtained by calculating the metabolite-digitoxin ratio for each tissue sample (Table 4). The high ratio for the liver

Table 4
METABOLITE-DIGITOXIN RATIO FOR VARIOUS TISSUES

Sample	Subject E. W.	Subject M. S.	Subject T. K.
Liver	8.1	12.7	6.1
Gall bladder contents	7.9	8.9	5.8
Jejunum	7.5	8.8	6.9
Jejunum contents	5.5	8.1	5.6
Ileum	6.2	8.8	7.1
Ileum contents	1.5	8.5	6.3
Colon	2.4	4.4	2.4
Colon contents	0.7	1.6	0.6
Kidney	2.0	4.8	2.5
Ventricle	1.7	2.5	2.3
Auricle	1.9	2.4	1.5
Spleen	17.2	11.2	18.9
Lung	1.8	2.2	1.6
Adrenal	3.0	2.6	1.8
Blood	5.0	3.5	3.6

indicates that this may be the major organ of detoxification. The high ratio for the spleen is difficult to explain because the spleen is not considered to be an important detoxifying organ. However, the high ratio may be due to the accumulation of metabolite-protein complexes by the reticulo-endothelial cells. The relatively high ratios for the contents of the gall bladder and for the small intestine may reflect the movement of digitoxin and its metabolic products from the liver without further degradation of the parent compound.

Evidence for the biliary excretion of the cardiac glycoside was indicated by its presence and that of its metabolic products in the gall bladder and intestinal tract. Both digitoxin and its metabolites are reabsorbed during their passage through the small intestine

as evidenced by their lower concentration in the ileum and its contents both on a tissue-weight and percentage basis. The higher concentration in the colonic content reflects their concentration through reabsorption of water by the colonic mucosa.

It is interesting to note the greater rate of reabsorption of metabolites by the small intestine as compared with the reabsorption of unchanged digitoxin. This is shown by the decrease in the metabolite-digitoxin ratio during passage of the labeled products from the small to the large intestine.

The metabolic fate of digitoxin in this series of patients is presented in the schematic diagram shown in Figure 1. Briefly, the metabolic fate of the glycoside may be summarized

Figure 1. Metabolic fate of radioactive digitoxin in human subjects.

as follows: after an intravenous injection of digitoxin, the drug disappears at a rapid rate from the vascular system with approximately 60 per cent of the dose leaving the circulating blood within 15 minutes after its administration.⁽⁴⁾ During this period and after the digitoxin blood level has reached equilibrium, some of the glycoside is metabolized by the liver, and the digitoxin and its metabolic products enter the gastro-intestinal tract by means of the biliary route. A major part of the metabolites and some of the unchanged digitoxin

are then reabsorbed by the small intestine and enter the entero-hepatic cycle. During this cycle, small quantities of the metabolic products and less of the unchanged drug are continuously removed from the vascular system by the kidney. Results from an earlier study⁽²⁾ indicate that of the original dose, approximately 71 per cent was excreted as metabolic products via the urine and only 8 per cent as unchanged digitoxin. In the feces, approximately 9 per cent was excreted as unchanged digitoxin and 8 per cent as metabolic products.

The cumulative action of the cardiac glycoside is borne out by the presence of unchanged digitoxin in the urine as long as 50 days after a single administration.

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THE ISOLATION OF THE NON-SPECIFIC HYALURONIDASE
INHIBITOR OF HUMAN BLOOD^{*†‡}

By

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SUMMARY

The purposes of this investigation were to develop a method for the isolation of a substance in normal human blood that inhibits the enzyme, hyaluronidase, and to learn more of its chemical nature.

A homogeneous substance that inhibits bovine testicular hyaluronidase was isolated from human blood by means of fractionation procedures. An investigation of its physical properties revealed that it contains amino acids and hexosamine and it gives the carbazole color reaction for uronic acid. The substance contains less than 0.1 per cent sulfur as sulfate, less than 0.2 per cent phosphorus, and less than 0.5 per cent lipid. There was no evidence that the inhibitor contained heparin. The molecular weight of the substance was estimated to be 100,000, its mobility in Veronal buffer at pH 8.6, $\mu = 0.1$, was -5.4×10^{-5} $\text{cm}^2 \text{sec}^{-1} \text{volt}^{-1}$, and the sedimentation constant, $s_{20,w} = 3.75$.

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‡ From the Departments of Pediatrics and Biochemistry and the Argonne Cancer Research Hospital, The University of Chicago, and the La Rabida Jackson Park Sanitarium, Chicago, Illinois.

THE USE OF Cr^{51} AND Fe^{59} IN A COMBINED PROCEDURE TO STUDY
ERYTHROCYTE PRODUCTION AND DESTRUCTION IN NORMAL
HUMAN SUBJECTS AND IN PATIENTS WITH
HEMOLYTIC OR APLASTIC ANEMIA^{*†}

By

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SUMMARY

Although the radioiron dilution technique⁽¹⁻³⁾ may be used to determine the rate of erythrocyte production and destruction in human subjects, the authors have found that the use of Cr^{51} and Fe^{59} in a combined procedure provides more accurate information erythrocyte survival in man. This method is also a simple and reliable means of measuring blood volume,⁽⁴⁾ which is necessary for calculation of the plasma turnover rate and the per cent of iron utilized in the formation of erythrocytes.

METHOD

The radioiron that was used was carrier-free Fe^{59} , obtained from Oak Ridge as FeCl_3 in 0.080 N HCl solution, with a specific activity of 4.57 mc per mg. It was adjusted to pH 6 and autoclaved. According to the method of Huff⁽²⁾ for determining plasma clearance and plasma iron turnover, 2 to 4 μc of Fe^{59} was added to about 20 cc of plasma. The mixture was incubated for 30 minutes at 37°C, and the radioactivity (cpm) in an aliquot was measured. The solution was then injected by means of a heparinized syringe and an 18-gauge needle into a large vein in the arm of the subject being studied. Thereafter, heparinized blood samples were withdrawn at appropriate intervals. The hematocrit of each sample was recorded after it had been centrifuged for 30 minutes at 2500 rpm. One-ml aliquots of plasma were counted in duplicate in a well-type scintillation counter.

The rate of clearance of Fe^{59} was calculated by plotting on a semi-logarithmic scale the total count per minute in the plasma of 3 ml of whole blood against time. If the data were corrected for the slight radioactivity in the red cells, there was no significant dif-

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ference in the half-time clearance from the plasma.

Plasma iron was determined by the method of Barkan and Walker,⁽⁵⁾ and iron binding capacity by that of Rath and Finch.⁽⁶⁾ Plasma iron values were found to range between 70 to 125 γ per cent and serum iron-binding capacity between 175 and 225 γ per cent.

Plasma iron turnover was determined by application of the following formula⁽²⁾

$$\frac{0.693 \times \text{plasma iron mg per ml} \times \text{plasma volume} \times 24}{\text{half-time in hours} \times \text{weight in kg}}$$

The quantity of Fe^{59} incorporated into newly-formed red cells was determined by withdrawing heparinized blood samples, usually every other day, starting on the morning after plasma clearance had been established. The hematocrit value of each sample was recorded. Three-ml aliquots of each blood sample were stored in duplicate in the cold. After several collections, the samples were counted. The radioactivity of red cells was calculated from the hematocrit values. Since no significant activity was found in the plasma after 24 hours, the radioactivity of the washed red cells was not measured.

The per cent iron utilization was then calculated as follows:⁽¹⁾

$$\frac{\text{cpm per ml red cells} \times \text{red cell volume} \times 100}{\text{total cpm injected}}$$

The radiochromium, as $\text{Na}_2\text{Cr}^{51}\text{O}_4$, obtained from Oak Ridge through Abbott Laboratories, had a specific activity of 1.66 mc per mg.

The following formula of Gray and Sterling⁽⁴⁾ was used to calculate blood volume:

$$\frac{\text{cpm per ml pre-injection sample} \times \text{ml blood injected}}{\text{cpm per ml post-injection sample}}$$

The post-injection sample was withdrawn at one hour. Red cell volume was determined by multiplying the total blood volume by the uncorrected venous blood hematocrit value. Normal values ranged between 50 and 80 ml per kg. Normal values for red cell volume ranged between 23 and 36 ml per kg and for plasma volume, between 27 and 44 ml per kg.

Erythrocyte survival was studied as described previously.^(7,8) The half time survival for normal human subjects was found to be 33 ± 3.2 days.

Ordinarily, the Cr^{51} -labeling procedure was initiated several days after the administration of Fe^{59} . After the administration of Cr^{51} , the radiation from both isotopes may be determined in a single sample by means of the formula

$$C_a = \frac{T_a - K_F T_\beta}{1 - \frac{K_F}{K_C}} \quad \text{and} \quad F_\beta = T_\beta - \frac{T_a - K_F T_\beta}{K_C - K_F}$$

or

$$F_{\beta} = T_{\beta} - \frac{C_{\alpha}}{K_C}$$

Where C_{α} is the portion of pulse height α due to Cr^{51} ; F_{α} that due to Fe^{59} ; C_{β} the portion of count at pulse height β due to Cr^{51} ; F_{β} that due to Fe^{59} ; T_{α} the total count at pulse height α ; and T_{β} that at pulse height β . Thus, $T_{\alpha} = C_{\alpha} + F_{\alpha}$ and $T_{\beta} = C_{\beta} + F_{\beta}$. By definition, $K_C = \frac{C_{\alpha}}{C_{\beta}}$ and $K_F = \frac{F_{\alpha}}{F_{\beta}}$. The constants K_C and K_F are found by counting Cr^{51} and Fe^{59} standards, and T_{α} and T_{β} are counts obtained using mixed samples.

Radioactivity of both isotopes was determined in a well-type scintillation counter with a thallium activated sodium iodide crystal. A scaler, which could be preset to select impulses only above a desired energy level and which eliminated background, was used. This discriminator was effective enough to permit differentiation between the emissions from Cr^{51} and those from Fe^{59} .

Using a Cr^{51} standard, a pulse height (α) was selected at which Cr^{51} could be counted with maximum efficiency. The radioactivity emitted by Fe^{59} was calculated by selecting a second pulse height (β) that was essentially free from Cr^{51} emissions.

RESULTS

Normal subjects: Representative curves for Fe^{59} plasma clearance, per cent Fe^{59} utilization, and erythrocyte survival are shown in Figure 1.

The half-time for Fe^{59} plasma clearance is shown to be approximately 1 hour, a value that is somewhat less than that found by other investigators.^(2,3) The per cent utilization curve (Figure 1) resembles those reported by Finch *et al.* In a series of 6 normal subjects, the maximum Fe^{59} utilization occurred within 7 days in 2 individuals and within 12 days in all of them. The maximum uptake was between 80 to 95 per cent of the injected iron in these persons. It is quite possible that the high specific activity of Fe^{59} used may account in part for the minor differences in the iron plasma clearance and per cent iron utilization.

The Fe^{59} plasma turnover ranged from 0.38 to 0.65 mg per kg per day in the normal individuals who were studied. These values are to be expected in view of the more rapid Fe^{59} plasma clearance data.

Hemolytic anemia: Data obtained from a patient with an acquired hemolytic anemia are shown in Figure 2. In our experience, the Fe^{59} plasma clearance in this disease state may occur so rapidly that no reliable information can be obtained from an attempt to measure this value or that of Fe plasma turnover. However, the per cent Fe^{59} utilization has been found to be reliable and reproducible.

The curve in Figure 2 shows that radioiron is taken up more rapidly than is the case in normal human beings but the uptake never reaches the maximum normal values.

Figure 1. Typical curves for Fe⁵⁹ plasma clearance, radioiron utilization by newly-formed erythrocytes, and survival time of a mixed population of Cr⁵¹-tagged erythrocytes in a normal subject.

Figure 2. Typical curves for Fe⁵⁹ plasma clearance, radioiron utilization by newly-formed erythrocytes, and survival time of a mixed population of Cr⁵¹-tagged erythrocytes in a subject with a hemolytic anemia.

Aplastic anemia: Data obtained from a patient with idiopathic aplastic anemia are given in Figure 3.

The delayed Fe^{59} plasma clearance, poor utilization of the Fe^{59} , and relatively normal red cell survival curve are in accord with the usual findings in patients with aplastic anemia.

Figure 3. Typical curves for Fe^{59} plasma clearance, radio-iron utilization by newly-formed erythrocytes, and survival time of a mixed population of Cr^{51} -tagged erythrocytes in a subject with idiopathic aplastic anemia.

It is often the case that patients with this disease have no demonstrable capacity for iron-binding. In such circumstances, the method to determine iron plasma clearance, which has been described, does not give accurate quantitative data. This is also true in cases of hemolytic anemia where the iron-carrying protein in the plasma is saturated. For the same reason, determination of the Fe^{59} plasma turnover rate by the method is unreliable.

The use of Fe^{59} and Cr^{51} in a combined procedure to determine respectively erythrocyte production and destruction has been found to be exceedingly useful in establishing courses of treatment for patients with hemolytic or aplastic anemias. The demonstration of accelerated red blood cell destruction is an indication for a therapeutic trial of cortisone or ACTH, and perhaps splenectomy. It must be emphasized however, that the accelerated curve may possibly reflect gastro-intestinal bleeding since such a condition is not uncommon in patients with aplastic anemia. Hence, efforts to diagnose this should not be overlooked.

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THE EFFECT OF BORATE AND SULFATE IONS UPON THE
ELECTROPHORETIC MOBILITY OF MUCOPROTEINS^{*†}

By

E. Goldwasser and M. B. Mathews[‡]

An appreciable increase in the electrophoretic mobility of the carbohydrate-containing protein that is a hyaluronidase inhibitor⁽¹⁾ was noted when sulfate or borate ions were present in the buffer solutions. Borate ions are known to form charged complexes with a variety of carbohydrates, and for this reason it seemed likely that the increased mobility was due to complex formation with the carbohydrate moiety of the mucoprotein. The action of the sulfate ion, however, was unexplained.

Crystalline bovine serum albumin was obtained from Armour and Company. Dr. R. J. Winzler of the University of Illinois supplied a sample of orosomucoid, a purified serum mucoprotein. The serum inhibitor of hyaluronidase had been isolated from human plasma during some earlier experiments.⁽¹⁾

Buffers of ionic strength 0.1 and pH 8.6 were used for electrophoresis (Table 1). Serum albumin and orosomucoid were studied electrophoretically in a Spinco model H apparatus with 11-ml cells at 0° and at field strengths of 4.5 to 5.2 volts per cm. A 2-ml semi-micro cell at a field strength of 0.75 volts per cm was used for the hyaluronidase inhibitor. The electrophoretic mobilities are given in Table 2.

Table 1
COMPOSITION OF BUFFERS, 0.1 μ AND pH 8.6

Component	Veronal-borate (molarity)	Veronal-sulfate (molarity)	Borate-sulfate (molarity)
Veronal	0.011	0.0037	--
Sod. Veronal	0.075	0.025	--
Boric acid	0.086	--	0.086
Sod. borate	0.025	--	0.025
Sod. sulfate	--	0.025	0.025

* Based on a communication submitted to the Journal of the American Chemical Society.

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Table 2
DESCENDING MOBILITIES ($\times 10^5 \text{ cm}^2 \text{ volt}^{-1} \text{ sec}^{-1}$) IN VARIOUS
BUFFERS AND RATIO TO MOBILITY IN VERONAL BUFFER

Buffer	Albumin [*]		Orosomucoid [*]		Serum inhibitor [†]	
	Mobility	Ratio	Mobility	Ratio	Mobility	Ratio
Veronal	-6.7	1.0	-5.2	1.0	-5.4	1.0
Borate-sulfate	-8.1	1.2	-7.1	1.4	-10.2	1.9
Veronal-borate	-6.6	1.0	-6.2	1.2	-7.5	1.4
Veronal-sulfate	-8.0	1.2	-6.8	1.3	--	--

* Concentration was 5 mg/ml.

† Concentration was 3 mg/ml.

The presence of borate ion in the buffer was found to increase the mobility of orosomucoid about 20 per cent and that of the hyaluronidase inhibitor about 40 per cent. There was, however, no measurable effect upon the mobility of the serum albumin. The presence of sulfate ion in the buffer had a pronounced effect on all of the proteins that were studied. A minimum of three moles of sulfate ion are bound per mole of serum albumin according to calculations based upon the charge-mobility relationship established by Longsworth and Jacobsen.⁽²⁾ Although dialysis equilibrium studies, in which methyl orange was used as the competing ion, did not indicate appreciable binding of the sulfate ion by albumin, such a method may be too insensitive for this type of assay.

It has been shown that mono- and oligosaccharide derivatives combine strongly with borate if a glycosyl hydroxyl group and an adjacent hydroxyl group are free.⁽³⁾ Borate may also combine, although less strongly, with carbohydrate when only vicinal groups are available.

Northcote⁽⁴⁾ found that at pH 9.2 the trans- and cis-vicinal hydroxyl groups of neutral polysaccharides complex with borate. Since the comparisons were not made in buffers of the same ionic strength, this conclusion has not been clearly established.

It is quite possible that the carbohydrate moiety of the mucoprotein contains vicinal groups that complex with borate ion. Complex formation, in the case of the hyaluronidase inhibitor, occurs without detectable denaturation.⁽¹⁾

Electrophoresis of mucoproteins in borate-containing solutions appears to be an excellent technique for the separation of these compounds and may provide information about their molecular structure.

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A NEW ROUTE FOR THE SYNTHESIS OF
OCTOIODOFLUORESCEIN^{*+}

By

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SUMMARY

The new method of synthesizing the 2',7'-diiodo-tetra-iodofluorescein offers a new route for preparing compounds with iodine present in the 2',7'-position of the fluorescein ring. The unequivocal preparation of octoiodofluorescein establishes the orientation of the iodine atoms and provides a simpler and more efficient route for the preparation of this highly iodinated fluorescein dye.

* Summary of a paper submitted for publication in the Journal of Organic Chemistry.

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