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The Effect of Hepatectomy on the Conversion of ^{14}C-Glucose to Amino Acids in the Brain of the Rat.

EUNICE V. FLOCK AND CHARLES A. OWEN, JR. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

Glucose-U-^{14}C was administered in doses of 10 or 20 μC by single intravenous injections to normal rats, to rats with low concentrations of glucose in the blood due to continuous infusion of insulin and glucose for a six-hour period, and to still other rats 6 and 18 to 20 hours after hepatectomy. The body temperature of the hepatectomized rats studied for six hours was maintained at about 33° C by external heating, while the temperature of those studied from 18 to 24 hours after hepatectomy was only a few degrees above room temperature. From 5 to 30 minutes after injection of ^{14}C-glucose, the rats were anesthetized with pentobarbital sodium (Nembutal) and 30 seconds later they were frozen in liquid nitrogen. The frozen brains were chiselled out of the cranium. Measurements were made of the concentration and radioactivity of glucose, lactic acid, and the individual amino acids in the brain.

The concentration of aspartic acid was increased somewhat in the brains of the insulin-treated rats and decreased in the hepatectomized rats. No change was found in the quantity of glutamine after injection of insulin, but there was a very marked increase after hepatectomy. A moderate decrease in concentration of glutamic acid was found in the brains of both insulin-treated rats and hepatectomized rats. The concentration of alanine increased greatly after hepatectomy and that of γ-aminobutyric acid changed very little.

More than 40 per cent of the ^{14}C in the brain was found in the amino acids five minutes after injection of ^{14}C-glucose and about 70 per cent was found 30 minutes after the injection. Higher percentages of ^{14}C were found in the amino acids when insulin was given to the rats and much smaller percentages of ^{14}C were in the amino acids when warm rats received ^{14}C-glucose by injection six hours after hepatectomy or cool rats received it 18 to 24 hours after hepatectomy.

Glutamic acid, the most abundant amino acid in brain, was the one synthesized the most rapidly from ^{14}C-glucose. Ten minutes after injection of glucose, the amount of ^{14}C in the glutamic acid in the brains of normal rats, expressed as counts per minute per gram of wet brain, exceeded that present in glucose or lactic acid and continued to increase greatly during the next 20 minutes, while that in glucose and lactic acid decreased. During this 30-minute period only about 30 per cent as much ^{14}C accumulated in glutamine and aspartic acid as in glutamic acid and even less was found in γ-aminobutyric acid. Very small amounts of ^{14}C-alanine were found in the brain. ^{14}C-glutamic acid was produced from glucose in the brain even more rapidly in rats treated with insulin and there was also a moderate increase in the rate of production of ^{14}C-glutamine and aspartic acid. More ^{14}C-lactic acid was produced in five minutes in insulin-treated rats than in normal rats, but it disappeared more rapidly. The most striking change in ^{14}C-uptake in the brains six hours after hepatectomy in warm rats was found in the much greater production of ^{14}C-lactic acid. This greatly exceeded the production of ^{14}C-glutamic acid which occurred more slowly than in brains of normal rats.

The production of ^{14}C-glutamine was similar in amount in brains of hepatectomized and normal rats for 15 minutes. It then increased more rapidly in the hepatectomized rats and approached 80 per cent of that of glutamic acid within 30 minutes after the injection of ^{14}C-glucose. The production of ^{14}C-alanine in the brain was increased and that of ^{14}C-aspartic acid was decreased after hepatectomy.

Determination of Renal Clearances by Diatrizoate ^{125}I and Orthoiodohippurate ^{131}I. CHARLES D. FARMER, W. NEWLON TAUXE, FRANK T. MAHER, AND JAMES C. HUNT. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

A technic for measuring glomerular filtration rates (GFR) and effective renal plasma flow (ERPF) based on a single intravenous combined injection of sodium diatrizoate ^{125}I (DTZ-^{125}I) and orthoiodohippurate ^{131}I (OIH-^{131}I) was compared with conventional inulin and para-aminohippuric acid (PAH) clearances determined simultaneously in 35 human subjects with various renal or renovascular problems. Solutions were assayed for purity by chromatographic technics.

Results indicate that by employing, as a model, an open two-compartment mammillary system similar to that suggested by Sapirstein, GFR could be calculated from the plasmatic disappearance curve of DTZ-^{125}I. There was excellent correlation with the simultaneously performed conventional inulin clearance. The mean clearance value of the group for inulin was 93.1 ml per minutes with a standard deviation of 29.2. The mean clearance value for DTZ-^{125}I was 97.3 ml per minute with a standard deviation of 28.4. The difference between the mean clearance values of the group obtained by the two methods was 4.2 ± 1.4. The standard error of the estimate was 8.30, and the linear regression equation, $C_{inulin} = 0.987\ C_{DTZ} - 2.86$.

Similarly, ERPF calculated from the OIH-^{131}I plasma curve correlated closely with simultaneously performed PAH renal clearances. The mean clearance value of the group for PAH was 378.8 ml per minute with a standard deviation of 124.6. This compared with a mean clearance value of the group for OIH-^{131}I of 364.4 ml per minute with a standard deviation of 108.5. The difference of the mean clearance values obtained by the two methods was 13.6±6.6; the standard error of the estimate was 36.9, and the linear regression equation, $C_{PAH} = 1.1\ C_{OIH} - 22.5$.

Isotope Renography: Assessment of Renal Function Before and After Surgery for Upper Urinary Tract Obstruction. GEORGE M. BERGER, W. NEWLON TAUXE, JAMES C. HUNT AND ORMUND S. CULP. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

Eleven patients with upper urinary tract obstruction were assessed by means of isotope renography before and after surgical relief of the obstruction. The standard procedure previously published by the Mayo Clinic group was used. The following parameters of the renogram curve were measured: peak activity, peak time, initial slope of the secretory segment, 20 and 30-minute values, and the drop (at 20 minutes) from peak activity. An improvement in the slope of the secretory segment in the early postoperative period of eight of the 11 patients was tentatively considered to be a reflection of increased effective renal plasma flow. This improvement was maintained in several follow-up renograms. The excretion of dye from the renal pelvis, as reflected by the terminal segment of the renogram, was decreased in the majority in the early postoperative period. Excretion from the renal pelvis improved with time and increased the flow of urine. Apparently the restricted drainage that occurred during early recovery from adequate surgical intervention was unlikely to result in continued impairment of renal function. Finally, the authors stressed the difficulties and pitfalls inherent in extrapolating from the normal renogram curve to interpret the pathologic changes.

Factors Affecting Thyroxine Exchange Between Rat Liver and Blood. **COLUM A. GORMAN, EUNICE V. FLOCK, CHARLES A. OWEN, JR. AND JAIME PARIS. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).**

The isolated perfused rat liver was used to study factors regulating the exchange of thyroxine between liver and blood.

Sprague-Dawley rats were injected intravenously with 50 to 100 mC (1.8 to 5 μg) of L-thyroxine (T_4) labeled with ^{131}I. At times ranging from 1 to 18 hours later, the livers were removed and perfused with nonradioactive rat blood. Radioactivity within the liver was followed by a graphic recording from a collimated scintillation crystal aimed at the liver. The perfusing blood was sampled periodically and analyzed for radiothyroxine and radioiodide. Bile was collected at hourly intervals, and its total content of radioactivity measured. Labeled thyroxine and its derivatives in blood, liver, and bile were chromatographically separated and quantitated.

Results showed that thyroxine could be released from the isolated liver at intervals up to at least 18 hours after T_4 had been given to the rat and that the thyroxine was returned to the blood in a chromatographically unchanged form. Outflow of thyroxine from liver to blood was diminished when sodium salicylate was added to the perfusate. When thyroxine within the liver and in the blood had reached an equilibrium, exchange transfusion resulted in a further outflow of thyroxine from the liver and the establishment of a new equilibrium. Use of ^{125}I and ^{131}I-labeled thyroxine in the same experiment enabled us to demonstrate movement of radiothyroxine from liver to blood and concurrently in the opposite direction, thereby illustrating the dynamic nature of the equilibrium. The addition of 2 mg of stable thyroxine to the blood reduced the hepatic release of labeled thyroxine to the blood and increased biliary excretion of the compound.

Livers from rats given radioactive triiodothyronine were compared with those given radioactive thyroxine, and further parallel studies were done in which labeled thyroxine or triiodothyronine was added to the blood perfusing a nonradioactive liver.

Splenic Sequestration of Erythrocytes in the Rat. **CHARLES A. OWEN, JR. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).**

There is abundant evidence in man that ^{51}Cr from labeled erythrocytes tends to concentrate in the spleen and liver. In certain hemolytic diseases the splenic-^{51}Cr predominates, a fact which may be of prognostic value when splenectomy is contemplated.

A study of splenic sequestration of labeled erythrocytes was carried out in normal rats. The cells were universally labeled *in vivo* with ^{59}Fe by twice weekly injections of ^{59}ferrous citrate, for 60 days (one red cell life of span), *in vitro* with ^{51}Cr by conventional technics, or by both methods. Disappearance of radioactivity from the body was assessed by a whole body counter consisting of two 5-inch sodium iodide (Tl) crystals facing each other. Small samples of blood were collected periodically for measuring ^{59}Fe, ^{51}Cr, or both. Animals were sacrificed regularly for quantitation of the distribution of the isotopes.

The ^{51}Cr disappeared from the body of normal, male white rats at the rate of about 1.3 per cent per day. The isotope disappeared from the bloodstream about three times faster. The spleen was the principal organ for sequestration of the ^{51}Cr, containing about two thirds of all ^{51}Cr in the body, other than that in the bloodstream. In splenectomized rats, sequestration of ^{51}Cr shifted to the liver but not to the extent that it concentrated in the spleen of intact animals.

The ^{51}Cr in the spleen did not represent intact erythrocytes, however, because much less ^{59}Fe accumulated in that organ that did ^{51}Cr. There is the possibility that the spleen trapped the cells, lysed them, split off the heme, and retained the ^{51}Cr-globin. This seems more likely than the possibility that ^{51}chromic ion was split from the erythrocytes and trapped by the spleen, because intravenous ^{51}Cr was almost quantitatively excreted in the urine. It also seems unlikely that hemoglobin, released by destroyed erythrocytes, was selectively trapped by the spleen since intravenously administered hemoglobin-^{59}Fe-^{51}Cr was not taken up by that organ.

Comparison of Insulin Protein Binding by Radioelectrophoretic and Radioimmunologic Technics. PASQUALE J. PALUMBO, W. NEWLON TAUXE AND GEORGE D. MOLNAR. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

The serum protein binding of exogenous insulin-^{131}I was studied in 54 subjects: 51 diabetic patients and three neonates of diabetic mothers. The 51 diabetic patients included eight menstrating and 13 pregnant women, three insulin-resistant patients, seven patients treated with nicotinic acid, phenformin, and adrenal steroids, and one patient treated by hypophysectomy for advanced diabetic retinopathy. Diurnal serum specimens were examined in 15 of these patients.

Two approaches were used for determination of insulin binding levels: combined radiochromatography and radioelectrophoresis (Berson-Yalow method) and the radioimmunologic method (Skom and Talmage).

With the former method, the range of insulin binding was 0.1 to 37 units per liter of serum in all cases except the insulin-resistant patients, in whom 36 to 97 units of insulin were bound per liter of serum. Electrophoretograms showed the binding of radioiodinated insulin applied *in vitro* to be between the beta-gamma and gamma globulin regions.

Seventy-nine of the serum specimens were examined independently by the radioimmunologic method by precipitation of the human serum gamma globulin-insulin-^{131}I complex by antihuman gamma globulin serum (Skom-Talmage method). In the insulin-resistant patients, binding values ranged from 37.5 to 77.5 per cent of radioiodinated insulin bound to gamma globulin. In all others, the insulin binding ranged from 3.1 to 21.8 per cent of radioiodinated insulin bound to gamma globulin.

The coefficient of correlation between the two methods was 0.86. The regression equation was $Y = 3.4 + 1.24X$, in which Y = the Skom-Talmage value and X = the Berson-Yalow determination. Under the various conditions studied, no significant change was noted in the serum protein binding of insulin-^{131}I in a given subject except for one insulin-resistant patient in whom the level of insulin binding decreased significantly 2½ months after administration of adrenal steroids.

Adjuvant Use of Radioactive Colloids in the Treatment of Carcinoma of the Ovary. MARGARET A. HOLBROOK, DONALD S. CHILDS, JR. AND JOHN S. WELCH. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

The definitive treatment of malignant lesions of the ovary is surgical. Postoperative radiation therapy is indicated in those cases with actual or potential spread to the peritoneal cavity. A cancerocidal dose of radiation can be delivered to minute masses of malignant cells on the peritoneal surfaces or in the peritoneal space by radioactive gold (^{198}Au) colloid instilled in the peritoneal cavity.

Between 1952 and 1957, 68 patients at the Mayo Clinic were treated with the instillation of 140 mC of ^{198}Au-Colloid into the peritoneal cavity in the early postoperative period. The overall 5-year survival rate was 63 per cent (43 of 68 patients). The 5-year survival rates according to extent of disease were as follows: Group 1 in which the tumor had spread to the surface of the ovary but was removed totally at operation, 82 per cent (nine of 11 patients); group 2, in which there was contamination of the peritoneal cavity by spill of cystic contents but with no visible spread beyond the ovary, 68 per cent (15 of 22 patients); group 3 in which there was gross spread to the peritoneum but all peritoneal spread was removed, 68 per cent (13 of 19 patients); and group 4 in which peritoneal spread was not completely removed at operation but no individual mass of remaining tumor was more than 2 mm in diameter, 38 per cent (six of 16 patients).

Serious complications were limited to intestinal damage, evidenced by intermittent or complete obstruction, and occurred in 7 of the 68 patients (11 per cent); five of these patients had had additional external irradiation to the pelvis.

In the published paper, results also are given according to other classifications which utilize the histologic type of tumor as well as the anatomic extent of disease.

Design of the Mayo Whole Body Counter. ALAN L. ORVIS. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

A preliminary survey of background levels in potential sites showed variation by a factor of four. Total counts in the range 0.05 to 2.55 MeV, measured with a 5-inch (o.d.) by 4-inch sodium iodide (T1) crystal were reduced from 939 to 10.4 counts per second by the steel room. Walls consist of 6-inch thick steel, lined with 1/16 inch of lead. The inside dimensions of the room are 6½ feet wide, 8 feet long, and 7½ feet high. The sodium iodide counter is used for spectrometry, but gross counting is done by a group of eight plastic detectors, 18 inches square by 6 inches thick. Each plastic detector is viewed by four EMI 5-inch photomultipliers. The plastic detectors are placed on movable mounts so that response characteristics as a function of geometric arrangement may be studied for different radioisotope distributions. Resolutions of the plastic detectors for potassium-40 is 12.5 per cent HWHM (half-width half-maximum).

Special nonoverloading amplifiers and a fast coincidence system are used to minimize pile-up effects, to evaluate noise reduction, and to investigate coincidence whole-body counting in special situations. Punched paper tape for computer processing (IBM 7040) of the analyzer data is to be available.

The Effect of Craniotomy on the Brain Scan. PETER D. VAN VLIET, W. NEWLON TAUXE AND HENDRIK J. SVIEN. (Mayo Clinic and Mayo Foundation, Rochester, Minnesota).

Postsurgical brain scans were studied with polyvinylpyrrolidone (PVP) labeled with ^{131}I in 32 patients (43 scans) to determine the effects of surgery on the vascular permeability of the brain. Increased accumulation of PVP-^{131}I was found in the craniotomy site in all patients scanned in the postoperative period between 8 and 79 days. The pattern of localization was distinctive and could be differentiated from the pattern shown by a tumor. In two cases slightly increased accumulation occurred after retraction of the cerebral cortex only had been carried out; scalp incision alone (for temporal artery biopsy) did not result in increased uptake. In patients who had no evidence of tumor recurrence, there was no abnormal localization in scans made more than 79 days after craniotomy.

The accumulation of scanning medium is presumably due to alterations in the blood-brain barrier. Whether this alteration is reflected in the postoperative clinical condition remains to be determined.