rather than by mechanically reducing outflow. Therefore, a reduction in water and salt secretion by whatever means can apparently produce proportional decreases in enzyme secretion.

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7. Flow during the first collection period of 2 to 5 minutes after 90 minutes of blockage was substantially augmented relative to prior control flow rates (2.7 times on the average). The total cumulative increase in flow over controls amounted to a mean increase of 95 μl. This suggests that the duct system has a substantial capacitance, most probably in the larger collecting vessels. The capacitance would be of about the same volume as that of the duct itself, as estimated by extracellular space markers in the free-flow situation [M. Rossier and S. S. Rothman, *Am. J. Physiol.* **228**, 1199 (1975)]. The presence of a capacitance can also be inferred in another manner. When an open cannula was raised to the column height at which flow just ceased, flow began again in a matter of seconds. The column was continuously raised in this manner, until flow stopped permanently. This is consistent with the gradual filling of a capacitance.
8. The more complex model of bidirectional exocytosis or exocytosis linked to endocytosis cannot explain the present results in a simple fashion. For example, in a bidirectional model, the reduced output would presumably result from increased reuptake of enzyme. However, increased reuptake would have to be regulated relative to flow in order to maintain the concentration of enzyme in the duct system constant over a wide range of flow and during total blockage; that is, it would have to increase in exact proportion to the reduction in flow. Furthermore, in order to explain the fact that the concentration of enzyme in the duct increases and decreases independently of flow under stimulated conditions, we would have to further hypothesize that the regulated reuptake could itself be regulated; namely, the relationship between protein efflux from the cell and its reuptake could be varied independently of flow.
9. These experiments also bear upon another aspect of pancreatic secretion. It has been proposed that cells lining the ducts, as opposed to or in addition to cells in or near the acinus, are responsible for the addition of a substantial proportion of the secreted fluid [I. Schulz, A. Yamagata, M. Weske, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **308**, 277 (1969); S. A. Mangos and N. R. McSherry, *Am. J. Physiol.* **221**, 496 (1977)]. If this is true, then the acinar secretion of digestive enzyme would be diluted by fluid as it traveled down the ducts. In this case, during a flow reduction produced by blockage or back pressure, the concentration of enzyme throughout the duct system would increase over time toward that at the site of enzyme secretion. Since the concentration of enzyme did not increase during 90 minutes of blockage, it can reasonably be concluded that an admixture of a significant amount of fluid by cells lining the ducts during spontaneous flow does not occur.
10. We thank Dr. J. Ho and R. Fielding for their valuable discussions. Supported by NIH research grant AM16990 and training grant GM00927.

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Tissue Perfusion Rate Determined from the Decay of Oxygen-15 Activity After Photon Activation in situ

Abstract. *Rates of cerebral perfusion were obtained from measurement of the disappearance (wash-out) of oxygen-15 after in situ tissue activation with 45-million-volt x-rays. In an anesthetized cat, typical values were 90 milliliters per minute per 100 grams of tissue, with 55 percent wash-out. In a specific radiotherapy patient, the value was 65 milliliters per minute per 100 grams of tissue, with 63 percent wash-out of oxygen-15 through incorporation into tissue water.*

Continuous perfusion of all body tissues by blood is required for their vitality. Measurement of the distribution of cerebral perfusion in patients with occlusive cerebral disorders can reveal the location of infarcted tissue and the degree to which blood flow to this tissue can be restored by surgical, radiotherapeutic, or pharmacological intervention. In cancer patients, the rate of tumor perfusion influences significantly the transport of both oxygen and drugs to malignant tissue volumes. Measurement of tumor perfusion and its manipulation by the introduction of heat, anesthesia, and vasoactive drugs can lead to improved radiotherapy and chemotherapy. The radioisotope clearance technique, which utilizes gamma and positron cameras to image the accumulation (wash-in) or removal (wash-out) of radioactivity in the region of interest after the intravascular injection or inhalation of radiopharmaceuticals, has been employed to study regional tissue perfusion in animal and human subjects (*1*, *2*). However, the technique has often been limited in its ability to quantitate blood flow in poorly perfused tissue. The signal obtained from the area of low blood flow can be substantially contaminated by detected emissions from adjacent, well-perfused tissue, with its correspondingly greater uptake of radioactive indicator (*3*). Where clinically feasible, injection of the indicator directly into the poorly perfused area can, in principle, overcome the above limitations, and has indeed been employed to study tumor perfusion by radioisotope wash-out (*4*). However, the results obtained by direct injection can be influenced significantly by the degree to which the microcirculation is perturbed by tissue pressure gradients, and by the failure of the injected indicator to adequately diffuse throughout the relevant volume prior to the initiation of radioisotope imaging.

Radioactive isotopes of carbon, nitrogen, and oxygen can be introduced directly, uniformly, and noninvasively into the tissue region of interest through activation of the tissue by high-energy radiation. Reports of activation of tissue elements in vivo by 33- and 45-MV x-rays (*5*, *6*) and 200-MeV protons (*7*) may be found in the literature. We report here the results of quantitative studies of regional tissue perfusion employing in situ activation by photons in a 45-MV clinical betatron x-ray beam. Interaction of beam photons with tissue element nuclei produces ^{11}C, ^{13}N, and ^{15}O, all of which decay by positron emission, with half-lives of 20.5, 10.0, and 2.0 minutes, respectively.

An emitted positron interacts with tissue atoms and, with an atomic electron, is quickly annihilated, producing two gamma-ray photons of energy 0.51 MeV, approximately 180° apart. After activation, the positron activity from a given volume of irradiated tissue is monitored as a function of time, employing standard coincidence counting apparatus and techniques to detect pairs of annihilation gamma rays. Analysis of the time dependence of positron emission from a static, tissue-like gelatin cylinder revealed that the separate contributions to the total activity from ^{15}O and ^{11}C could be identified, and that the contribution from ^{13}N was negligible. The analysis also established that, during the first few minutes after the completion of activation, nearly all of the coincidence signal was due to the decay of ^{15}O in the activated volume. Therefore, in our studies of tissue perfusion, the radioactive indicator must be ^{15}O, and the rate of tissue perfusion must ultimately be determined from the measured half time for wash-out of this radioisotope from the imaged volume of interest.

The time dependence of the measured decay of ^{15}O activity from a volume of living tissue is governed by the fate of this radioisotope after it is produced. The ^{15}O nuclei emerge from photonuclear reactions as recoil ions moving through the tissue medium. A certain fraction of these "hot" ions will attach to cell proteins (or protein fragments) and, as the labeled macromolecules cannot diffuse through capillary walls, the decay of the signal from such a "fixed fraction" will be purely physical, with a half-life of 2.0 minutes. The measure of

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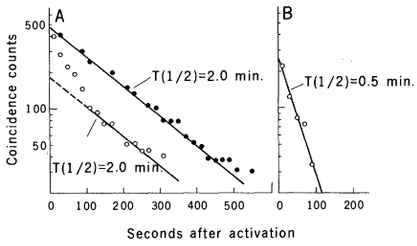

Fig. 1. Photon activation-^{15}O decay studies of cerebral perfusion in a cat. The maximum radiation dose to the irradiated brain tissue was 100 rad. The imaged volume was about 5 cm^3. Each data point represents coincidence counts registered in a 20-second interval. (A) Total signal spectra. Open circles are data from a live cat, anesthetized with Nembutal. Closed circles are data from the same animal after its heart was stopped by an overdose of Nembutal. The indicated half times of 2.0 minutes identify the purely physical decay of ^{15}O and, therefore, the portion of this induced radioisotope fixed in tissue. (B) Spectrum due to the decay of mobile ^{15}O, incorporated into tissue water in the live cat. The decay half time of 0.5 minute identifies the half time for the physiological wash-out of ^{15}O to be 0.7 minute, corresponding to a cerebral perfusion rate of 90 ml/min per 100 g of tissue (*16*).

tissue perfusion is obtained from the complementary "mobile fraction" of ^{15}O, which is incorporated into tissue water ($H_2{}^{15}O$). The radioactive tissue water is washed out of the imaged volume through transcapillary exchange with water in plasma, and it is from the half time for such wash-out that the rate of tissue perfusion is determined. Studies in our laboratory with irradiated, packed red blood cells and also with irradiated rat hearts have established that substantial wash-out of ^{15}O, consistent with the above interpretation, can be expected after in situ photon activation of living tissue (*8*). Thus, from the data collected from gelatin and living matter, it is assumed that for several minutes after activation, the time dependence of positron emission from an activated tissue volume may be adequately described by two exponential terms, representing, respectively, signal decay from fixed and mobile fractions of ^{15}O. The contribution to the total signal from the ^{15}O fixed in tissue is identified from the latter portion of the spectrum and is subtracted (over the entire range) from the total signal, revealing the decay of the signal from ^{15}O incorporated into tissue water. The half time for decay of the mobile fraction is then determined from the remaining exponential. The reciprocal of this half time is the sum of the reciprocals of the physical half-life of ^{15}O and the half time for wash-out of ^{15}O in tissue water. The rate of tissue perfusion (that is, of capillary blood flow expressed in milliliters per minute per 100 grams of tissue) averaged over the imaged volume is very nearly equal to the reciprocal half time for ^{15}O wash-out multiplied by 69.3 (*9, 10*).

We have employed the photon activation-^{15}O decay technique to study tissue perfusion in animal and human subjects. The results of studies of cerebral blood flow in a cat anesthetized with Nembutal are presented in Fig. 1. Activation was produced by a 2.5-cm-diameter beam directed through the brain from above, and the activity was detected by two sodium iodide detectors (2.5 cm in diameter, 2.5 cm thick), operating in coincidence, on either side of the cat's head (*11*). The dose given to activate the brain tissue was 100 rad. About 20 seconds after the completion of activation, the detection equipment was turned on and the coincidence counts per 20-second interval were recorded for the next 10 minutes, at which time they approached background. In Fig. 1A, the open circles represent raw data obtained from the living, anesthetized animal. The closed circles represent data obtained from the same animal, after the cat was given an overdose of Nembutal (intravenously) and the heart had stopped beating. Data from the live and dead animal were taken with the head activated in exactly the same position and receiving exactly the same dose.

Data from the live cat reveal a rapid disappearance of activity for the first 2 minutes, and then a slower rate of decay, characterized by a 2-minute half time for the remainder of the collection interval. This slower constant rate of decay is that of the fixed (or immobile) ^{15}O discussed earlier. The rate of decay of ^{15}O in $H_2{}^{15}O$ is obtained by subtraction (Fig. 1B). The measured resultant half time of 0.5 minute identifies the half time for wash-out of radioactive tissue water to be 0.7 minute, corresponding to a rate of cerebral perfusion (averaged over about 5 cm^3) of 90 ml/min per 100 g of tissue. This value compares favorably with those obtained in animal studies employing other techniques (*12*). Comparison of the counts obtained from the fixed and mobile fractions of ^{15}O during the 20-second interval immediately following activation (determined by extrapolation) indicates that 55 percent of the induced activity washed out of the imaged volume while 45 percent was immobile. The 45 percent not washing out presumably is partially accounted for by nonperfused regions such as cerebrospinal fluid (which washes out very slowly) and bone, and the remainder is due to ^{15}O chemically fixed in the tissue.

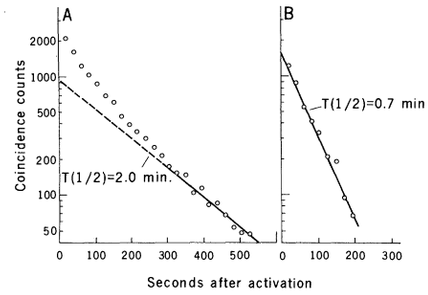

Fig. 2. Photon activation-^{15}O decay study of cerebral perfusion in a cancer patient receiving therapeutic radiation to the brain. The given dose to the irradiated tissue was 100 rad. The imaged volume was about 15 cm^3. Each data point represents coincidence counts registered in a 20-second interval. (A) Total signal spectrum. The indicated half time of 2.0 minutes identifies the decay of ^{15}O fixed in tissue in the activated (imaged) volume. (B) Spectrum due to decay of mobile ^{15}O, incorporated into (diffusible) tissue water. The measured half time of 0.7 minute identifies the half time for ^{15}O wash-out to be 1.0 minute, corresponding to a cerebral blood flow of 65 ml/min per 100 g of tissue.

As indicated earlier, the closed circles in Fig. 1A are data obtained from the dead cat. It is observed that the initial count rate is essentially that obtained with the live cat, but that the decay is characterized over the full counting interval by a single half time of 2.0 minutes. Since none of the ^{15}O produced can now be removed from the activated (imaged) volume by wash-out, the indication of a purely physical decay of the ^{15}O signal is exactly as expected.

The data from photon activation-^{15}O decay studies of cerebral perfusion in a cancer patient receiving 45-MV x-ray therapeutic radiation to the brain are presented in Fig. 2A. Activation was produced by a 7 by 7 cm^2 beam incident anteriorly, perpendicular to the patient's forehead (*13*). The detection equipment was as described earlier, and the maximum dose to the irradiated volume was 100 rad. As in the studies on the cat, each data point represents a counting interval of 20 seconds. The data reveal a rapid disappearance of activity for about the first 3 minutes, followed as before, by a slower rate of decay characterized by a 2.0-minute half time. Extension of the "fixed fraction" decay back to the initial counting interval and subtraction from the total spectrum yields the decay of the "mobile fraction," that is, of the ^{15}O in tissue water (Fig. 2B). The half time of this decay is 0.7 minute, identifying the half time for wash-out of ^{15}O in tissue water to be 1.0 minute. The latter half time corresponds to an average rate of cerebral perfusion in the imaged volume (about 15 cm^3) of 65 ml/min per 100

g of tissue. This is within the range of values of cerebral blood flow obtained by other methods (*14*). Comparison of the counts from fixed and mobile fractions during the 20-second interval immediately following the completion of activation (determined by extrapolation) indicates that 63 percent of the induced activity washed out of the imaged volume and 37 percent remained immobile. As indicated in the discussion of the animal data, the immobile fraction is most likely due to the incorporation of ^{15}O in slowly diffusing cerebrospinal fluid and in bone, and also to chemical attachment of ^{15}O to tissue protein.

Preliminary data obtained from the application of the above technique to studies of perfusion in mammary tumors in C_3H mice and tumors (rhabdomyosarcoma) in WAG/Rij rats indicate, as expected, that the rate of perfusion varies widely from tumor to tumor. Our data also indicate that the rate of perfusion for a given tumor can be significantly affected by anesthetization of the animal subject. Such observations are consistent with those reported by other investigators (*15*).

We are developing the photon activation–^{15}O decay technique for the more precise, noninvasive measurement of regional tissue perfusion in animals under a variety of physiological and pharmacological stresses. We are also employing the technique to investigate perfusion rates in human tumors being treated with radiation. As applied to radiotherapy patients, this technique is truly noninvasive, since the radiation dose to the irradiated site is determined solely from therapeutic considerations. When the signal-to-dose ratio has been optimized by hardening the activating beam and by utilizing a positron camera for detection of pairs of annihilation photons, we anticipate extension of the photon activation–^{15}O decay technique to the study of other pathological conditions (such as cerebral ischemia) where the gravity of the prognosis will justify the radiation dose.

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Effect of Fluorine Substitution on the Agonist Specificity of Norepinephrine

Abstract. *Substitution of fluorine for hydrogen in position 2, 5, or 6 of the aromatic ring of norepinephrine markedly alters the α- and β-adrenergic agonist properties of norepinephrine. The 6-fluoro isomer is an α-adrenergic agonist with virtually no β agonist activity, while the 2-fluoro isomer is a β-adrenergic agonist with little α activity. The 5-fluoro isomer is equipotent with norepinephrine as an α agonist and significantly more potent as a β agonist. The possible physiochemical basis for these differences is discussed.*

We have been investigating the effects of fluorine substitution on the aromatic ring in a series of ring-hydroxylated biogenic amines. Our hope was that increased phenolic pK_a, altered hydrogen-bonding properties, and enhanced lipophilicity would produce altered but ultimately predictable behavior with respect to transport mechanisms, enzyme active sites, and receptor recognition. To this end, we prepared a series of ring-fluorinated tyramines (*1*), dopamines (*1*), and 5-hydroxytryptamines (*2*). These analogs were evaluated in a number of systems, and the results suggested that they will be of considerable value as biochemical and pharmacological tools (*3*). We therefore extended our synthetic efforts and recently synthesized the three isomeric ring-fluorinated analogs of norepinephrine (NE), the primary adrenergic transmitter. We report that the site of fluorine substitution dramatically affects the specificity of these NE analogs and suggest that the present findings may have considerable significance regarding our knowledge of receptor-agonist interactions.

The synthetic route involved side-chain elaboration of the isomeric fluorinated 3,4-dimethoxybenzaldehydes prepared from the corresponding diazonium fluoroborates by our photochemical fluorination procedure (*1*). Cyanohydrin formation with trimethylsilyl cyanide (*4*), lithium aluminium hydride reduction, and boron tribromide demethylation afforded the desired ring-fluorinated (±) norepinephrines, isolated as the hydrochloride salt (*5*) (Fig. 1). The stability of the fluoroderivatives was similar to that of NE. Stock solutions in 0.001*M* HCl were kept at −20°C for several weeks without loss of biological potency.

The effect of fluorine substitution on the α-adrenergic agonist properties of (±)NE was determined in the isolated aortic strip of the guinea pig (Fig. 2A) and the β-adrenergic agonist properties in the isolated guinea pig atrial preparation (Fig. 2B) (*6, 7*). As indicated by the dose-response curve of aortic strip contraction (Fig. 2A), 5- and 6-fluoronorepinephrine were equipotent with (±)NE [median effective concentration (EC_{50}) = 2.6 μ*M*] as α-adrenergic agonists. The α-adrenergic activity of 2-fluoronorepinephrine (EC_{50} = 110 μ*M*) was marginal, with the compound exhibiting a potency only 0.03 times that of (±)NE. Pretreatment of the aortic strip with the α-adrenergic blocking agent phentolamine (10 μ*M*) completely blocked the response to both (±)NE and the fluorine derivatives, whereas pretreatment with the β-specific blocking agent propranolol (10 μ*M*) had no effect. The β agonist potencies are indicated by the dose response for the increase in rate of contraction of the atrial preparation shown in Fig. 2B. 2-Fluoronorepinephrine was equipotent with (±)NE (EC_{50} = 0.7 μ*M*) in this regard, whereas the 5-fluoro derivative (EC_{50} = 0.1 μ*M*) was approxi-

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