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RADIOSCINTIGRAPHIC STUDIES OF ^{11}C DISTRIBUTION IN CATS GIVEN 1-^{11}C-ETHANOL

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In vivo studies of ^{11}C isotope distribution in cats given 1-^{11}C-ethanol show accumulation of radioactivity in liver. Redistribution of radiolabel occurs after ethanol loading. Results indicate that some aspects of the metabolism of ethanol in specific tissues can be assessed by gamma-ray scintigraphy.

Many studies in both animals and humans show that ethanol has a number of direct effects upon intermediary metabolism. However, it is not known how these effects contribute to the development of the chronic dysfunction of organs such as the brain or liver or to the behavioral changes that are seen in ethanol addicts. Metabolic studies in man are not easily achieved since sampling of tissues must be limited to accessible organs where the risk of sampling injury is low. Thus, investigations on the critically ill patient or the study of vital organs such as the brain usually are not feasible.

We are exploring the possibility that useful information about the effects of ethanol can be obtained from radioscintigraphic studies in which labeled metabolites are given to the intact animal. Carbon-11-labeled compounds are used in these studies to take advantage of the central role of carbon in human metabolism and of the fact that carbon isotope substitution does not alter the behavior of small organic compounds. Carbon-11 has a short half-life (20 min) and decays by emitting a positron, which on annihilation results in the production of a pair of 511-keV gamma rays. It has not been used widely as a research or clinical tool because an accelerator is needed for its production and methods for the production and detection of compounds labeled with this radionuclide have not been generally available. Recently, however, a number of laboratories have made considerable progress toward the production and use of ^{11}C-labeled compounds. This includes the synthesis of compounds such as ^{11}C-cyanide, ^{11}C-dopamine, and a number of biologically active amino acids and carboxylic acids (*1–6*).

Since there is significant interest in the development of new high-energy linear accelerators that can be used for radiation therapy, the reaction for producing ^{11}C by linear accelerators reported in this paper can be used for hospital production and thus make this ^{11}C technique more widely available. The close proximity of the Stanford Linear Accelerator to the Division of Nuclear Medicine has allowed the development of methods to use this isotope. We now report the results of preliminary tests that are done in cats with 1-^{11}C-ethanol. This compound has been given in both "high" and "low" specific activity doses to assess the effect of acute expansion of the body ethanol pool upon radionuclide distribution.

EXPERIMENTAL

Production of $^{11}CO_2$. The $^{11}CO_2$ used is obtained from the Stanford Linear Accelerator Center where

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high-energy electrons and positrons (2–22 GeV) dissipate their energy in water targets or beam dumps. Radiation effects and collisions with atomic nuclei result in formation of an electromagnetic cascade (e^-, e^+, photons, etc). This cascade interacts with target nuclei which emit one or more nucleons producing isotopes of the same or of different elements of lower atomic number. Carbon-11 is produced by photospallation of oxygen ${}^{16}_{8}O(\gamma - 2p3n){}^{11}_{6}C$ by high-energy photons and appears as $^{11}CO_2$. This $^{11}CO_2$ is carried by an air stream flowing at 6 to 9 liters/min into 100 ml of 4N NaOH. Yields of 100–500 mCi of ^{11}C are easily retrieved in the form of $Na_2{}^{11}CO_3$ by this method since the total inventory of ^{11}C at saturation in the water target used is of the order of 1 Ci/kW.

1-^{11}C-ethanol synthesis. The solution of 4N sodium hydroxide containing the $Na_2{}^{11}CO_3$ was transferred with nitrogen under pressure to a releasing flask that was connected through an acetone-dry ice-drying trap to a separatory funnel containing methyl magnesium bromide (Grignard reagent). The $^{11}CO_2$ was released from the sodium hydroxide trap solution by slowly dropping sulfuric acid (25 ml concentrated acid plus 150 ml water) from a separatory funnel into the releasing flask. The releasing flask was heated to insure the complete liberation of $^{11}CO_2$. Nitrogen gas was used during the heating to flush the $^{11}CO_2$ into the Grignard reagent (10 ml of 3M ether solution of Grignard reagent plus 50 ml of freshly distilled anhydrous ether). After 10 min the carbonated Grignard solution was allowed to flow into a three-necked distillation flask containing 1 gm of $LiAlH_4$ in 5 ml of anhydrous ether (freshly distilled and dried over $LiAlH_4$). A stream of nitrogen was continuously bubbled through the reaction mixture while being stirred by a magnetic stirring bar. After 5–10 min the reaction mixture was hydrolyzed by slowly adding 10 ml of water from a separatory funnel that had been previously attached to one of the necks of the flask. The resulting aqueous solution of ethanol (6–8 ml) was separated from the by-products by fractional distillation.

Gas-liquid chromatography of the distillate showed it to contain 0.16% by weight of ethanol and 0.07% by weight of diethyl ether in water. Representative samples showed a radioactivity in the ethanol of 2–5 mCi of ^{11}C/ml. All the radioactivity was associated with the ethanol peak. The total time from the start of synthesis to the injection of labeled compound into the animal was 60 min. The ratio of the radioactivity of the tagged ethanol to the total activity of sodium hydroxide trap was 1/10. Hence, 1 mCi of tagged ethanol is produced from a sodium hydroxide trap containing 10 mCi of ^{11}C.

Gamma-ray scintigraphy. The ethanol solution was made isotonic with sodium chloride and transported to the Division of Nuclear Medicine of the Stanford Medical Center. Here an anesthetized cat ("Pentasol" 0.44 ml/kg) was positioned under a pinhole-collimated gamma camera (Searle Radiographics Pho/Gamma III Scintillation Camera System Model 6403) equipped with supplementary 5-cm thick lead shielding. Anesthesia was necessary to immobolize the cats so that interpretable scintigraphs could be obtained. Although anesthetics have an effect on alcohol metabolism, the comparative results obtained are still valid in development of scintigraphic techniques using ^{11}C-ethanol or other ^{11}C compounds. The isotope (2–20 mCi) was injected through a catheter in the cat's femoral vein. Eight cats were used (four in high-specific activity studies and four in low-specific activity studies). In the high-specific activity "trace studies" the dose of ethanol was less than 10 mg/kg. In the low-specific activity studies the radioactive tracer was mixed with non-radioactive ethanol to give a total dose of 300 mg/kg. Injection volume was 3–8 ml. Data from the gamma camera were continuously collected and stored on the disk of a Hewlett-Packard Model 5407 computer. This portion of the study was terminated after 90 min. Fifty microcuries of ^{99m}Tc-sulfur colloid and/or ^{131}I-macroaggregated albumin were then given intravenously. The selective accumulation of these isotopes in the liver and lung of the animal was then recorded. At the completion of these studies, the data were displayed in a 64 × 64 channel matrix on the computer oscilloscope screen. The image thus obtained was divided into regions of interest as follows: the liver and chest areas were defined by the respective accumulation of ^{99m}Tc-sulfur colloid and ^{131}I-macroaggregate in these organs (*7,8*); the area of the heart was defined as the major location of the ^{11}C isotope in the chest within the first minute after the bolus injection; the remaining areas such as the head and peripheral tissues were defined as outlined in Fig. 1. This done, the ^{11}C radioactivity in each area of interest was then reported (corrected for decay) as a function of time.

RESULTS

The results of all studies appear in Fig. 1. Here the distribution of radioactivity at each 1-min interval of the study is reported as a part of the total activity present in the animal. The cumulative contribution of ^{11}C in each region to total activity is given as a percent by the ordinate. Over 50% of the activity is present in the liver and lungs. The scintiphotos of Fig. 2 show the typical distribution of ^{11}C in a "tracer study". The data of both Figs. 1 and 2

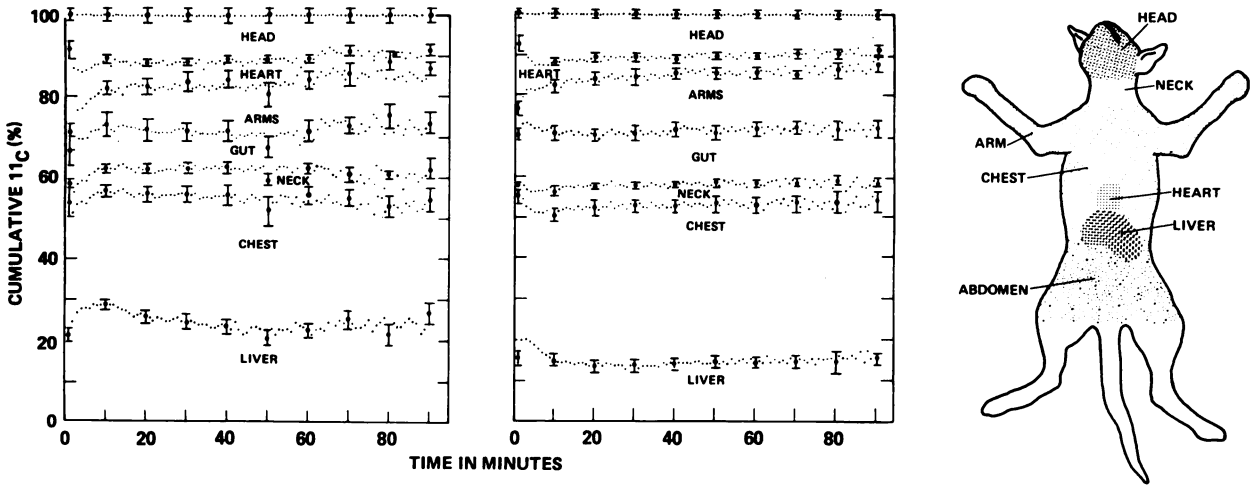

FIG. 1. Carbon-11 distribution in each region of cat is given by areas between data points. Activity in each region is added to that in next region and is expressed as percent of total activity. Thus it is possible to observe changing activity in each region of cat as function of time and total activity. Four cats were used in each study. Results shown in graph on far left were obtained from trace cats and those given in center graph were obtained from cats given an ethanol load. Bars indicate standard deviation. Carbon-11 loss as CO_2 is not shown. Note relatively large amount distributed to liver of trace cats. Loading with ethanol increases activity in chest but does not change fraction of total activity in head region.

indicate that the radiocarbon is in general uniformly distributed to all regions of the animal's body with the exception of the liver (which is easily identified because of a relatively high activity) and the extremities (which are not easily seen). It would appear that equilibration of isotope is remarkably delayed in both of these areas. Activity is continuously accumulated in the "arms" for at least the first 50 min of the study while it is first accumulated, then released, and then possibly again very slightly increased in the liver. Delayed equilibration of isotope in peripheral tissues such as the arms has been reported by investigators using different techniques. This has been related to skeletal muscle content of bound water and/or to relatively lower blood flow to the tissues (*9,10*). Ethanol is mainly oxidized in the liver at least for the first step of the oxidation (*11*). It is oxidized primarily by alcohol dehydrogenase to acetaldehyde and then to acetate or its activated form, acetyl coenzyme A, by aldehyde dehydrogenase (*11*). It is presumed, therefore, that the differences in isotope turnover in the liver obtained in the present studies involve these enzymes and metabolites. The exact nature of the involvement is not known at this time. This rather unique behavior of the liver is not surprising. This organ has relatively larger amounts of alcohol dehydrogenase in contrast to all other regions such as the brain or peripheral tissues (*11*).

Figure 1 also shows that there is a relative de-

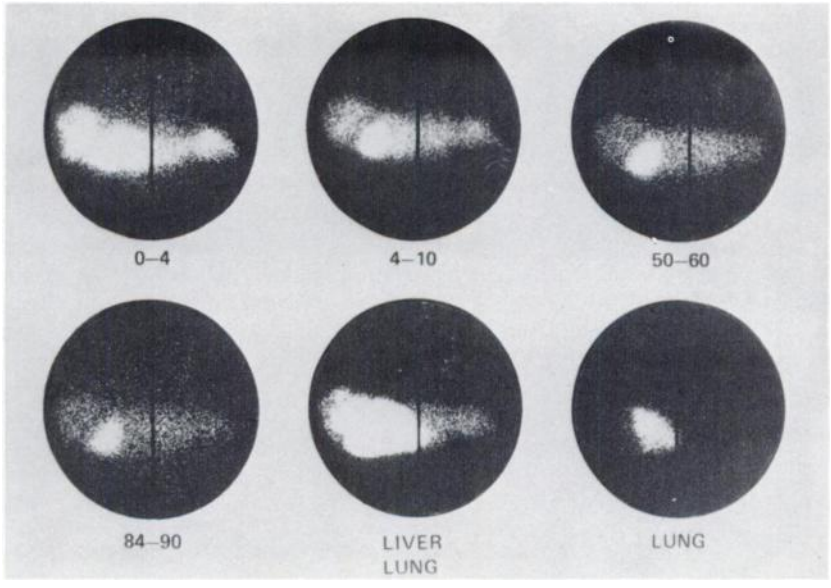

FIG. 2. Scintiphotographs of ^{11}C distribution in "trace cat" taken 0–4, 4–10, 50–60, and 84–90 min after injection of 1-^{11}C-ethanol. Scintiphotographs labeled liver/lung and lung were obtained with standard ^{99m}Tc-sulfur colloid and ^{131}I-macroaggregate to indicate position of these organs relative to ^{11}C distribution. Animal head is to right. Limited detector field prevented visualization of hind paws.

crease in the amount of ethanol in the liver in the low-specific activity "load" studies. This change is associated with an increase in activity in the "lung" area. The reduction in the liver area most likely occurs because alcohol dehydrogenase displays zero order kinetics for the metabolism of ethanol to acetaldehyde (*11*). However, it is also possible that ethanol may have an additional direct effect on the liver or that cardiac output may have been reduced so as to cause an increase in lung water (*12*). There is also some increase in radioactivity in the arms and "gut" areas. However, no change is noted in the distribution of label into the brain. Also, our findings give direct support to the generally held notion that although equilibration is delayed in peripheral tissues, ethanol is present in most organs (particularly the brain) in direct proportion to the amount of ethanol ingested.

We conclude that it is possible to use ^{11}C-labeled compounds to obtain useful in vivo information by gamma scintigraphy that should be explored further for possible use in studying the effects of ethanol in metabolism. Indeed, the unique character of isotope turnover in the liver suggests that special metabolic functions of specific organs may eventually be detected. It should be understood that ^{11}C distribution and not ethanol distribution is measured in this study. The information obtained is thus a complex measure of the movement of carbon atoms in the pathways of ethanol metabolism. Parallel studies with ^{14}C compounds now under way in our laboratories show that this type of measurement can be clinically useful (*13–15*). For example, measurements of the clearance rates of $^{14}CO_2$ into expired air have been used for diagnosis of hematologic disorders and for the evaluation of the effects of drugs (including ethanol) upon human metabolism. We are now planning double-isotope studies in which appropriate additional radionuclides will be used simultaneously for measurements of isotope distribution into the blood or urine compartments. We are hopeful that such subsequent studies will lead to a new and direct approach in the evaluation of drug kinetics in man.

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