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TRIP REPORT - LOS ALAMOS SCIENTIFIC LABORATORY ON 14 JANUARY 1971

A meeting was held in the H-4 Division building of Los Alamos Scientific Laboratory on 14 January 1971 at the suggestion of DBM. The purpose of the meeting was to explore the possibility of inter-laboratory collaboration on a ¹³C clinical demonstration program.

The meeting was attended by the following personnel: From LASL, D. G. Ott, C. T. Gregg, E. C. Robinson, N. A. Matwiyoff, and B. Tolbert (presently a LASL consultant from the University of Colorado). From the Medical Department of BNL, W. W. Shreeve. From BIM of Argonne National Laboratory, P. Klein. From DBM, as observer, D. C. Borg.

The meeting was informal in character and grew out of the express need of the LASL group and of DBM to establish a firm market for ¹³C-labeled products, such that there would be sufficient assured utilization of ¹³C over the next few years to justify the setting up and operating of a ¹³C production plant (either by private industry or by AEC) whose volume output would be sufficient to keep the cost of ¹³C to the research community adequately low: i.e., in the range of \$5 to \$10 or lower per gram of 90% enriched ¹³C. The LASL group and Klein have explored a number of other potential uses for ¹³C on a large scale, such as the labeling of petroleum products from refineries or the labeling of lots of narcotic or other registered drugs; but despite some expressed interest in these potential applications, there was no indication of prompt sponsorship of development programs in this area. The general outlook for a demonstration of the utility of ¹³C in an application that would have moderate-to-large volume consumption upon its adaptation for production use seemed most promising in the area of clinical medicine. This conclusion had also been reached by the Monsanto Company group who were evaluating the feasibility of Monsanto's setting up a commercial production plant for enriched ¹³C. The general feeling seemed to be that clinical applications were immediately apparent, and if sufficiently reliable and inexpensive ¹³C detectors could be put in the hands of clinicians or clinical centers, and if there were clear demonstrations of diagnostically useful ¹³C applications, that than the medical community - as distinguished from medical research groups - would develop a requirement for ¹³C that would amount to hundreds of kilograms a year. This, in turn, would clearly justify the commercial production of ¹³C; and once this was established, the likelihood of commercial houses developing catalogues of relatively cheap ¹³C labeled compounds for laboratory application would be very great. Malinekrodt has already

The Malinskrodt group also reported that they were interested in subsidizing a ^{13}C clinical demonstration experiment. Their inquiries have been directed at a collaboration at the University of Texas, but the details of the Malinskrodt plan were not recalled by the LASL people. Apparently the target disease here was rarer than that being considered by the Monsanto combine, because full utilization of the diagnostic tests that might derive from the demonstration experiment would develop a market of only five kilograms-per-year.

There was then some general discussion of the present status of the pursuit of various applications of ^{13}C at the market level. Clinical uses did appear most promising, because the tracing of oil spills with products labeled appropriately with ^{13}C at the refinery was not being considered seriously by any group in a position to fund the initial experiments. True, such a use would generate a phenomenal market for ^{13}C ; but some of the government agencies that had discussed this matter pointed out that the tracing of minor oil spills is not a great problem, whereas the tracing of major oil spills need hardly be done by a sophisticated physical technique since the identity of major oil leaks was not hard to establish. Hence the present outlook for the use of ^{13}C in the tracing of oil spills is quite uncertain. Most other large scale environmental tracer projects would be "one shot" deals and hence would not provide a continuing market for ^{13}C . Following an initial lead from DBM, Klein has been in contact with the Bureau of Narcotics and Dangerous Drugs of the Department of Justice concerning the question of coding of drug lots with enriched nitrogen, oxygen, and carbon isotopes in order to trace their passage in illicit commerce. Klein reports that the Bureau expressed real interest in exploiting such a possible labeling procedure, but it had not yet concluded that the matter was pressing enough to warrant their subsidizing initial demonstration experiments.

There was then some discussion of the present status of production activities and plans with regard to enriched isotopes of common elements. Robinson pointed out that the LASL group conceives of production plants where nitrogen-oxide distillations would be the core facilities. For example, an ICONS production facility would involve an NO distillation, to produce enriched ^{17}O , ^{18}O , and depleted ^{16}O , as well as ^{14}N and ^{15}N . A CO distillation plant for the production of enriched ^{13}C and ^{12}C then could be set up as a secondary facility. ^{13}C produced in this way would be almost "free," because the main operating costs for such a setup would be for liquid nitrogen to power the NO facility; and the CO plant could be put on-line downstream with an insignificant increment in liquid nitrogen utilization.

Robinson pointed out that there is already a very large market for enriched ^{16}O and that the CNC-4 group at LASL (formerly CMF-4) had already supplied

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126 million dollars worth of this isotope to the DMA. In addition to tracer applications in weapons diagnostics, etc. ¹⁶⁰ depleted in ¹⁷⁰ and ¹⁸⁰ is desired for use in plutonium oxide-fueled nuclear energy sources, such as heart pumps and high power-density SNAP reactors. This is because the (α , n) reaction on ¹⁷⁰ and ¹⁸⁰ gives a significant neutron radiation background unless the plutonium oxide is depleted in these isotopes. Conceivably the ¹⁶⁰ market would support the ^{13C} production as a by-product.

Another interest in stable isotope applications has been enthusiastically recognized and supported by Dr. Stout, of the ACBM. He feels that there is a sizeable market for isotope studies using enriched ^{15N} and depleted ^{14N} in various agricultural investigations.

The discussion then turned to problems of ^{13C} detection. At the level of research instrumentation that would be used for the detection of ^{13C} in pilot experiments, the use of mass spectrometers, with or without on-line gas chromatography, was recognized as the most obvious system for use. In addition, this appears to be the most sensitive detection system now available. On the other hand, should routine clinical applications for ^{13C} detection develop, cheaper and more rugged detection systems would be required. Infra-red detectors look promising in this regard. Infra-red detection using carbon monoxide or carbon dioxide (or even carbonate) can detect natural background ^{13C} at the present time. H. A. Gebbie at the National Bureau of Standards Boulder Laboratory has been doing Fourier transform far infra-red interferometry, and his explorations suggest that it may be possible to build a simple and rugged ^{13C} detector that might cost as little as about \$1,000 on a production basis. However, this device would require access to a computer that was able to do the Fourier transforms that are necessary to convert interferometric data to spectral displays that can be quantitated.

Increased sensitivity of mass spectrometer detection of ^{13C} is being explored at LASL. In the CNC-4 group the molecular beam entering the mass spectrometer is chopped, and detection is then phase-locked to the chopping frequency. Of course, this markedly increases signal-to-noise ratios, and in one case, using bromine isotopes, a signal-to-noise of ten was achieved with a 1 to 10,000 dilution. In principle, this phase-locked mass spectrometer system can be applied to other isotopes, but actual demonstrations are not yet in hand. Robinson pointed out that two beams in such a spectrometer, chopped at different rates, could be analyzed simultaneously so that controls and samples of isotope-enriched materials could be detected at the same time and their differences compared directly.

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Other research developments underway involve using light from Lyman alpha emissions to ionize molecular beams, rather than using approximately 70 electron-volt electrons, as in present spectrometers. The electron ionization procedure yields many fragments from any given complex organic molecule, while the Lyman alpha emission at approximately 10 electron-volts gives rise to a much simpler ion-fragment pattern, thereby simplifying the detection problem and adding to the sensitivity of detection of a particular species.

R. McDowell of CNC-4 is working with continuous infra-red analysis of carbon dioxide gas that is well dried, and this apparently will provide precision of $\pm 2\%$, with a sensitivity good enough to detect natural abundance ^{13}C . A commercial device might cost \$5,000 to \$6,000, but it is not clear that the clinically useful dilutions of 1 in 1000 to 1 in 10,000 could be detected in this way. Robinson then pointed out that for a special-purpose detector of mass ratios 44/45 in a single substance, such as CO_2 from the breath, leak detectors might be modified cheaply as special-purpose mass spectrometers. Many years ago Robinson had such an experience when leak detectors were modified to detect deuterium/tritium ratios in a weapons diagnostic experiment in which the mass spectrometer was consumed in the fireball and it was desired not to use an expensive instrument. It appeared likely to Robinson that the concept could be engineered up to produce a special-purpose detector for masses 44/45.

A proposal for a laser raman detector for ^{13}C used in clinical applications had been submitted to DBM by the Stanford Research Institute. Both Gregg and Tolbert had seen the proposal as referees, and they were both unimpressed. They felt the sensitivity of the device was borderline, that it required solidifying the carbon dioxide (which would be a major complication in the continuous analysis of breath carbon dioxide), and it would cost \$10,000 to \$15,000 on the production level.

Matwiyoff then pointed out that the detection of ^{13}C in liquids may be more promising than initially conceived. By looking at ^{13}C -proton sidebands in proton magnetic resonance, cheap NMR special-purpose spectrometers might be quite applicable to solutions that have modest concentrations of ^{13}C and are not too complex in terms of their molecular components.

Following the background discussions regarding ^{13}C production and detection, Shreeve commenced a review of promising clinical applications of ^{13}C . The discussion was extensive and cannot fully be reviewed here. However, it was based upon a brief outline compiled by Shreeve, a copy of which is enclosed. Following the initial review, there was a re-evaluation of the attractiveness of various proposed tests for the purpose of a pilot clinical demonstration program. The group felt that the factors to be

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optimized in making such a selection included: obvious clinical significance, application to a clinical condition of widespread incidence (rather than to a rare disease), and - of course - consideration of the practical problems of preparation of ¹³C materials required and of detection from some biological product. For a widely applicable clinical screening test, it was thought desirable to avoid a requirement for venepuncture, either for administration of materials or for the collection of biological samples. Thus oral administration of the labeled compound and collection of breath or urine would appear to be much more attractive prospects for widespread application to large numbers of individuals. Since there are very simple techniques for the collection of carbon dioxide in expired air, and since Shreeve and others already have documented with ¹⁴C studies certain metabolic defects which alter the appearance of carbon tracers in the breath, clinical tests involving ¹³C analysis in expired carbon dioxide were viewed as the first choice. Within this category, Shreeve's applications numbered 1, 2, 3, 5, and 6 receive the most serious consideration.

Program 2, absorption and oxidation of lactose as a test for lactose intolerance, was thought to be especially attractive. Lactose intolerance is seen in many non-Caucasians as a genetic factor, and pediatricians have a great need to establish the presence or absence of this intolerance in infants with diarrhea associated with mild milk intolerance. At present there is no practical test that can clearly resolve this diagnostic question, and most pediatricians are forced to ignore the matter or to lead their patients through cumbersome and unpleasant therapeutic trials. On the other hand, there is not presently available in Shreeve's clinical program a contact with patients of this sort. However, Tolbert was aware of an interest in lactose intolerance among some physicians at the University of Colorado Medical School, and he stated that he would pursue the possibility of a clinical study with them.

The oxidation of uniformly labeled ¹³C galactose as a test for galactosemia or early diabetes, Shreeve's application number 1, was also discussed at some length. Since galactose intolerance is a well recognized test for hepatic functional incompetence, the test could also serve a role in liver function determinations in medical diagnosis. Galactose oxidation to carbon dioxide is also remarkably sensitive to ethanol levels in the blood, such that a drink of alcohol drops breath ¹⁴C carbon dioxide from a ¹⁴C galactose load by 50%. (The reason for this is that a high liver balance of NADH/NAD markedly inhibits a liver epimerase enzyme that converts galactose-6-phosphate to glucose-6-phosphate, and ethanol rapidly increases liver NADH.) An especially attractive feature of this clinical application is that the ¹⁴C data already in hand show a specific activity dilution of carbon from galactose, as determined in expired air, of only 150 to 250

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times; and this is very favorable from the detection point of view. On the other hand, uniformly labeled ^{13}C -galactose is required, and about 18 grams of the material is needed for a single test.

There then followed some discussion of the advantages and disadvantages of using clinical loading tests with stable isotopic tracers. It was pointed out that with radioactive labels, such as ^{14}C , detection sensitivity permits very high dilution, so tracer studies that do not involve metabolic loads are often desirable. On the other hand, with stable isotopes a high metabolic dilution of labeled material makes detection exceedingly difficult, if not impossible; and therefore loading studies look very favorable for the application of stable isotopic labels in clinical tests.

Shreeve's application number 3 (the absorption and oxidation of unsaturated fatty acids labeled with ^{13}C as a test of faulty fat absorption in steatorrhea) also appeared to have a potential for wide clinical application. Although this application appears to be a straightforward extension of the kind of study now done with radioactive labels when there is a diagnostic need, there is no ongoing clinical program in this area; and considerable preliminary clinical documentation would be required before a ^{13}C demonstration could be effected.

Oxidation of glucose-1- ^{13}C to breath carbon dioxide as a test of early adult diabetes, or of the diabetic diaphesis in blood relatives of diabetic patients, appeared to have much promise in terms of clinical utility. Shreeve's studies with ^{14}C materials indicate that the suppression of labeled carbon excretion in the breath of obese patients with diabetic tendencies is about as sensitive an indicator of their pre-diabetic condition as is the most specific clinical test now known, namely the intravenous glucose tolerance test. However, whereas the intravenous glucose tolerance test requires repeated venepunctures (and therefore is not favorable in terms of a screening test that could be carried out with untrained personnel and that would have high patient acceptance), a ^{13}C screening procedure that involved only the ingestion of a nonradioactive oral load of sugar followed by the collection of some breath samples about an hour later might have great value in clinical medicine. Clinically the program has some advantage for a pilot study in that Shreeve has a patient study actively underway, using ^{14}C and tritium labels. Hence the ^{13}C experience could be compared directly with ^{14}C data in the same patients, and the additional ^{14}C clinical correlations would then serve to verify the diagnostic utility of a statistically small number of ^{13}C studies. Although the loading studies require approximately 40 grams of glucose per square meter of body surface, and the specific activity dilution in the test is of the order of 1 to 1,000 (and these features are not as favorable as the corresponding ones for clinical application number 1), the ^{13}C -labeled test material required

for this application need be labeled only in one carbon position, namely C1. Furthermore, the LASL group stated that they were aware of a reasonably attractive procedure for the direct synthesis of labeled glucose.

The last attractive clinical application was number 6, the oxidation of intermediary carbohydrates (such as pyruvate, acetate, lactate, and glycerol) to breath carbon dioxide as another test of early adult diabetes, as well as of some other less common clinical conditions. Shreeve has an active program going in this area, and he pointed out that the decrease in ^{14}C -oxidation of these intermediary carbohydrates is depressed in diabetes even more than is ^{14}C -glucose oxidation. However, the studies with ^{14}C have been tracer studies not involving loading. In principle, loading studies would also be useful clinically, and - as pointed out several paragraphs above - loading studies are highly desirable from the point of view of avoiding excessive dilution of stable isotope. However, in the absence of clinical data regarding the diagnostic interpretation of loading studies with intermediary carbohydrates, it would not be possible to demonstrate the clinical applicability of a pilot ^{13}C experiment without the prior documentation that loading studies with carbohydrate intermediates actually could be correlated with the diabetic diaphesis, as plausible as this extension of the tracer data might seem.

For all of these reasons, it appeared that the clinical study that was most attractive for immediate investigation was number 5, oxidation of glucose-1- ^{13}C to breath carbon dioxide. With regard to the preparation of ^{13}C materials for such a study, Ott and Gregg pointed out that there is presently no ^{13}C synthesis method for glucose and galactose. Since ^{13}C acetate already is in hand, ^{13}C -glucose-1-phosphate, glycerol, pyruvate, lactate, and - of course - acetate might be made more available by biosynthesis; and it was also likely that algae could be grown very rich in ^{13}C fatty acids. However, some algal biosyntheses for ^{13}C -glucose can be proposed, although they have yet to be checked out. But 1- ^{13}C -glucose could be made more directly, with the carboxyl group being labeled from ^{13}C carbon dioxide. If all the labeled carbons were useful for detection, uniformly labeled ^{13}C glucose might be a more efficient way to utilize the limited stock of ^{13}C , but in a case where detection is dependent upon the metabolism only of one specific carbon fragment - such as in the glucose-1-carbon test of Shreeve's clinical application number 5 - uniformly labeled material would be wasteful of scarce ^{13}C , and a specific label would be better. The LASL people felt that the use of ^{13}C for clinical applications would be limited for some years yet by the yearly production of seven kilograms total, some of which is committed already for other purposes. Therefore for the initial demonstration clinical experiments, selections should be made of tests that avoid

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excessive dilution of the label, and - as noted - only loading studies appear attractive from this point of view.

The matter of processing and collecting breath carbon dioxide samples appeared very simple. It has been demonstrated that one need only collect breath carbon dioxide in an alkaline solution, with the patient breathing out through a straw. Carbon dioxide may be collected to a desired total amount, as determined by an indicator in the solution. Then the alkaline solution containing the carbon dioxide as carbonate may readily be shipped to the detection facility. Acidification to gasify the carbon dioxide can then be done before detection. In the case of the pilot demonstration experiments, detection will be by mass spectroscopy, using Klein's apparatus at Argonne National Laboratory.

A discussion between Robinson and Klein concluded with the agreement that a carbon dioxide inlet for Klein's mass spectrometer could readily be made, and Ott had on hand packing material for gas chromatography columns that allows holdup of carbon dioxide. Hence it was thought likely that detection would involve mass spectroscopy following gas chromatography, which would be preceded by a cold sulfuric acid wash to trap water and other organics present in the gas which could interfere with the mass spectrographic determinations. Final analysis would then be made by a determination of mass ratios 44 and 45 on the mass spectrometer. Klein was confident that an isotopic enrichment of .01% could be detected using his apparatus in this manner. However, to get very fine sensitivity, much better counting statistics on the spectrometer could be achieved if carbon dioxide were first collected in a reservoir and then slowly bled into the mass spectrometer through a fine capillary. In this way the mass spectrographic analysis could continue for a long period of time, rather than being confined to the period of elution of carbon dioxide from the gas chromatographic column, which would be the limiting feature with the present setup. Klein was given some very fine brass capillary tubing by Robinson, and he was given some ^{13}C -enriched carbonate by Gregg. Klein said he intended to make an immediate conversion of his spectrometer so that it would have a high resolution capability for masses 44/45. He expressed enthusiasm at the prospect of being able to increase the sensitivity of ^{13}C detection in expired carbon dioxide in this way so that quantitative detection limits might be improved by a factor of 10 to 100 or more, over the approximately .01% isotopic enrichment limit of detection now available. Should this prove possible, then the amount of ^{13}C required for each clinical demonstration experiment would be correspondingly reduced, and the available ^{13}C supply could support a much larger clinical program than now appears possible.

On evaluating the requirements for a single clinical trial, using $1\text{-}^{13}\text{C}$ -glucose, it was estimated that 80 grams of total material would be

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required, with approximately 7% of this (5-6 grams) being ^{13}C . At the 1-to-1,000 specific activity dilution expected in breath CO_2 on the basis of Shreeve's ^{14}C studies, the breath would then show an isotopic enrichment of approximately .1% in its carbon dioxide component, assuming approximately 95% enriched starting material. The breath CO_2 enrichment is approximately ten times greater than the conservatively estimated safe detection limits noted in the preceding paragraph. Since, at this degree of enrichment, detection of ^{13}C by infra-red spectroscopy also seems feasible; it was proposed that duplicate breath samples be sent to Los Alamos for infra-red analysis.

Summary:

1. The "best" clinical demonstration proposal (based on considerations of patient availability, prior documentation with ^{14}C studies, diagnostic usefulness, a high incidence of the clinical condition being studied, the availability of an appropriate ^{13}C labeled material for a loading study, the efficiency of utilization of the ^{13}C label, and the ease and facility of obtaining samples for detection) was concluded to be the test for early adult diabetes based upon ingestion of approximately 80 grams of 1- ^{13}C -glucose, with collection of breath samples approximately one hour later (with the patients breathing out through a straw into an alkaline trapping solution). Shreeve would propose to carry out the studies with a patient group obtained from his present clinical program at BNL or his affiliations with the Meadowbrook Hospital on Long Island. Each patient would undergo parallel studies with ^{14}C -labeled glucose, and intravenous glucose tolerance tests and other documentations would also be carried out to insure the greatest possible clinical relevance of the limited number of ^{13}C studies that could be done.
2. Five to six grams of ^{13}C as 1- ^{13}C -glucose would be required for each patient study, with a total glucose load of approximately 80 grams. Studies to undertake the synthesis of this material would be in the hands of the LASL group, Gregg and Ott. Best estimates indicated that with the available ^{13}C for this use and an efficiency of approximately 40-50% in the glucose labeling step, sufficient material for 5-10 clinical studies could be made available.
3. The collection of samples appeared to be a trivial matter, as already noted. As alkaline solutions carefully sealed off from CO_2 exchange with the atmosphere, the samples could be shipped from Brookhaven to Argonne by commercial means without difficulty. Duplicate samples could be sent to LASL.

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4. The primary detection would be with a gas chromatograph/mass spectrograph apparatus in Klein's laboratory at ANL. Modifications to increase the sensitivity of separating mass ratios 44 and 45 will be undertaken directly by Klein, and if preliminary tests indicate that significant gains in detection sensitivity may be confidently expected, the requirements of ^{13}C in each clinical trial can be reduced accordingly, and additional patient studies may be possible with the same amount of available ^{13}C .
5. Although the final course of action would await further discussions with DBM, it appeared that a useful first step would be for each of the separate investigating groups at BNL, ANL, and LASL to draw up a separate 189 budget form to request support for its participation in the overall ^{13}C clinical demonstration experiment. Each laboratory's application would extensively cross reference that of the other participants so that DBM could evaluate the entire inter-laboratory collaboration as one program.
6. The participants were well satisfied with the day of exploratory study, several of them coming away with high enthusiasm. It was the general consensus that an attractive, workable pilot study had been roughly sketched out, that it involved a useful application of the special programs and facilities of each of the participating laboratories, and that the understanding and blending of the many considerations necessary to design an optimum pilot experiment could not have been achieved without the meeting.

Donald C. Borg, M.D.
 Biophysicist, Biology Branch
 Division of Biology and Medicine

Enclosure:
 As stated

cc: C. W. Edington
 Betty Hower

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