AN INSTRUMENTAL INSTALLATION FOR THE PUBLIC TO ACQUIRE DATA FOR MONITORING PERSONAL HEALTH AND THE QUALITY OF MARKET PRODUCTS

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Abstract: Currently Nuclear Magnetic Resonance Spectroscopic Technique has advanced to such an extent, the utilization of the potential of this technique can be for the public by the public at large. By this, it is meant here that acquiring data with this technique is no longer the prerogative of only the scientific community preoccupied with advanced scientific research and development. For the science to benefit the community, the initiatives need not be only from the scientists. The prevailing technological advances have enabled the common public to avail the research findings by reaching upto the scientists with data acquired with the advanced instruments in a convenient format that enables the professionals to interpret the individual data conveniently and quickly, particularly monitoring health trends and categorise them as personal traits or potential epidemics. Also, quality control by consumers of the market products, particularly the nutrition related parameters of food products would be possible. These data acquired as a matter of routine by everyone in the public can be compiled appropriately and conveniently owing to the rapid progress in the dedicated systems for digital formatting and storage and retrieval of data for information processing. What is required for all such public involvements is an installation of scientific instruments and maintenance personal. Typical example of such an installation is the X-ray and MRI scanner facilities which have made huge strides as part of the clinical laboratories, where people can submit samples of biological fluids or acquire images of specific organs, parts of human body as per prescription. It is hereby envisaged that a routine data acquiring by people (voluntarily and not necessarily by only on prescription from a Qualified Physician) for health statistics is possible by MRS, Magnetic Resonance Spectroscopy, NMR Spectroscopic Technique in particular. Then the first step would be to realize an enterprise, organization (not necessarily a research establishments or an institution),. This paper contains an enumeration of the typical criteria for the considerations of such a social, may be a non government organization involving public private partnership.

1. INTRODUCTION

When a knowledgeable common man has to be convinced about the advantages of seeking to get an instrument installed in the public interest, first and foremost it is necessary to know what could be the purpose and how it would be useful in the long run to put in efforts and spend time on such an enterprise. Enterprise could mean it is meant for earning a living by a qualified unemployed, or a voluntary service by a privileged for the benefit of the public at large. This article is intended more for the later kind of the above two categories of activities. Thus in the early parts of this paper, a description of what this Magnetic Resonance Spectroscopy (*usually it is a technique used along with the Magnetic Resonance Imaging technique, by abbreviation spelt as MRI and MRS*) is about so that a common man would get enough familiarity with the intended activity. Typically to mention from the utility point of view, it is like an MRI scanner centre which many people are aware about. The X-ray since a very long time and MRI facility which is much more popular nowadays, are the facilities to cite in this context. In fact, the MRI is a facility, the principles of the technique being Magnetic Resonance. Magnetic Resonance Spectroscopy is the technique how the magnetic resonance phenomena soon after its discovery became a useful method in physical sciences and now it is an integral part of the analytical methods in Chemistry. Nuclear Magnetic Resonance technique is that aspect of the Magnetic Resonance Phenomenon which reveals the electronic structure in a molecule around a specific nucleus of the atoms which are bonded to make up the molecule. By specifying Nuclear Magnetic Resonance (NMR), the Nuclear ***Spin*** Magnetic Resonance is referred to, and the implications are to the well known Electron ***Spin*** Magnetic Resonance (ESR). The ESR and NMR constitute the main subject matter of Magnetic Resonance Spectroscopy. Because of the fundamental differences between the nature of Electron and atomic Nuclei, the technology related to detection fo ESR and NMR signal vastly differ, while both the spectroscopic techniques basically are describable in terms of principles of magnetic resonance phenomenon. The name Magnetic Resonance arises from the fact that the electrons and nuclei posses electrical charges associated with spin property. The consequence of spin is that there is a magnetic moment associated with these sub atomic particles. This characteristic magnetic moment of individual particles interact with an externally applied magnetic field, intensity of which is several orders of

magnitude larger compared to the inherent magnetic moment of the individual subatomic particle. Thus a Magnet system to apply strong magnetic fields is an integral part of the NMR spectrometer system. Even while these atoms are bonded to form a chemical molecule, their central nuclei retain their specifications as much as in the free atoms. The nuclei remain unaffected while the atom is bonded to other atoms in a molecule and hence the bonding in molecules are essentially the redistribution of electronic charges by sharing of the electrons appropriately by the atoms. Thus when the electrons of the atoms undergo a rearrangement to enable a stability of the molecule, the atomic nuclei remain almost without any change. It is the changes in electron charges in the outer most shell of atoms that causes an NMR spectrum possible. in the Since Nuclear Magnetic Resonance is more used in several of the contexts, in this paper to begin with, the Nuclear Magnetic resonance is considered. Among the several nuclei which have characteristic Spin value, Proton (Hydrogen), Carbon, Nitrogen and Phosphorous are the atomic nuclei which are mostly used in this context as would be described in the remaining part of this paper. The scope of NMR spectroscopy can be assessed by a glance through the contents of documentation and books on NMR typically as in Ref 5.

Having thus found useful for small molecule applications particularly in organic chemistry, scientists soon started on biological applications and realized its potential for *in-vitro* and *in-vivo* contexts. The applications to biology and macromolecular characterization grew faster because of the Technology which advanced at much faster rates and started producing Spectrometers which had such capabilities features which the developmental stage was not available. The result is that biological macromolecules could be characterize much more simply than even the details which the small molecules would require to be obtained only after detailed processing and interpretation of spectral data. If the characterization does not require such details for processing which only experts look for, then it becomes simple enough for many to avail this characterization by NMR (specifically Proton NMR). Coincidentally the small molecular spectral features well documented and available in the form of databases enables the usage simply a matter like acquainting with a PC unit which receives data from spectrometer ouput terminals. Such a black-box kind of spectrometer operation requires a lot of automation in the spectrometer operation to make it all a high-through put efficient. It is in this context that in this paper the possibility of a facility for the public utility is envisaged. There is a recently created internet webpage which has been published (Ref 1) to the internet by this author on how to introduce the Magnetic Resonance Phenomenon, instrumentation and Spectroscopy ins and colleges. With this kind of documentation, an effort can be made to orient students even at the formative years for a science education with environmental awareness and availing these kind of centres envisaged in this paper, for a routine monitoring of personal health linked to social welfare.

2. OBTAINING PROTON NMR SPECTRA

Obviously availability of an NMR spectrometer is the first requirement. To begin with required spectra can be obtained from any of the Sophisticated Analytical Instrumentation Facility (SAIF) located in some of the National Laboratories, Institues and Universites can be helpful. These National /Regional /Institutional facilities are funded by DST for the use by scientists on working on research projects and students working for their respective degrees. The procedure consists of submitting a sample dissolved inappropriate solvent, with a requisition form for the required spectral features. This form is to be signed by the scientists concerned and the Head of the Section/ Division/ Department where the scientific work is carried out. The picture below (Fig 1) is an NMR spectrometer **Magnet** inside which the sample is being positioned. This is a picture of the NMR spectrometer housed in the SAIF of NEHU (Ref 2).

Fig. 1

After inserting the sample and ensuring proper positioning, the spectrometer console (consists of required RF radiation sender, receiver for NMR signal and the computer to monitor and display data). can be used for setting up the NMR experimental parameters to let the experiment start. Further there would be a provision of software to process data and tabulate NMR spectral parameters; finally the

spectrum can be obtained in a graphical plot with the plotter connected to the computer. Such Instrumentation facilities were established with a full time operator and maintenance staff when the instrumentation transcended the stage

Fig 2

of home-built spectrometer uses and the Scientists themselves operating the instruments.

. The spectrometer operations could enable a job for full time operators and a centre for housing the instruments because the instrumentation itself became a profession and the advances could justify the necessity for a black-box provision to scientists, to get reliable spectra routinely without knowing much of what entails the spectrometer instrumentation and operation. The sample submission from a window and the spectra gathered from delivery window was good enough to find the spectra reliable and consistent for scientific interpretations (Ref 2). This is a much more popular version of the NMR facility, on a footing similar to that of MRI scanners. With such a NMR facility what the public can be engaged with is dealt with in the article cited in reference (Ref 3). A typical NMR system is sketched in Fig 3.

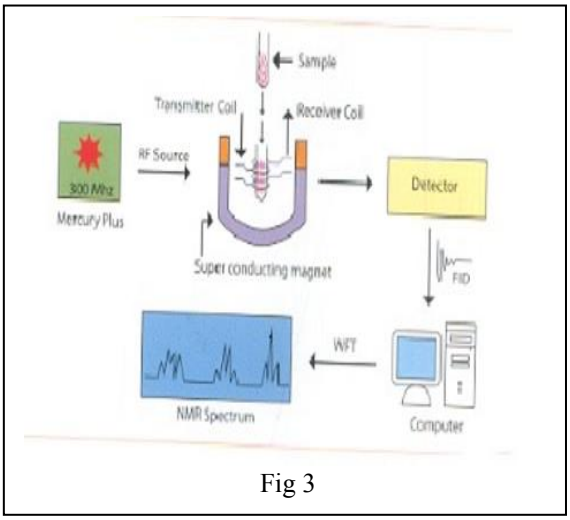

Fig 3

3.

The samples to be used are to be primarily the human biological fluids. How these biological fluids contain metabolites is hat the living systems are networks of biological processes (in-vivo biochemical reactions) during which several products are formed and in several ways these products get distributed in the living system, by which it is meant that metabolic products are necessary in several human organs to enable them function. And the excesses may get eliminated as excretion. Or appropriately extracting these semi liquid samples from appropriate organs and well predetermined times during the biological routines, these metabolites can be obtained as samples for NMR monitoring. A metabolism typically consists of reversible processes the forward process (Fig 3) may be called CATABOLISM and the backward process ANABOLISM. The catabolism is a process by which large size (*Mol.wt in several Kilo Daltons*) macro biological molecules get degraded by biochemical processes into smaller size (*Mol.wt of the order of a Kilo Dalton*) molecules, consequently energy is released. The entire set of molecules from smallest to the largest molecule encountered in the biological processes are inclusive in the inventory of Natural Products, and, may be specified as biologically relevant natural products. The overall energy changes which occur get used up in additional processes that may be occurring if there is generation of energy. The specified network requires input of energy; it must be usually supplied from some other network that generates energy. While in the balancing phenomenon the energy remains conserved mostly within the system, and the dissipation usually is in terms of chemical matter rejected by the system from the inputs conducive for consumption. Thus keeping track of what happens to the chemical matter consumed as food and inhaled as air (pollution again

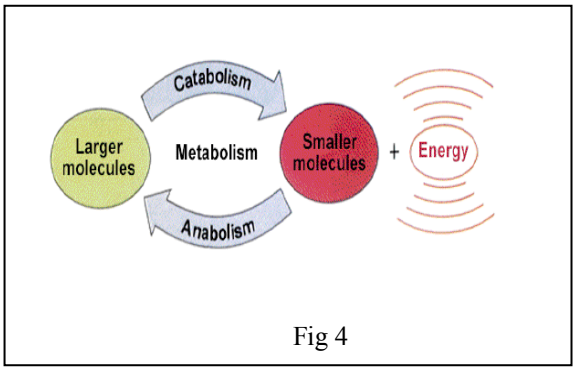

Fig 4

being an unwanted chemical) is the main task of chemists while noting the energy requirements and accounting for the same. It is these molecules which are produced as a consequence of metabolic process in living systems which are called metabolites. When these molecules are monitored by their characteristics and estimated for the amount produced and present in the system of a living object as and when it occurs, then it is referred to as *in-vivo* study. When these molecules are isolated and in a laboratory, and study undertaken, then it is referred to as in-vitro study. or the molecules are synthesized in the laboratory, then

it is a synthetic product and not a natural product. It is these kinds of products (molecules) that are called METABOLITES since the interest is in following the metabolic processes to infer on the mechanisms and efficiency of the process indicating the health status of the living systems. Thus excretion in living systems that routinely occur to evidence that the system is undergoing changes all the time and it si the dynamics in the network of these processes that makes "living" possible. It is the intake of food which supplies the stock of macro molecules to te=he living system which eventually undergoes a catabolic process to release the energy. The anabolism results in production of macromolecules in the system. By routinely monitoring these metabolites present in the living system health trends become evident. For example milk is a product which is yielded by cows, the living species, routinely consists of metabolites and monitoring these metabolites in a milk can reveal a wealth of information pertaining to nutrition factors and the health of the animal that is optimum for yielding good quality milk in larger quantities. Thus it is simultaneously a food product and the natural product yield from a living animal species. Thus in the next section, the metabolic profile studies reported in the literature would be discussed.

4. NMR SPECTRA

A beginning can be made with the considerations on the biologically relevant small molecules. There are 20 amino acids which are the small molecule building blocks for macro molecular proteins and enzymes. Most of the metabolites are some kind of proteins, carbohydrates, lactates, and so on. The proteins are the most often encountered metabolic products and these protein molecules can break up to smaller peptides. The molecular units that are responsible for biological macromolecules – the proteins – are amino acids. The smallest in the analogue of amino acids are the two amino acids by name: Glycine and Alanine. In Fig 5 and Fig 6, the molecular structure and the proton NMR spectra of these two amino acids are given: There is only one line seen in the spectrum corresponding to the CH_2 group and the protons of NH_2 group and the OH group are not appearing in the spectrum. While such dynamic situations not appearing in spectra is quite common, it is necessary at the moment to recognize the Glycine NMR by a single line at the specific X-axis value called ***chemical shift(Ref .*** In Fig 6 for alanine two Proton NMR lines can be noted, unlike the single line for Glycine. In this case as well the two proton lines with their corresponding chemical shift values characterises the presence of Alanine. These chemical shift values under various conditions for these amino acids have been tabulated and available in databases. By recognizing such features the presence of these two amino acids can be identified. However when these are existing as peptide residues in a Protein the spectra can be

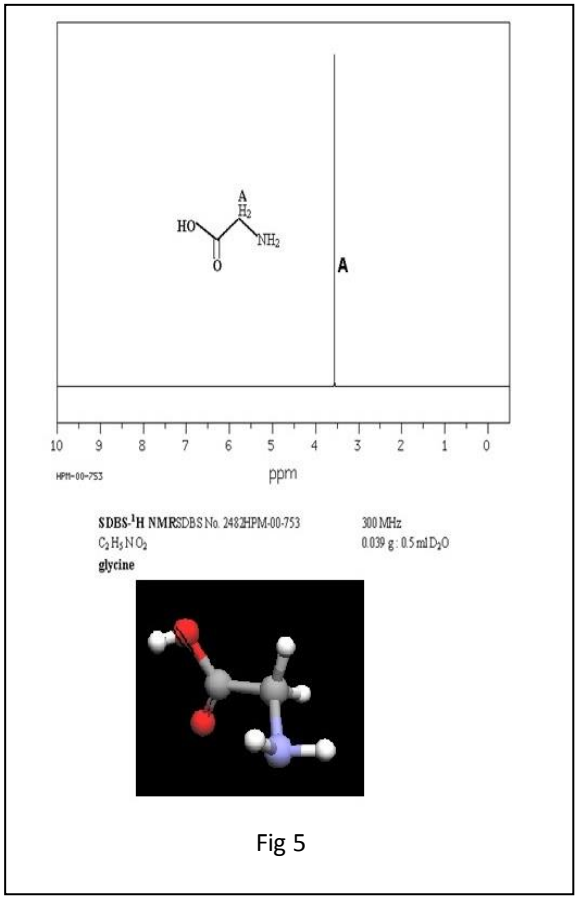

Fig 5

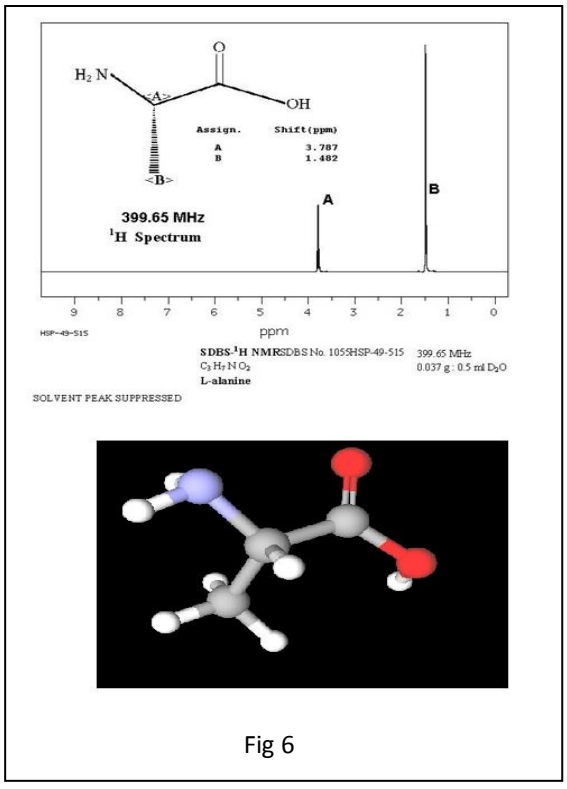

Fig 6

different, and all such variations have been notified in the databases with appropriate remarks. This can enable a sequence of amino acid residues in protein molecule and the entire set of identified lines can mark the presence of characteristic protein / macro molecular entity which are obtained as metabolites. Proteins are only a class of biological macro molecules and all kinds of metabolites that have been found in various contexts are available as databases to refer and identify them. When the similar bio fluids are gathered from different members and as a function of time, small changes on the x-axis positions and the y-axis parameter called intensity are to be recorded and monitored.

In fact at the early part of this article, a mention was made that there are 4 important elements which occur biological molecules and these four nuclei have isotopes with reasonable natural abundances (Ref 8), Hence the NMR of all these four nuclei can be obtained by the current models of NMR spectrometers commercially available.

Metabolite profiling makes it possible to regularly aquire data NMR data of bio fluids and watch out for the characteristics of the NMR lines (X-axis Chemical shift value of each line and the corresponding Y-axis intensity value of that line), which can be analysed for qualitative and quantitative changes in the NMR spectra. This monitoring can be done specifically after the regular use of a drug for cure of diseases, and the changes data of days prior to administering drug, can be indicative of the metabolic changes and hence mechanism for the cause and subsequent cure. Several such efforts are going on with animals and human patients, by metabolic profiling. MRI and MRS combined seem to be giving more details to make possible the precise diagnosis. Thus the metabolic profile turns out to contain symptoms of diseases.

5. METABOLOMICS OF MILK

In the milk (from cows or buffalos) is typically a bio fluid and happens to be a food material. From both points of view, bio fluids and food material, monitoring milk routinely can be helpful in pointing out the health conditions of cows and buffalos as well as nutritious contents for the human systems. By appropriate excerpts from published data (Ref 9) the typical names for metabolites and the way the data is documented would be illustrated in a typical case and this should enable the initiators to know what kind of digital storage facilities could be thought of for these tasks at various stages of the project to get an installation going. This paper in the Journal of Food Composition and Analysis, gives a clear exposition of the NMR application, using the Phosphorous (isotope mass-31) for analysis of milk to characterize the milk variety and relate it to the various consequences of milk as food. Hence in the following few lines the figures and tables from the paper would be used liberally to make it even simpler for the common public to appreciate the advantages of metabolic profiling as a public amenity. As in Fig 7 the ^{13}P NMR spectrum has only

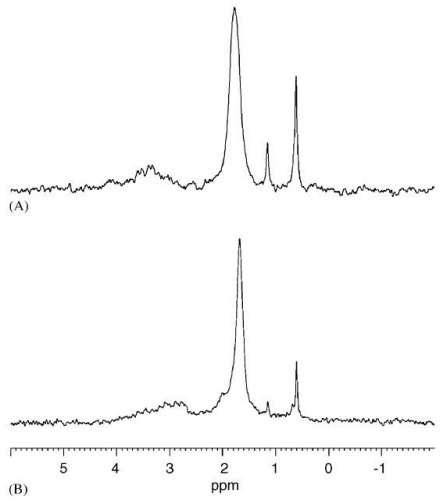

Fig. 1. ^{31}P-NMR spectra of buffalo (A) and cow (B) milks. The spectra were referenced to phosphocreatine at −2.45 ppm. Sharp signals were assigned as follows: inorganic phosphate (1.77 ppm), glycerophosphorylethanolamine (1.18 ppm), and glycerophosphorylcoline (0.63 ppm). Phosphoproteins in casein micelles appeared downfield with respect to the inorganic phosphate as a broad signal between 2.5 and 4.0 ppm.

Fig 7

a few lines, typically for a (solution) liquid state High Resolution NMR spectrum, some of them are broad (considering the width of the lines as measure) and some of them narrow lines. As mentioned earlier, the X-axis value corresponding to the central peak of the lines is a characteristic parameter measured in ppm Parts per million) units which is dimensionless constant value assigned in this particular spectrum characteristically. It is important to realize that all the NMR peak values are assigned as a difference from the line position of a conveniently chosen reference compound. In the spectra above the reference chosen is ***phosphocreatine*** line position set at -2.45 ppm value. From the appearances of lines displayed in the two spectra of Fig 7, it is possible to discern the similarities in the two spectra may be taken to characterize milk, and the differences between the two spectra may be useful for recognizing the different sources, the buffalo and the cow. Whether such a conclusion from the first instance exposure can have a general validity or not would be borne out by collecting samples from a variety of species, and assigning the spectral patterns to the characteristic source, and recognizing these trends dependably to know the differences in biological processes that could be responsible. Thus generating a database and providing software features to analyse these data to assign these parameter trends to specific processes in specified species.

Table 1
Phosphorilated compounds identified in ^{31}P-NMR spectrum of milk ultrafiltrate

Compound (peak number[a])	(mol%)[b]	
	Buffalo	Cow
Phosphocreatine (**1**)	2.2	1.0
Glycerophosphorylcholine (**2**)	9.5	10.7
Glycerophosphorylethanolamine (**3**)	2.5	1.7
N-acetylglucosamine-1-phosphate (**4**)	4.3	6.7
Galactose-1-phosphate (**5**)	1.9	3.0
Inorganic phosphate (**6**)	57.0	50.7
Phosphorylcholine (**7**)	6.7	1.7
Phosphorylethanolamine (**8**)	2.8	0.9
Glycerol-1-phosphate (**9**)	0.8	1.6
Glucose-6-phosphate (**10**)	1.3	1.4

[a] According to Fig. 2.
[b] A preliminary estimation was performed as follows: all of the peaks between 3 and 6 ppm in Fig. 2 were simulated, and the calculated area of each identified compound was used to determine the relative concentration. The total area was used as normalization parameter. Each value is a mean of two different samples.

Fig. 8

In Fig 8 above is a table consisting of the names of molecules identified, which are in general referred to as metabolites. The tabulation in Fig 8 refers to the spectra in Fig 9. Again note the X-axis chemical shift 'ppm' values, and their corresponding Y-coordinate value. The chemical shifts are the parameters which characterize the specific compound identified, and the Y-axis relative value is a quantitative comparison helpful to differentiate, when samples from two different sources happen to have the same characteristic compound.

In Fig 9, the peaks are numbered in sequence and attributed to a specific named compound. The source of the milk samples in two spectra also are spelt out. Further the authors could point out such features as is relevant for the finding out the nature of these sources of the milk samples and the substances occurring in them. To quote from the authors:

"Phosphocreatine, glycerophosphorylethanolamine, phosphorylcholine, phosphorylethanolamine were more abundant in buffalo than in cow milk, while N-acetylglucosamine-1-phosphate, galactose-1-phosphate, and glycerol-1-phosphate were more abundant in cows than in buffalo sample."

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Such are the methods, and simple enough to evolve a system for monitoring, provided there is an installation of the instrumental facility. In Ref 3 of the list of references included at the end of this article, more examples are given with appropriate references to consider the suggestions as mooted here in.

Other animal bio fluids have been subjected to such studies and analyses have proved the potential of such health statistics based compilation of data. Before the experts go through the compiled data to derive information, it is necessary to collect such data in large scale appropriately categorized, which cannot be happening only at the instance of a disease or ailments when doctors prescribe the tests. This should be a routine voluntary activity and in such a way that

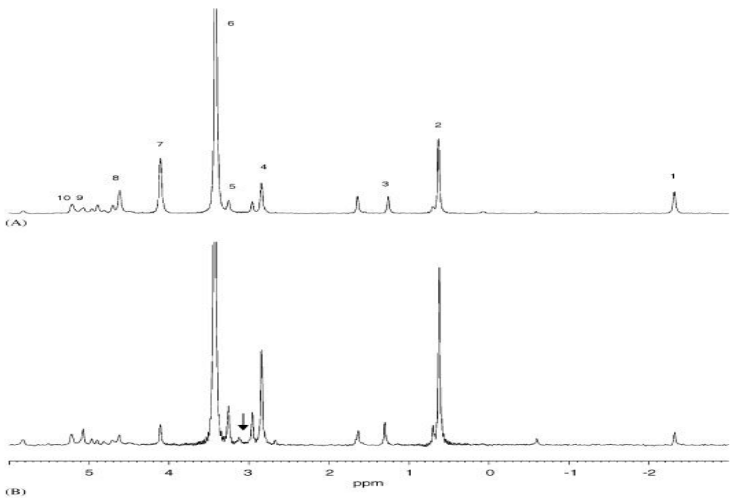

Fig. 2. ^{31}P-NMR spectra of a tenfold concentrated buffalo (A) and cow (B) milk ultrafiltrates at pH 9.4. The assignments were as follow: phosphocreatine (used as an internal reference), −2.32 ppm (peak **1**); glycerophosphorylcholine, 0.62 ppm (peak **2**); glycerophosphorylethanolamine, 1.26 ppm (peak **3**); *N*-acetylglucosamine-1-phosphate, 2.83 ppm (peak **4**); galactose-1-phosphate, 3.25 ppm (peak **5**); inorganic phosphate, 3.44 ppm (peak **6**); phosphorylcholine, 4.11 ppm (peak **7**); phosphorylethanolamine, 4.63 ppm (peak **8**); glycerol-1-phosphate, 5.09 ppm (peak **9**); and glucose-6-phosphate, 5.21 ppm (peak **10**). The arrow in panel B indicates lactose-1-phosphate and/or glucose-1-phosphate.

Fig. 9

soon every citizen finds it a responsibility and to contribute to the collection of data. in order to compile materials for this article no special effort is made to find standard books or library resources. The approach has been to go by the appropriate internet searches, almost like searching through data bases, but of more open resources than of secured servers. It is the effort to ensure that as much a s possible all these contents are within the reach of common public to further explore and mobilize support from much privileged lot who do not probably had the means to acquire necessary skills by appropriate training or proper education.

6. METABOLOMICS IN HUMAN NUTRITION

This section contains an elucidation of the discussions in the review article of Ref 10. In this review article published in the year 2005, the author begins the article with these lines:

> *"Metabolomics has been widely adopted in pharmacology and toxicology but is relatively new in human nutrition. The ultimate goal, to understand the effects of exogenous compounds on human metabolic regulation, is similar in all 3 fields. However, the application of metabolomics to nutritional research will be met with unique challenges."*

and the first illustration in the review article is typical proton NMR spectrum of human urine (generally a bio fluid). Further to quote from the authors as observed:

> *"The first challenge must be to identify all the chemicals in different bio fluids that are linked to the human nutrition metabolome, and the priority must be to gain a consensus for the definition of a metabolome in human nutrition. The second biggest challenge associated with the large NMR and MS outputs is how to work with these large total data-capture data sets in which many compounds remain unidentified."*

Thus the magnitude of effort required in generating data bases of bio fluids from living beings to go about making use of this technique of metabolic profiling for concerns of social welfare probably cannot come about by simply instances of ailing people acting under doctor's prescriptions. A voluntary social activity must be the way; but it requires an installation on the footing as if it is being a part of a research organization. The author discusses several factors (Fig 11) related to human health and possible inferences from metabolomics studies. The article is simple enough for beginners to follow what is implied at this stage by pleading for an installation of this type. The crucial stage is to mobilize resources and start up an enterprise of the type which is a non-profit NGO. To the extent that it requires trained persons and a consultancy with experts makes it to be a an innovative endeavour involving huge costs. Maintaining a super conducting magnet system without accidents requires extreme caution. But the consoling factor is the several clinical laboratories are run particularly using similar systems with regard to MRI scanning facilities but till now MRS centres were not thought of and it is not too early to take initiatives.

The references cited at the end of this article can be leading more people to consider the establishment of such instrumental utility. And how much can this be a entirely initiative of social organizations, and the extent it will have to opt the institutional facilities are the main considerations.

7. CONCLUSION

As enough introductory notes have been included in this paper all along, it is probably too early at this stage to discuss more of the advances made in this area, and the kind of research activities pursued in institutions in India and other countries. Hence in summary, it is high time now consider a social venture involving huge investments financially, and a monumental responsibility by a voluntary participation by a cross section of the public to undertake a task in the that is challenging to venture into. In the webpage of this author (Ref 11) at the end, a variety of materials are included to evidence the potential use of Metabolite profiling. The link to a published article "PlantMetaboliteProfiling.pdf" is an introductory article on Metabolite profiling which has a comparative report on the use of Mass Spectroscopy and NMR Spectroscopy.

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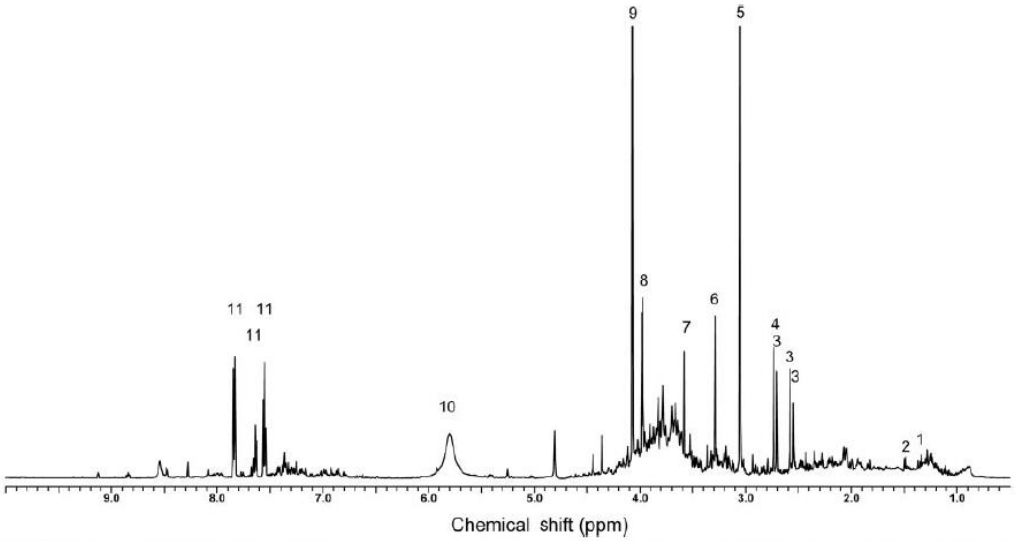

FIGURE 1. A typical 500-MHz ^{1}H nuclear magnetic resonance spectrum of human urine. The identification of the major metabolites is highlighted. 1, lactate; 2, alanine; 3, citrate; 4, citrate and dimethylamine; 5, creatinine; 6, trimethylamine-*N*-oxide; 7, glycine; 8, hippurate; 9, creatinine; 10, urea; 11, hippurate.

Fig 10

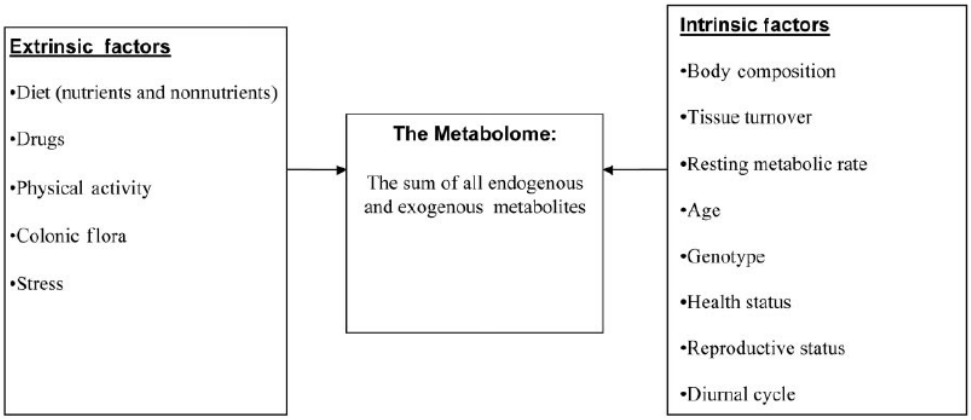

FIGURE 2. Exogenous and endogenous factors likely to influence the human nutritional metabolome.

Fig 11

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