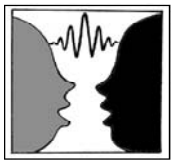
Face to Face



This section features conversations with personalities related to science, highlighting the factors and circumstances that guided them in making the career choice to be a scientist.

Of Mechanisms, Microscopes and Methyl isocyanate

Dr S Sriramachari talks to Sujata Varadarajan

Pathology, the science of the causes and effects of diseases, is a branch of medicine that involves laboratory examination of samples of body tissue for diagnostic or forensic purposes. Pathologists, the 'backstage workers' of medicine, are an essential part of the system, for their work confirms the diagnosis and ultimately suggests the direction of treatment. It is rare to find a pathologist with experience in diverse areas of the field and even rarer to find someone who has moved from description and diagnosis to research.

Dr. Sriramachari has delved into, and left his mark on, a range of areas such as Hepatic pathology, Nutritional pathology, Neuropathology, Osseo- and Muscle pathology and Clinical Toxicology. He appears to have effortlessly made the transition from general medicine to pathology at a young age. Working as an independent research student at a time when medical research was uncommon, he learnt hepatic pathology by reading, and practicing liver biopsies in the mortuary, before performing them on patients. He was practically the first in India to do these biopsies and at this time he also developed a new method employing water soluble glycol waxes for embedding and staining tissues for lipids and enzymes. This was just the beginning of a long and productive research career. Over a period spanning more than five decades, he has proposed, and proved several pathbreaking theories, using a combination of human pathology and animal studies. He has also developed (and patented) several appliances and methodologies dealing with various kinds of photography and microscopy for mass production of color atlases of pathology at low cost.

He is often found on the scene when widespread illness or death in the country requires specialized pathological opinion. He, along with Dr. Patoria, helped resolve the mystery of the sudden deaths of children in Nagpur in summer months; he was a part of the pathology team in

Bhopal after the leak of Methyl isocyanate (MIC) occurred. He also served as an Observer on behalf of the Government of India for the autopsy studies of the Air India 'Kanishka' crash and during the Bangalore episode of Sivarasan, who was involved in Rajiv Gandhi's assassination.

Not surprisingly, he has had a large array of degrees and academic honors conferred on him (including Padmashri, in 1985). More importantly, he has directly or indirectly touched the lives of many in this country, through his research, his interest in developing affordable medical educational aids and his considerable administrative skill.

His unassuming (and extremely courteous) manner belies his extraordinary breadth and depth of knowledge. His deep thoughtfulness is apparent from his conversations and style of tackling research problems, for he considers not just the problem in isolation, but also the causes and consequences in a scientific and social context. He cheerfully brushes all compliments aside, saying, "There's no place for pride or show in learning."

He is currently based at the Institute of Pathology-ICMR (of which he was the Founder Director), as an INSA Honorary Scientist. Now in his eighties, he is still actively involved in science, despite health constraints.

SV Could you tell us about your early years and your educational background?

SS We are Tamils (not Tamilians) settled in Andhra Pradesh for 300 years, who can talk pidgin Tamil, but not read and write. My father was an Ayurvedic physician and also a great scholar in Sanskrit and could off-hand quote from many Sanskrit texts. But he had a modern outlook as well. He enrolled me and my brothers in a convent school – and that gave us a sort of a lead. My father had two friends, who were elderly teachers. Every day on our way to school (or before going to school for a 'special tuition' of 'Gurukula type'), we also used to take to their house a small vessel of milk. At that time, I used to think of them as incarnations of Vasishta and Vishwamitra. Eventually, one of them, Sri Nishtala Seetharamayya, when he was about 95 odd years, came to stay in our house at the fag end of his life. I will never forget his attitude towards philosophy of '*niṡhkāma karma*'.

After high school, I passed matriculation, in the thirteenth year. Our English standard was good and our second language was Sanskrit, so it all went off very well. Though I was given a seat in the Medical College, my name was struck off because I was 'under aged'. So I had to go to nearby Vizianagaram and do a BSc. That was a transitional period, peculiar historically. That was the time when Vishakapatnam town was bombarded by the Japanese. There was a mass migration of people because that part of

the country had not experienced war before. Our father was also very sick and we really thought he would never survive; therefore we went to our mother's place – Zulakallu in Palnad Taluq of Guntur district. And at that time another big tragedy struck our family. Our young sister – she was the youngest and the darling of the house – suddenly died. We were all completely shattered. When we returned (to Vishakapatnam), because of all the difficulties, I got a standard third class BSc. But based upon my marks in Intermediate, which were quite good, I got a medical seat. Having joined the medical college, I had my regular study and was also active in political work – that was the pre-Independence era.

SV How was your transition into research?

SS I was studying in Andhra Medical College, Vizag from 1943 to 1948. After graduating, because I did not come within the top four ranks, I had to work as an unpaid House Surgeon. However, my idea was to train myself for private practice in a rural area, Yadavolu, West Godavari district. But at the same time, I thought, "Why not I try to see if I can do a post-graduate degree?" This was very difficult in those days. Luckily, another important person who came in my life's way was a teacher by the name Dr. M D Ananthachari (also referred to as MDA).

An extraordinary case really changed my course and career. One hefty businessman collapsed in the judge's court and was brought unconscious to the Casualty Department of K G Hospital (KGH). At that time I was 'on duty' and I did every possible investigation and wrote a detailed 'Case Report'. When he was admitted, his temperature was about 99 ^{o}F, but later he developed very high temperature of 105 ^{o}F and died in the night. Dr. Ananthachari sent for me early next morning and questioned me firstly whether I was aware as to what happened to the patient in the night. I was frightened that I must have made some blunder, but I answered the questions and described all that I had done. Finally, he said, "Oh! You'll go a long way!" I felt relieved, and was also impressed by his extraordinary, thoughtful approach and appreciation whenever correct work was done by the juniors. I also remember him complimenting me for doing a correct diagnosis of a case of meningitis due to *H. influenzae* (bacillary and not viral). A six month old baby was brought late in the evening to the Casualty. I did a lumbar puncture[1] which stained positive and started the treatment. It was confirmed later by the Department of Bacteriology. Dr. MDA asked me to present the case, on his behalf, in the KGH Clinical Meeting!

[1] A technique of withdrawing cerebrospinal fluid from the spinal canal.

I then applied for an unpaid Senior House job, but because of my political affiliations, I was asked to leave. When Dr. Ananthachari asked me about my interests, I told him that I was 'interested in research'. He queried "What do you mean by research? Do you want to get a Nobel Prize?" I replied: "Not at all, Sir. I just want to learn to do the work in a scientific way". He said, "You've got the right attitude".

He seemed to approve/welcome my desire. As advised by him, I wrote an application to undertake studies on 'Liver Function and Liver Structure in Malnutrition'. Based on some pamphlets and literature that I had collected, I applied for, and was awarded the Lady Tata Fellowship. The grand monthly remuneration of Rs. 250 was without any other contingency grant. But when I requested permission to do the work in the KGH, the Medical Superintendent said "You are no longer a student here. Sorry, I can't do anything for you."

Then, on the advice of Dr. Ananthachari, I went to Dr. N G Pandalai, the Principal of AMC (Andhra Medical College). He said, "I never realized you are interested in research. You can come and work with me in my IRFA Project on 'Culture of leprosy bacillus'. After some hesitation, I said "I would like to pursue my studies on the liver disease." In those days, malnutrition was rampant in India and many people had liver disease. The Principal said, "Even if you don't come to my Department, it doesn't matter, as long as you are interested in research". And he wrote a note to the Medical Superintendent to provide me necessary permission to carry out 'clinical research'. He quoted that I, a student of AMC, was awarded the Lady Tata Fellowship, seventeen years after Dr. M V Radhakrishna Rao got it at AMC, when he did really pioneering work on ICC (Indian Childhood Cirrhosis, known at that time as IBC or Infantile Biliary Cirrhosis) under Dr. T S Tirumurti.

And so I started. I never realized that I was virtually the first person to do liver biopsies in India. I collected a lot of information from books and journals. I did biopsies on all types of people including malnourished beggars, especially alcoholics. But it was like a 'Damocles Sword', because everybody thought "Oh, he's going to kill patients." So I used to take help or assistance of a 'mortuary attendant'. I practiced doing the liver biopsies on unclaimed dead bodies that he used to draw out from the morgue. As a token of appreciation, I used to give him about 4 Anas every time, for his immediate needs (including toddy)!

In those days it was very difficult to procure special laboratory reagents. So I used to go to the abattoir and collect sheep brains and prepare the Cephalin–Cholesterol Emulsion. I've done over 200 odd cases of liver biopsies and liver function tests. I

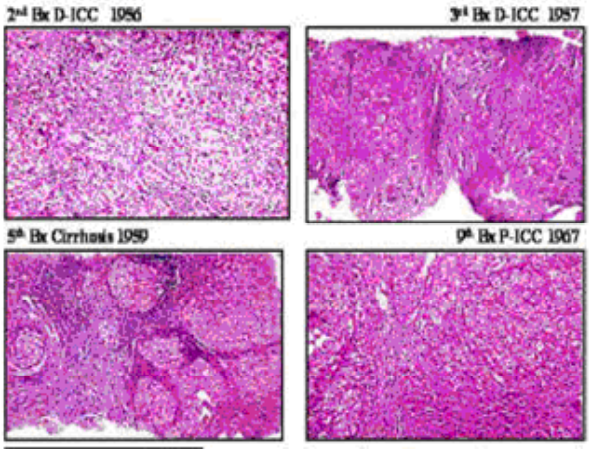


Figure 1. Biopsies of a patient with Indian childhood cirrhosis, monitored through infancy and childhood (1955 to 1967) with a series of liver biopsies, showing the various stages of the disease, and its reversibility. The 2nd biopsy shows widespread ballooning of liver cells. The 3rd biopsy is marked by cellular damage, inflammatory changes and fibrosis of typical cirrhosis. These decreased in the 5th biopsy. In the 9th biopsy, except for ballooning, it reverted to nearly normal.

always recall this adventure as an inspiration. As a result of the work, I also got the Lady Tata Fellowship for the second year. But I declined it because of an offer from Nutrition Research Laboratories of ICMR (Indian Council of Medical Research) at Coonoor. But I continued to work with Dr. MDA; we printed the Report in my uncle's ancient Arsha Press of Vizag. Eventually, after a lot of entreaties Dr MDA agreed to be a co-author of the work that I had done under him. It was finally published in the *Indian Journal of Medical Sciences* in 1954 [1].

My Professor (Dr. MDA) had an original, inquisitive mind. Once I showed him a liver section under the microscope, and told him, "Sir, here is fatty change". He said, "Show me the fatty change… My dear man, why do you want to draw inferences from empty holes? Why can't you stain for fat?". The routine staining methodology at the time involved treating the section with alcohol, xylol, paraffin and in the process the fat is removed. As luck would have it, that very afternoon I went to the Staff Library and came across an article on the staining of intracellular lipids in the *Journal of Stain Technology*. Immediately I wrote a letter to the author (Chifelle Putt) in USA, got the details of the compounds and methodology and eventually demonstrated the presence of fat in the formalin-fixed liver sections processed in polyethylene glycols (carbowaxes).

SV In which direction did this research lead you?

SS By word of mouth, people came to know that I was doing liver biopsies. I was offered a job of Assistant Research Officer in the Nutrition Research Laboratories in Coonoor. However, due to bureaucratic lapses it was delayed by several months. I joined the Department of Pathology under Dr. V Ramalingaswami, who was the Research Officer.

In the post-war period, malnutrition was rampant in the children of plantation workers in the Nilgiris. They had swollen feet and bodies, with reddish discolored hair (Kwashiorkor). I used to perform liver biopsies and would examine the tissue using my new histochemical methods. The first publication on Kwashiorkor in India is by Sriramachari and Ramalingaswami, in 1952 [2]. The methodology of demonstration of fat that I had done in Vizag, was published in the *British Journal of Clinical Pathology* [3]. So as it happened, my initial work had its impact on understanding the changes associated with fatty liver under conditions seen locally, and it was also acceptable for publication. In those days we never even realized the importance of publications.

The liver of the children with Kwashiorkor is laden with fat, the structure is altered almost completely, and there is very little fibrosis. With my interest in chemistry, I jumped on to Histochemistry i.e. the demonstration of chemical constituents in the histological tissues. The alcoholic fatty liver (showing cirrhosis[2]) was different from the Kwashiorkor fatty liver (mild fibrosis[3], no cirrhosis).

We were also doing animal studies. In course of time, Dr. Ramalingaswami postulated a theory on the genesis of fatty livers, but I did not believe it to be correct and declined to be part of the publication. After doing several such experiments, I asked to be excused from this work. At that stage a flash of an idea came to me: what is it that mobilizes fat (releases it from the liver)? For that you need protein and a lipotropic factor (lipotropic factors are part of the vitamin B complex series) in conjunction with the fat. By changing the diets of rats accordingly, I was able to produce two kinds of changes – periportal (as in Kwashiorkor livers) and centrilobular (as in alcoholic livers). When the fat content was more, there was fibrosis, but when I kept these animals for longer periods, fibrosis disappeared. I was amazed! I thought the experiments went wrong. Further experiments revealed that fibrosis was not a permanent feature. I put up a hypothesis that the growing rat is utilizing its own fibrous tissue for its nitrogen requirement – a form of auto-cannibalism.

In the meanwhile, I went to America to be trained in Neuropathology, came back, did my MD in Vizag, and later registered for a DSc degree. At this time, I applied and was selected for a job in NIMHANS in Bangalore – then called AIIMH (All India Institute of Mental Health). I told the people there that my work was not complete and I would not be able to join. But, the authorities were prepared to wait for me, if I was really interested in the job. Very few trained neuropathologists were there at that time – I'm

[2] Degeneration of tissue in an organ. [3] The thickening and scarring of connective tissue.

the second man to be trained in India, so they waited for me until I completed my work on the phenomenon of 'Reversibility of Fibrosis and Cirrhosis'. This work was recognized by pioneering experts in the area such as Sir Roy Cameron and Dr. Robb Smith of Oxford and Dame Sheila Shylock (two of my examiners).

SV Did you continue your work in hepatic pathology subsequently?

SS I worked on one or two small problems later. But more importantly, I was drawn by the study of Indian Childhood Cirrhosis by S T Achar's group. The work which I have recently completed is on Indian Childhood Cirrhosis. It is a caste and community-based regional disease, unique to India for over a century. Many children used to die of this disease. What always worried one is why should there be cirrhosis only in the better fed communities (viz. Brahmins, Vaishyas and Khatris)?

Even since 1955, I was drawn towards the studies of Prof. S T Achar, Dr. V Balagopala Raju and Dr. N Sundaravalli. I studied the liver biopsies for them. And that was a great opportunity – I have examined about 2500 liver biopsies and have been involved in this for over 50 years. Nobody knew the cause of this condition. Over the past 100 years, there were continuing disagreements about the disease. In 1979, a Britisher by the name Tanner, came to Poona and observed that affected people prepared food in brass utensils (which contain copper) and stated that the disease was occurring due to 'dietary copper toxicity'. However, Dr. Ramalingaswami, who was DG, ICMR approached it through a Multi-centric National Collaborative Study in different areas, using a standard methodology. Meanwhile, Dr. Nayak, the Coordinator, retired and went abroad. So the responsibility of completing the report fell on my shoulders. It took quite some time to study the entire material and to reexamine the data. At last, we have succeeded in deciphering the spectrum of the disease and are able to classify each stage [4]. Out of the 748 cases that we finally included, 120 cases had repeat biopsies (done over a period of time). We also found that copper only increased in the end stages, and not in the initial stages. Further, we carried out BTEA (Biological Trace Element Analysis) by the Graphite Furnace Atomic Absorption Spectrophotometry technique at BARC, Bombay, for 156 samples and found that both copper and non-toxic zinc levels had increased. Therefore I said, "This is not in favor of copper toxicity. It is an incidental or associated phenomenon". The study aroused a series of doubts.

Then what is the cause? Are there any peculiarities in the afflicted homes? It has occurred to me that every home had been using indigenous domestic therapeutic remedies. There is a certain similarity between traditional medicines of Bengal,

Andhra Pradesh, Tamil Nadu, Maharashtra and Uttar Pradesh. I am trying different combinations in rat studies and have got some preliminary clues attributable to potentially harmful home medications. Meanwhile the disease has virtually vanished. Why? The middle class seems to have become wiser and changed its attitudes and customs; many are depending on hospitals for medication instead of home deliveries.

Has it got any application? We are using herbal formulations without subjecting them to critical appraisal and rigorous tests for toxicity. Even our scientists are not applying their minds to these basic issues. The common 'Alcoholic Fatty Liver and Cirrhosis' could also be the effects of herbal substances used in manufacture of different brands of alcohol.

SV What areas of neuropathology did you work on?

SS In those days, my Institute (NRL) was interested in Neuro-lathyrism[4]. Therefore, I was sent abroad for training in neuropathology at the Armed Forces Institute of Pathology (at Washington DC, USA) – the Mecca of Pathology. For a short time, I worked under Dr. Webb Haymaker (a remarkable, world famous neuropathologist) and his colleagues, especially Dr. Leo Krainer, both of whom I consider as my mentors. Since my training was for just a short period of six months, I equipped myself by reading and working late into the nights as the subject was a very complex one and the time very limited. I saw the thoroughness with which the Americans diagnosed the specimens. The idea that diagnosis is an end in itself and it must be completely unassailable was the lesson I learnt. I concentrated on understanding the fundamental principles of neuropathology, and when I returned, I joined AIIMH and published an original paper on Neuro-lathyrism in *Nature*. Later on I was sent on a WHO fellowship to England, where I visited and studied in several important Centres of Neuropathology, especially Dr. Greenfield's collection at Queen Square, London, through the courtesy of Prof. Blackwood. I also worked in Neuropath Labs in Germany, the fountainhead of neuropathology. In AIIMH, I developed the postmortem room and conducted autopsies and surgical neuropathology.

Suddenly in 1962, Dr. C G Pandit brought me to the ICMR to initiate High Altitude Research Projects in the wake of the Sino–Indian conflict. I was subsequently invited by the All India Institute of Medical Sciences (AIIMS), New Delhi to help them in the diagnosis of brain tumors, when there was no department of neuropathology as such. I

[4] A diet-induced neurological condition characterized by severe muscular rigidity and paralysis of the lower limbs.

worked for Dr. P N Tandon, Dr. Baldev Singh, Dr. Medha Pathak and others. Then I also developed an interest in muscle pathology and worked with Dr. Virmani, and Dr. Indira Narayanan. I gave training in neuropathology to Dr. Sarla Das, who incidentally joined my ongoing work on topo-optical properties[5] of glial filaments[6] of brain tumors, as compared with other bio-filaments like collagen and reticulin.

In those days there was an argument about children dying in summer months – whether it is due to encephalitis (an infection) or heat hyper-pyrexia[7] or any other factor. To solve this problem, Dr. Baldev Singh as the leader and myself as the pathologist, along with Dr. Patoria of Nagpur did a thorough study of 14 such cases in Nagpur. Dr. Patoria did the autopsies and I got a total of 14 brains. I examined them in a thorough manner, as I had learned from Robb Smith and others at Oxford. I found that none of the brains had any evidence of infection. But they were not normal; they were showing severe edema[8] which affected several areas of the brain similar to the classical descriptions of heat hyper-pyrexia by my former chief, Dr. Haymaker. Based on our work we published a detailed report attributing Nagpur encephalopathy to 'heat hyper-pyrexia'.

Then arose the question – how to prove the point? I had been working on experimental models for head injuries, I thought, "Let me use that knowledge for simulating this problem". Due to the basic differences in brains of different animals, I decided to use a primate model. Dr. Balani, my student, carried out the work by subjecting the primates to raised temperatures in incubators and examining the brains in detail.

This work was important not only to recognize how it happened but also why. We see it is happening in cities in Maharashtra, Uttar Pradesh, where roads are getting tarred, buildings are expanding. A few years ago, around 6000 people died in Andhra Pradesh alone. So the problem is firstly (avoiding) exposure, and then prevention by timely action. In Israel, they have passed a law that nobody shall die of heat hyper-pyrexia and they are using techniques to ensure this. Today the hazard of global warming is a matter of great concern.

SV Could you describe your work on head injuries?

SS Head injuries is a phenomenon on the road. Whenever anybody is knocked down,

[5] Topo-optical properties of tissues are studied using specific reagents that alter the natural birefringence of the tissue constituents, enhancing their visualization under the microscope. [6] Connective tissue of the nervous system.
[7] Abnormally high body temperature. [8] Abnormal accumulation of fluid causing swelling or distention.

there'll be compression, concussion – hemorrhages, swelling and other types of changes. What are the types of changes and how to recognize them? I decided to study it in a systematic, standardized way. Dr. Balani and I simulated these changes in primate models, studied different combinations, and recorded the physiology and pathology changes for each animal. We studied in all about 400 monkey brains. We sectioned each brain and studied in detail 15 blocks per brain, by a wide array of staining procedures. We did a tremendous amount of work using classical neuro-pathological techniques based on 'paraffin and celloidin blocks'. We have shown not only the presence of severe edematous changes but also methods to correct it and restore normality. It was a very taxing, time consuming job, and my colleague and PhD student was finally rewarded with the Khanolkar Prize of the Indian Association of Pathology and Microbiology during its Silver Jubilee Session in 1975.

They were followed by difficult times. I also had a series of personal tragedies, such as my wife's death and attendant problems, of old parents and children's education and unsympathetic bureaucracy. I thought that I may never have a chance to fulfill my scientific mission.

SV What were your experiences of the Bhopal gas disaster?

SS This was perhaps the world's greatest Chemical Disaster in terms of the death and disability amongst the survivors and the information that we collected was really enormous and phenomenal [6].

Actually it was the year 1984, December 3rd, when this tragedy took place. I was about to leave Delhi and go to Belgaum on 'sabbatical leave' to undertake some studies on fluorosis and the protective role of tamarind, etc., when the Bhopal disaster involving the leak of the deadly gas methyl isocyanate (MIC) from the Union Carbide Plant was announced. I thought, "It's an extraordinary challenge for the scientific community and why not I remain here". As soon as we heard about it, we thought of going to Bhopal, but Dr. Ramalingaswami, chief of ICMR, happened to be abroad and so, by the time we reached Bhopal it was the 11th of December. By the time great damage had already taken place. Dr. S Varadarajan had moved into the field and had done remarkable work in his 'Operation Faith'. At that time the story was so dispiriting and horrible – several thousands of people had died and mass cremation was done. There was continuing damage to the eyes and lungs of people, and the bodies were scattered round the Medico Legal Institute (MLI) – one of the most extraordinarily equipped Forensic Laboratories in the country, of which my friend Dr. Heeresh Chandra was the

Director: he was an outstandingly dynamic and committed scientist.

From the third day onwards, a total of 730 bodies had come to MLI and more than 150 autopsies were done, partially or completely by the team of doctors at the MLI. The autopsies revealed an intense red discoloration of the viscera, especially the lungs, trachea and upper respiratory tract and my friend coined the term 'cherry red discoloration'. The liver, spleen and kidneys were also reddish. Mouth and nostrils were laden with heavy whitish foam. With the help of my colleague, Dr. H M K Saxena, we managed to carry out detailed histopathological study of over 150 autopsies and established the full range of acute and chronic damage of the lungs and other organs. The brains were very much edematous and swollen, and several individual nerve cells had dropped off (or detached). There was hemorrhage both on the surface and the substance of the brain. Death was very sudden and was due to lack of oxygen reaching the respiratory centres.

Another important feature was the involvement of the eyes – more than 98% of the people had ocular manifestations. They could not open their eyes; in many of them the eyes were either clouded or red in color.

A scientist by the name Max Daunderer had come from Munich, and he had brought a big collection of injectable sodium thiosulfate, on the suspicion that there could be cyanide toxicity. Heeresh Chandra caught up the idea and advanced it further and very convincingly announced that the changes are due to 'cyanide toxicity' and therefore we should give thiosulfate injections. I also met Max Daunderer. I was impressed, but the people, probably at the instigation of the Union Carbide, tried to play down the whole thing. In a matter of a few days he was asked to leave the country. This sometimes shows how the so-called influential people can affect the treatment. But Heeresh Chandra persisted with this work.

Regarding my contribution – I had a flash of an idea: If thiosulfate is injected and if it is giving relief to the survivors, then there must be a metabolic waste product also (due to an interaction between thiosulfate and suspected cyanide toxicity). Therefore, we must examine urinary thiocyanate excretion. If it is elevated, it would be a proof of cyanide toxicity. At that time, Dr. Varadarajan who was in overall charge of all the field operational procedures, had set up a 'Wireless Station' near the Union Carbide factory for quick exchange of informatiom. I requested him to get some detailed information on method of estimation of thiocyanate in urine, based on the earlier studies in Coonoor on 'Extra Cellular Space in Kwashiorkor'. We got the information in less than 24

hours. Just as we were waiting for it, the autopsy of an elderly man of 72 years was in progress. I noticed that his bladder was enlarged. I said, "This liquid is precious. Let us collect it and keep it in the refrigerator". That was the starting point for the work on urinary thiocyanate excretion. We demonstrated that there is indeed an increase in levels in this sample. I suggested to my friends, "Let us examine the excretion in the dead bodies as well as the surviving patients". Lo and behold, our initial observations were confirmed, by a three to four-fold elevation of urinary thiocyanate levels.

I then thought, "If we are doing the thiosulfate treatment, let us not forget the estimation of the urinary thiocyanate in the patients (during the course of the treatment)". We standardized the methodology for the estimation and did 'Double Blind Clinical Trials' and established the therapeutic rationale of sodium thiosulfate. We studied about 19,000 samples over a period of time. Meanwhile, people got relief from the injections – most importantly, the difficulty in breathing, inability to walk, etc., was overcome. We estimated urinary thiocyanate levels before and after the injections and were convinced. But to our strange surprise and disappointment, the same individuals had recurrences in symptomatology, and there could not have been further inhalation of cyanide. Therefore, doubts arose in our own minds (as to) whether we are dealing with abnormal cyanide toxicity, and recurrence phenomena became a big headache for us.

In that transitional stage, I put forward another hypothesis, about the possible role of binding of MIC itself (in the blood). Meanwhile Union Carbide was sending messages that MIC cannot cross the alveolar lung-blood barrier and will not enter the blood-stream because it will be broken down into methyl amine (MA) and dimethyl urea (DMU), and that all this Indian work cannot be relied upon.

If that be the case, I thought we must have some method of demonstration of the transport of MIC into the bloodstream and from the bloodstream into the tissues. I thought if we collect samples of blood and preserve them, we may be able to demonstrate the presence of "blood contaminated by MIC". Knowing that the lifespan of an erythrocyte[9] is 120 days, we (realized that we) had little time. I did not know how to proceed. But in the course of intense search, I read about 'formal titration' [10]. The free amino groups of the hemoglobin molecules could be determined (by this technique) – and if there is binding of MIC, then the 'formal titration' value would be decreased. On my urgent request, work was done by Prof. Ramaiah and his student Dr. Roman Reddy in the Department of Biochemistry at AIIMS. They found 30-40%

[9] Red blood cell – this contains hemoglobin, a protein involved in oxygen uptake and release.

[10] Formal titration is a titration using formalin.

reduction of the formal titration values by the TNBS technique (using Tri Nitro Benzene Sulfonic acid).

Further work was done by P K Ramachandran and his associates in Gwalior (DRDO). They synthesized MIC and did experiments, first using rat blood, followed by blood of Bhopal victims. Here the reduction was only 2-3%. To explain the anomaly I thought that may be there is binding of MIC to glutathione. Unfortunately, since we did not succeed in demonstrating it, we had to stop it. Later on, Baillie and Slatter from Seattle, USA confirmed the idea.

However, after studying more than 59 samples of blood and several cryo-preserved tissues of the post mortem organs, we were able to show the binding of MIC to the amino acid of amino-terminal valine residue of hemoglobin by a process *N*-Carbamoylation. This indicated that oxygen release is not taking place as it should. Such a phenomenon also could be responsible for the cherry-red discoloration. Realizing the value of the hypothesis, Heeresh Chandra followed it up and showed that 7 or 8 different amino-terminal amino acids of proteins in different organs were *N*-carbamoylated.

At the height of the disaster, with the help of Sridharan and Patil of Defence Institute of Physiology and Allied Sciences (of DRDO), we also managed to get blood samples tested for 2,3-di-phosphoglycerate (2,3-DPG)[11]. Lo and behold, the values were increased to abnormal levels, as if people had gone to a height of 14,000 feet and above. The same individuals were re-examined after 3-4 months, when fresh hemoglobin was generated. The values had returned to 'normal'. Therefore, we were able to successfully overrule and disprove the hypothesis advocated by the Union Carbide and their associates that MIC could not cross the 'alveolar-capillary barrier'.

SV What have the longer term studies revealed?

SS One of the problems worrying me is the exact mechanism of 'Chronic or Recurrent Cyanide Toxicity'. I have hit on a new idea of the possible role Chloro-derivatives of MIC. Work is continuing on the interaction of chloroform with MIC, resulting in Chloro-derivatives (which might have been generated in the tank in Bhopal), and their

[11] Hemoglobin is present in two forms – oxyhemoglobin (bound to oxygen) and deoxyhemoglobin (devoid of oxygen). 2,3-DPG, a molecule found in red blood cells, promotes oxygen release (from oxyhemoglobin) in tissue capillaries by stabilizing the deoxyhemoglobin form. An increase in 2,3-DPG causes hemoglobin to unload oxygen more efficiently at lower atmospheric levels of oxygen. The body responds to an immediate decrease in perceived oxygen pressure (for example at high altitudes) by increasing levels of 2,3-DPG.

possible toxic effects. This is being done along with Drs. S K Bhattacharya and Vijayaraghavan at DRDO Gwalior.

In the victims, mercifully, the edema is reversible, and the neurological manifestations which we were worried about have not progressed. The opacification of the cornea and lenses was also found to be reversible. There were a lot of miscarriages, abortions, etc., and people attributed it to the immediate toxic effect of the gas. But detailed retrospective studies showed that it might be due to psychological factors, fear etc. The perpetual question – even now unsolved is about risk of cancer in the Bhopal victims. The story is not yet over, but it seems unlikely that it has caused an increased incidence of cancer. I suggested that we study the survivors and their next of kin for two generations, and try to characterize the genetic abnormalities, if any. That kind of work will be useful for science in general, India in particular and the world at large.

SV How do you think science could be made more accessible to young people?

SS My exhortation for the future generation of Indian scientists is to imbibe a true spirit of science of pioneers of bygone days like Sir C V Raman. In 1954 when I visited the Neuropathological Laboratories of Dr Cyril B Courville at Los Angeles, he gave me a gift, of a book which he published in 1934. He had translated, edited and annotated the book entitled *Precepts and Counsels on Scientific Investigation – Stimulants of the Spirit* by S. Ramon Y Cajal of Spain written in 1893! Ramon Cajal was one of the earliest Nobel Laureates. Ever since, I have venerated it as my 'Daily Bible'.

I sincerely feel that extracts of similar accounts should be periodically published in *Resonance* and in the DST Publication *Dream***.** Thereby we would be promoting scientific curiosity and stimulating Doubting Thomases**.** There is an urgent need to popularize 'Frontiers of Science', by people like Prof. D Balasubramanian and Dr R Ramachandran.

There must be Science Clubs and Science Magazines for school children, where various subjects can be discussed. There should be debates on scientific and practical aspects of life – nutrition, clothing, environment, and health. The consequences and side effects of various issues must be brought to their active notice and they must be encouraged to think about ways of overcoming them (the undesired aspects).

Lastly, we must encourage computer-based learning through CDs, or DVDs in every village and hamlet, aided by the State, social activists, constructive workers (who work) along Gandhian lines and philanthropists.

Figure 2. (left) Dr. Sriramachari lecturing to students, during a workshop on scientific microscopy, at the Indian Registry of Pathology (now called the Institute of Pathology), in the early seventies. (right) In the Institute of Pathology, 2007.

SV What are your other interests?

SS Apart from my scientific pursuits, I have very limited interests. My main interest is the study of the Indian political scenario – I have a large collection of books written by political leaders. Microscopy, photomicrography, photography have been a passion for me. I was interested in establishing a museum of microscopes. I had every conceivable microscope with me, but of late I am out of touch with it. I am interested in science – and in studying the liver, lung and kidney in particular. My current interest is in Voice Recognition Systems such as Dragon Naturally Speaking, as they will reduce dependence on stenography and typing. The future seems to be in this area.

I am not very much interested in religion, but I am interested in understanding the underlying moral principles of it. I am also interested in a different type of social work. If anybody really needs medical help, I am ready to go out of the way to help them, especially the underprivileged, in spite of my limited contacts with medical men and institutions.

Suggested Reading

[1] **S Sriramachari and M D Ananthachari, Malnutrition and Cirrhosis: Study of Hepatic Structure and Function. *Ind. J. Med. Sc.*, Vol.8, pp.31–40, 1954.**

[2] **S Sriramachari and V Ramalingaswami, Liver changes in Kwashiorkor, *Ind. J. Paediat,* Vol.20, pp.1–6, 1952.**

[3] **S Sriramachari and V Ramalingaswami, Polyethylene Glycols as embedding media in Histochemical work. *J. Clin. Path,* Vol.5, pp.346–359, 1952.**

[4] **S Sriramachari, (Editor-in-Chief), *MNCS Report on Indian Childhood Cirrhosis (ICC),* ICMR- October 2006.**

[5] **S Sriramachari and N K Patoria, Pathology of Acute Encephalopathy Syndrome in Children in Summer, *Ind.J.Med. Res.*, Vol.64, pp.296–313, 1976.**

[6] **S Sriramachari, The Bhopal Gas Tragedy: An Environmental Disaster, Current Science, Vol.86, pp.905–920, 2004.**

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