Life and Work of Sambhu Nath De

A Sen and J K Sarkar

"When discoveries are published in scientific literature, they are presented in a form which does not tell us very much about how things really happened…" (Arne Tiselius in 'Priorities in Scientific Research' Commentarii, Vol.11, N.26) quoted by S N De in his opening remarks at the 43rd Nobel Symposium, on 'Cholera and related diarrhoeas', 1978.

Amitabha Sen was formerly Professor and Head of the Department of Chemistry and Emeritus Scientist, Bose Institute, Calcutta.

Jaladhi Kumar Sarkar was formerly Professor of Virology and Bacteriology, Director and Emeritus Scientist, School of Tropical Medicine, Calcutta.

Many individuals of this country who have risen to high distinction in life were born and nurtured in peaceful and natural surroundings of the Indian village. One such village, called Garibati is located on the west bank of Ganga at a distance of about 30 km north of Calcutta, near the former French colony of Chandannagar. Sambhu Nath De was born in this village in 1915. His fater, Dashurathi De and mother, Chattesweri belonged to a large joint family whose immediate forefathers with a flourishing business were reduced to penury due to heavy losses resulting from floods. De's grandfather died young leaving his widow, minor sons and daughters. The elder son (De's father) had to earn from his childhood as a shop assistant while the younger son was educated up to college level. Later De's father ran a small business but being a devout Vaishnav, he was not much inclined to run the business profitably. De, being the eldest child, was brought up with much affection. His uncle, the only educated member of the family, took special care of his nephew's education which laid the foundation of his later educational career. From the Garibati High School, De passed the Matriculation examination with a District scholarship which enabled him to study at the Hooghly Mohsin College. De secured a DPI scholarship in his Inter-Science examination and was selected for admission in the Calcutta Medical College. But the scholarship money alone was not enough to meet his expenses at Calcutta. A benevolent gentleman of the locality, Sri K C Sett came forward with the offer of free boarding, and lodging in his office-cum-residence at

Reproduced with permission from Special Issue on S N De and Cholera Enterotoxin, *Current Science*, Vol.59, Nos 13 & 14, pp.630–636, 25 July 1990.

Keywords
Vibrio cholerae, cholera toxin, enteropathogenic *E. coli*.

Figure 1. Sambhu Nath De.

Calcutta as long as necessary. However, in the following year and subsequent years, De secured a college scholarship and also free studentship and could afford hostel accommodation.

De's performance soon drew the attention of his teachers, especially of Professor M N De of Pathology and Bacteriology who was a well-known research worker of the time and author of standard treatises on bacteriology. He not only kept a watchful eye on the progress of his student but being greatly impressed by his performance and nature, later gave away his elder daughter, Torubala in marriage despite wide differences in social and financial status between the two families.

De passed his M B examination in 1939 and took the Diploma in Tropical Medicine in 1942. In the same year he joined Calcutta Medical College as a Demonstrator of Pathology under Professor B P Tribedi and published a few papers with him during the following years. He also carried on private practice in clinical pathology (allowed in the job) to augment his income as he had to shoulder the burden of maintaining the family of parents, and several minor brothers and sisters.

Figure 2. S N De (right) with Sir Roy Cameron, London, 1962.

De's father-in-law, Professor M N De, by that time Professor of Medicine at Calcutta Medical College was a far-sighted man. He could foresee the promise in his young son-in-law and played a key role in moulding his future career. Having had contact with Professor G R Cameron, FRS (later Sir Roy Cameron) of the University of London, he arranged, after the World War II, De's admission under him as a PhD student in 1947.

De joined the University College Hospital Medical School in London in 1947 to work on the problem of changes in the brain caused by experimental hydrocephalus. In the initial stages he confronted great difficulties in the production of experimental hydrocephalus in rats as most of the

animals died of respiratory distress accompanied by frothing at the mouth and nostrils soon after being injected with a fibrin forming mixture in the 4th ventricle. De was totally upset at the failure of these experiments in his first three months in London. He was seized with acute mental depression and decided to discontinue work and return home at the first opportunity. However, Professor Cameron, who was to become De's mentor, friend and the main source of inspiration for all his future work, recognized the importance of the accidental finding and advised him to work on the formation of pulmonary oedema (respiratory distress, etc.) which was killing the rats. These works were published in papers on pulmonary oedema (Cameron & De, 1949) and on experimental hydrocephalus (De, 1950). Professor Cameron in a letter to Professor M N De in early 1948 wrote: "Dr. S. N. De… passed through a bad period of depression where nothing seemed to go right… he has regained confidence. He is doing excellent work." De was awarded the PhD degree of the London University in 1949.

De returned to Calcutta in 1949, and those of us who knew him from his student days were struck by the complete transformation in his outlook towards research which from now on was to become his *yoga*. He started experimental work on the pathogenesis of cholera even before joining Nilratan Sircar Medical College and was without employment, with one of his former colleagues (JKS) at Calcutta Medical College, and communicated his first work on the action of cholera toxin (De *et al.* 1951b). Apparently he had conceived the idea of working on cholera while in England as he brought with him from London small appliances for measuring blood pressure of cats, etc. In this preface to his book on cholera (De, 1961a) he wrote that while working in the University College Hospital Medical School in London he had developed a keen interest in experimental bacteriological pathology as he watched the course of such a study on dysentry toxin by a fellow worker. When he took up the Chair of Pathology at the Nilratan Sircar Medical College at Calcutta where the attached hospital catered to most of the cholera cases in and around the city, cholera

came to be his chief subject of study. Here De began his studies on the remarkable pathological changes in the kidneys in cholera cases which showed renal shunt mechanism in operation – a feature of many other toxic conditions. He published a number of papers on this topic between 1950 and 1955.

In the paper (De *et al*. 1951b) on experimental study of the action of cholera toxin referred to above, De wrote: "… the toxic appearance of the cholera patient… suggest that the disease in man is primarily an intoxication and the organisms multiply in the lumen of the gut, undergo autolysis and liberate their endotoxin which acts locally and also absorbed, producing systemic effects". Being a non-believer in the poison theory of Koch, he was interested in looking into the mode of local action of the toxin in the lumen of the gut. His problem was to devise a suitable animal model for reproducing symptoms of cholera. Within the following two years his success came in finding the animal model which was described in the paper entitled 'An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane' (De & Chatterjee, 1953). This paper has been designated as a Citation Classic in Current Contents of February 9, 1987, being a widely cited paper since 1955.

Diphtheria exotoxin was discovered within four years of the discovery of the bacillus, tetanus exotoxin within six years and botulism toxin at the same time as the organism. It has taken seventy five years for cholera exotoxin to be discovered.

As to how he was led to do these experiments has been best told by De himself in his 3rd Dr. B. C. Roy Memorial Oration at the Calcutta Medical Club (De, 1980b). He said: "*Vibrio cholerae* has now been promoted to the rank of few exotoxin producing bacteria. However, diphtheria exotoxin was discovered within four years of the discovery of the bacillus, tetanus exotoxin within six years and botulism toxin at the same time as the organism. It has taken seventy five years for cholera exotoxin to be discovered in 1959 after the organism was discovered by Robert Koch in 1884… . He did think of the possibility of the existence of a cholera poison. He argued, the group of symptoms in a regular attack of cholera, usually thought to be the consequence of the loss of fluid and thickening of blood should really be considered as the effect of a poison which is absorbed and which acts on the circulatory systems and paralyses it."

De pointed out that efforts would probably have been made to pay attention to the intestine if Koch's authority did not decree that the cholera poison acted systemically. Instead every conceivable preparation of *V. cholerae* was injected parenterally – and not enterally – by different workers into a wide variety of animals. The effects observed had no relation to the disease. Many experimental methods on a large variety of animals were used by Koch, who concluded that none of these animals on which one could do experiments was susceptible to cholera, and a true cholera process cannot be reproduced in animals.

De further said in the above lecture (De, 1980b): "We entered the cholera field in early 1950. . . . In our first experiments heavy cultures of cholera vibrio were introduced into the lumen of the small intestine of rabbits after opening the abdomen under local anesthesia. The animals had no diarrhoea but they were dead by three or four days – just as Robert Koch and other early workers had noted. However, at autopsy on these animals, we found that the huge caecum of these rodents, which normally contain pasty semisolid material, was full of semiliquid faecal matter from which *Vibrio cholerae* could be recovered. We argued that in these experiments, fluid is poured out in the small intestine which accumulates in the caecal backwater and cannot find its way out to manifest as diarrhoea. So we next by-passed the caecum, isolated a four inch segment of small intestine by two silk ligatures – introduced a loopful of *V. cholerae* mixed with one c.c. of peptone water medium – killed the animals the next day. We were happy to see that the trick worked and we had a suitable animal model. The loop was distended with about 15 c.c. rice-water fluid while the control loop receiving the sterile medium was collapsed. This represents cholera localized to a small segment of the intestine".[1]

In the following years, using his rabbit ileal loop technique, De with one of us (JKS) among his coworkers made some pioneering studies on enteropathogenic activity of cultures of *E. coli* isolated from cases of acute diarrhoea in Calcutta. He found that such organisms frequently caused intestinal secretion which resembled

[1] This was a reinvention of ligated intestinal loop of Violle and Crendiropoulo who published their work (*C R Soc. Biol.*, Vol.78, p.331, 1915) in a short paper which had rapidly sunk into obscurity. De noticed it while writing his book on cholera and reported it in a footnote (De, 1961a).

that induced by *V. cholerae*. He also made the very important observation that a classical enteropathic serotype is not necessarily pathogenic since identical serotypes could be isolated from healthy persons or from water sources which lacked the ability to induce intestinal secretion in rabbit gut loop (De *et al.* 1956; De, 1980a).

In 1955 De went to England for a few months with a Nuffield Foundation-Royal Society Bursary and presented his work on cholera and *E. coli* before the Pathological Society of Great Britain, which was highly acclaimed. His teacher Professor Cameron in a letter to Professor M N De in August 1955 wrote: "There is no doubt about it – he is one of the most outstanding of young men I have had through my hands and I am prepared to believe that he is probably the best of the experimental pathologists in India…. I am confirmed in my belief by other people's opinion… he is now primed up with plenty of ideas which he should be helped to pursue at all costs. He proposes writing a book on cholera which I think will be of great value".

In the same year De joined Calcutta Medical College as Professor–Director of Pathology and Bacteriologist to the Government of West Bengal. In spite of the burden of heavy teaching and administration he vigorously continued his researches mostly outside working hours. He gave up private practice by opting for a nonpractising post. His aim now was to isolate the toxin to prepare a toxoid. Research facilities for isolation of the toxin were not available at the Medical College. So at the Bose Institute where De had been an Honorary Worker since 1954, he worked with one of us as his colleague (AS) with biochemical and chemical facilities available there in the physical and protein chemistry laboratory. His work in right earnest began in 1957 and by 1960 he established the existence of cholera exotoxin. During these years, when he was endearingly called "Daktar Babu" by everyone in the Bose Institute, the doors of the laboratory were opened for him at all hours of the day. It was a familiar sight to see him daily, (even on Sundays) coming from Medical College after working hours in his own car with various materials for treatment

and analysis and working till late hours in the evening.

At this time De was labouring under the age-old belief that cholera vibrio produced an endotoxin. So around the middle of 1958, when he got the evidence of enterotoxicity of sterile culture-filtrate of *V. cholerae* in rabbit loops, he was greatly surprised and spent a few months to convince himself by repeating, critically examining and discussing his data before communicating the paper to *Nature* in December 1958, entitled 'Enterotoxicity of culture-filtrate of *Vibrio cholerae*' (De, 1959a; 1959b), which has been termed as a classic by van Heyningen (1983). At the time of communicating this note, De already had the evidence of the absence of any enterotoxic activity of sterile extracts of sonicated, washed vibrios in rabbit loops, i.e., the absence of any role of the endotoxin in producing symptoms of cholera. In his note to *Nature* (De, 1959a) he wanted to focus on the evidence of the role of exotoxin only. Fuller details of the properties of the exotoxin from the culture-filtrate and the endotoxin were published in the following year (De *et al.* 1960a; 1960b). De also showed (De *et al.* 1962) that the production of exotoxin required the correct choice of medium, its pH, temperature, nature of components, etc. He said (De, 1980b): "… we went to see whether bacteria-free product prepared from the vibrio, i.e., whether any cholera toxin could swell the rabbit loop. The idea of endotoxin was in our mind. So we disintegrated washed vibrios, suspended in saline with ultrasonic vibrations – centrifuged and filtered to remove residual bacterial bodies and tried the filtrate on isolated rabbit loop. The results were negative. We concluded that no endotoxin is related to the outpouring of fluid as seen in cholera. Then we turned to exotoxin. We grew the vibrio in a variety of liquid culture media and tried the sterile filtrate till we stumbled on 5% Difco bactopeptone medium which was successful …. we soon proved that the toxin produced by young cultures of *V. cholerae* in the special medium had all the characteristics of an exotoxin and is responsible for outpouring of the fluid in the intestine – the fundamental pathology in cholera and we called it cholera enterotoxin."

De's desire to carry on further with the purification of toxin did not proceed very far although latest protein purification techniques were then available at the Bose Institute. About his failure he said (De, 1980b): "It is worth emphasizing that we worked with classical cholera vibrio which was prevalent at that time. By 1963–64, we were faced with disappointment. A new variety of *V. cholerae*, the *El Tor* biotype which was so long prevalent in SE Asia especially the Celebes Island of Indonesia made its appearance in Calcutta and in the course of a year, completely replaced our classical cholera vibrio. No filtrable exotoxin could be found in this new arrival. The classical strains which we had, soon lost their capacity of toxin production and we did not know, nor had we the equipment for preserving the strains freeze-dried. I was forced to discontinue my work and lost all interest in cholera." He continued: "… After 1963, the year when *El Tor* biotype first arrived, no epidemic has been declared in Calcutta and in 1979 there has been no cholera death in Calcutta. The health authorities take credit to themselves, the physicians think they have designed a new treatment in oral fluid therapy which suffices in most cases eliminating the need for intravenous saline. But the truth is that *Vibrio cholerae* deserves most of the credit for having lost its toxin."

De, sorely disappointed, nevertheless continued to publish papers on cholera, the *El Tor* biotype and on other topics. A rude blow came with the death of Sir Roy Cameron in 1966, his teacher and constant source of inspiration, whom he had met in 1960 while on a visit to England with a Wellcome Foundation Fellowship and again, for the last time in 1962, at the time of receiving the DSc degree in Physiology of the London University. Lack of proper recognition of his work also disheartened him.

Cholera toxin which was purified after several years by workers abroad is one of the few toxins whose structure and mechanism of action are now well understood.

Cholera toxin which was purified after several years by workers abroad is one of the few toxins whose structure and mechanism of action are now well understood, and has found wide applications in a variety of investigations. Extensive literature now exists on the subject which show how much we have progressed in 30 years since De's discovery of cholera enterotoxin in 1959.

De retired from Medical College in 1973 at the age of 58. He did not ask for the customary 2-year extension, nor was he interested in the Principalship of the college which would have taken him in due course to the post of Director of Health Services. His inability to take his researches further as he desired, because of circumstances beyond his control, made him feel frustrated, and he lost interest in continuing teaching and in the administrative field. He began private practice by starting a clinical pathological laboratory in his residence to keep himself fit and occupied.

As a teacher who took particular care in preparing his lectures and as an administrator of a large department, De was held in high esteem by students and colleagues.

In 1978, several years after his retirement, The Nobel Foundation sought him out (their first letter did not reach him) requesting him to participate in the 43rd Nobel Symposium on Cholera and related diarrhoeas. De's talk in this Symposium dealt with his resarches on the serotyping of *E. coli* to which we have referred earlier (De *et al*. 1956; De 1980a).

As a teacher who took particular care in preparing his lectures and as an administrator of a large department, De was held in high esteem by students and colleagues. A softspoken, unassuming and dignified person, reserved by temperament, De never went out of his way to enhance his position in academic or professional bodies in which he was known for his work. He maintained a distance from centres of power in such bodies – a fact which may account for the absence of any recognition to him by way of Fellowship of an academy, awards or honours excepting the Coates Medal awarded by the Calcutta University in 1956 for outstanding research. For research, his grants were meager and he, very often, spent his own money. After he returned from the Nobel Symposium, he was keen on resuming his researches on nonenterotoxigenicity of the *El Tor* biotype. This needed an institutional attachment and the Bose Institute welcomed him as an Emeritus Scientist by a special resolution of the Council.

Though initially delayed, De's work as a major breakthrough became increasingly recognized abroad from the late sixties. The impact of his contributions has been discussed in detail in excellent reviews on cholera by van Heyningen and Seal (1983) and

Reviews of S N De's Work

- **W E van Heyningen and J R Seal, *Cholera: The American scientific experience (1947–1980)*, Boulder, Co, Westview Press, p.60, 1983.**
- **E Garfield, Mapping cholera research and the impact of Sambhu Nath De, *Current Contents*, Vol.14, pp.3–11, 1986.**

Garfield (1986). These writings have brought about in this country an awareness and interest, though belated, in De's pioneering work.

De was not the type who would enjoy large gatherings, seminars and conferences. He was happy in a small and intimate circle of friends and professional colleagues who were almost members of a family. If a friend was away, he would enquire after his family members and bring gifts for children. Though he never practised medicine, he had an amazing clinical diagnostic eye and helped many patients with advice. He died on 15 April, 1985. A few hours before his death, when he was in a state of coma, a letter received from S Arunachalam, Editor, *Indian Journal of Technology*, requesting him to get in touch with Eugene Garfield, Editor of *Current Contents* who was interested to know his biodata and professional contributions. But De could not be informed of this. In *Current Contents* of April 7, 1986 in an article entitled 'Mapping of cholera and the impact of Sambhu Nath De', Garfield paid a tribute to De whose work on cholera, he said paved the way to a more effective strategy for treatment and control.

Publications of S N De

[1] B P Tribedi and S N De, Thyroid tissue in ovarian dermoid, *Calcutta Med. J.*, Vol.41, pp.31–33, 1944.

[2] B P Tribedi and S N De, Observations on racial immunity to Tuberculosis in the Bengalees as seen in autopsy studies, *Calcutta Med. J.*, Vol.42, pp.97–100, 1945.

[3] S N De, B P Tribedi and M N De, Studies on fevers due to paratyphoid organisms and *bact. enteritidis*, *Indian Med. Gaz.*, Vol.82, pp.3–22., 1947.

[4] G R Cameron, G H Cooray and S N De, The effect of castration on the action of some barbiturates, *J. Pathol. Bacteriol*, Vol.60, pp.239–246, 1948.

[5] S N De, Streptomycin in the development of hydrocephalus in Tuberculous meningitis *Br. Med. J.*, Vol.ii, p.214, 1949.

[6] G H Cooray and S N De, Red cell changes in burn and acute anhydraemia, *J. Pathol. Bacteriol.*, Vol.61, pp.541–550, 1949.

[7] G R Cameron and S N De, Experimental pulmonary oedema of nervous origin, *J. Pathol. Bacteriol.*, Vol.61, p.375, 1949.

[8] S N De, A study of the changes in the brain in experimental internal hydrocephalus., *J. Pathol. Bacteriol.*, Vol.62, pp.197–208, 1950.

[9] G R Cameron, S N De and A H Sheikh, Cardiovascular responses in air embolism., *J. Pathol. Bacteriol.*, Vol.63, pp.181–194, 1951a.

[10] S N De J K Sarkar and B P Tribedi, An experimental study of the action of cholera toxin., *J. Pathol. Bacteriol.*, Vol.63, pp.707–717, 1951b.

[11] S N De, K P Sen Gupta, K Bhattacharyya and N R Konar, Observations on the mechanism of haemolysis in blackwater fever, *J. Indian Med. Assoc.*, Vol.21, pp.95–98, 1951c.

[12] S N De, K Bhattacharyya and P K Roychaudhury, In-vitro study of the action of terramycin and chloromycetin against *Vibrio cholerae*, *Calcutta Med. J.*, Vol.49, pp.360–361, 1952.

[13] S N De, K P Sengupta and N N Chanda, Haemoglobinuria in a case of cholera, *Br, Med. J.*, Vol.ii, pp.22–26, 1952.

[14] S N De, K P Sen Gupta, Shunting in the human kidney, *Lancet*, Vol.2, p.42, 1952.

[15] S N De, Cortcomedullary diversion and its possible role in acute renal failure, *J. Indian Med. Assoc.*, Vol.23, pp.60–68, 1953.

[15] S N De and D N Chatterjee, An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane, *J. Pathol. Bacteriol.*, Vol.66, pp.559–562, 1953.

[16] S N De and K P Sen Gupta, Shunting in the human kidney, *Lancet*, ii, p.681, 1953.

[17] S N De, K Bhattacharyya and P K Roychaudhury, The haemolytic activities of *Vibrio cholerae* and related vibrios., *J. Pathol. Bacteriol.*, Vol.67, pp.117–127, 1954.

[18] S N De, K P Sen Gupta and N N Chanda, Intravascular haemolysis in cholera, the effect of oxytetracycline, *Lancet*, Vol.I, pp.807–809, 1954.

[19] S N De, K P Sen Gupta and N N Chanda, Renal changes including total cortical necrosis in cholera., *A.M.A. Arch. Pathol.*, Vol.67, pp.505–515, 1954.

[20] S N De, K P Sen Gupta and N C Ganguli, Histochemical observations on the suprarenal glands in cholera, *Lancet*, Vol.I, pp.1043–1045, 1955.

[21] S N De, S N Bose and A Mondal, Observations on blood count and blood sugar in cholera., *Br. Med. J.*, Vol.ii, p.1065, 1955.

[22] S N De, K Bhattacharyya and J K Sarkar, A Study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis, *J. Pathol. Bacteriol.*, Vol.71, pp.201–209, 1956.

[23] S N De, Technical Report of the scientific Advisory Board, Indian Council of Medical Research, I.C.M.R., New Delhi, 1956.

[24] S N De, K K Bhattacharyya and A Mondal, A study of the factors in the spread of cholera in Calcutta, *J. Indian Med. Assoc.*, Vol.28, pp.514–519, 1957.

[25] G R Cameron, S M Hassan and S N De, Repair of Glysson's capsule after trangential wounds of the liver, *J. Pathol. Bacteriol.*, Vol.73, pp.1–10, 1957.

[26] S N De, P K Lahiri Choudhury and A Mondal, Observations on epidemic and non-epidemic cholera in Calcutta, *Trans. R. Soc. Trop. Med. And Hyg.*, Vol.52, pp.349–356, 1958.

[27] S N De and M L Ghose, False reaction in ligated loop of rabbit intestine, *Indian, J. Pathol. Bacteriol.*, Vol.ii, pp.121–128, 1959.

[28] S N De, Enterotoxicity of bacteria-free culture filtrate of *Vibrio cholerae*, *Nature*, Vol.183, pp.1533–1534, 1959a.

[29] S N De, Activities of bacteria-free culture filtrates prepared from *Vibrio cholerae*, *Annual Report of the Bose Institute, Calcutta, 1958–59*, pp.30–31, 1959b.

[30] S N De, M L Ghose and A Sen, Activities of bacteria-free preparations from *Vibrio cholerae*, *J. Pathol. Bacteriol.*, Vol.79, pp.373–380, 1960a.

[31] S N De, Activities of bacteria-free preprations from Vibrio cholerae, *Annual Report of the Bose Institute*, Calcutta, 1959–60, p.34, 1960b.

[32] S N De, *Cholera, its pathology and pathogenesis*, Oliver and Boyd, Edinburgh, 1961a.

[33] S N De, How does the cholera vibrio produce cholera, *Calcutta Med. J.*, Vol.2, p.184, 1961b.

[34] A R Dutta, A Mondal and S N De, Some observations on the pathology of cholera intestine, *Indian J. Med. Res.*, Vol.52, pp.902–907, 1964.

[35] S N De, B Chowdhury, S N Sen, S Mukherjee and M A Venkatachalam, Hepatic necrosis with haemorrhage fever, *J. Indian Med. Assoc.*, Vol.42, pp.557–560, 1964.

[36] S N De, C R Ghosh and B Mukherjee, Interesting variation observed in a strain of *Vibrio cholerae*, *J. Indian Med. Assoc.*, Vol.44, pp.520–524, 1965.

[37] S N De, B Mukherjee and A R Dutt, Is *El Tor* vibrio in Calcutta. *J. Infectious Diseases*, Vol.115, pp.377–381, 1965.

[38] B Mukherjee and S N De, Observations on some newer differential features of the *El Tor* vibrio and *Vibrio cholerae*, *J. Pathol. Bacteriol.*, Vol.91, pp.257–263, 1966.

[39] S N De, P Sen Gupta, S K Datta and B Ganguly, Morphoea (circumscribed scleroderoma or Addisons's Keloid). *J. Indian Med. Assoc.*, Vol.49, pp.182–185, 1967.

[40] S N De, Tetanus prophylaxis, *Bull. Medical College and Hospitals, Calcutta*, Vol.2, pp.171–173, 1968.

[41] S N De, Tetanus prophylaxis – a dilemma of the day, *J. Indian. Med. Assoc.*, Vol.51, pp.407–410, 1968.

[42] B Mukherjee, K K Bhattacharyya and S N De, Observations on experiments on infant rabbits with *Vibrio cholerae*, *Indian J. Med. Res.*, Vol.57, pp.2205–2212, 1969.

[43] S N De, T N Sen Gupta and P C Sen Gupta, Observations on synovial sarcoma in Indians, *J. Indian Med. Assoc.*, Vol.52, pp.357–362, 1969.

[44] S N De, P Sen Gupta and P K Roy Palodhi. Rhinosporidiosis in West Bengal, *J. Indian Med. Assoc.*, Vol.57, pp.321–323, 1971.

[45] S N De, The value of serotyping of *E. coli* isolated from cases of acute diarrhea in "*Cholera and related diarrhoeas: Molecular aspects of a global health problem*", Eds O Ouchterlony and J Holmgren, 43rd Nobel Symposium, Stockholm, 1978.

[46] S N De, *Cholera Exotoxin – a delayed discovery*, Dr. B.C. Roy Memorial Oration, Calcutta Medical Clud (unpublished), 1980b.