BOSE INSTITUTE: SOME LANDMARK DISCOVERIES

PRADEEP PARRACK*

One hundred years in the life of a research institute is a long time indeed. Established by the legendary polymath Acharya J.C. Bose, Bose Institute has in this period (1917-2016) harboured hundreds of inquisitive minds, each searching for truth in his or her own way, alone or in a collective fashion, and revealing some unknown facet of Nature. Some of their results got included in the mainstream of scientific endeavour. They are the ones that have stood the test of time, and qualify as landmark achievements in the history of scientific research. Such landmarks are necessarily few.

In this article, I shall discuss some such achievements from Bose Institute, and try to impress upon the reader the significance of each of these. The work of D.M. Bose is excluded from this list, since a separate article in this issue (by Singh and Roy) has covered his work. Five such landmark discoveries (by J.C. Bose, Shyamadas Chatterjee, Gopal Chandra Bhattacharya, Sambhu Nath De and Birendra Bijoy Biswas) have been chosen, which stand out as truly pioneering breakthroughs in their respective fields. The first three are discussed in some detail, since the original publications for these are not available very easily.

J.C. Bose : The First Quantitative Study of Photosynthesis and the C_4 Pathway

At the time that Bose Institute was started (1918), the creative genius of J.C. Bose (JCB) was craving to engage himself in the exciting world of plants, glimpses of which had started revealing itself to his unorthodox, self-designed equipments. Indeed, he carried out experiments in diverse areas of growth and response of plants under a variety of conditions, the results of which appeared in the form of books or in a series of papers in the *Transactions of the Bose Institute*. The latter journal was perhaps started for this very purpose. Nevertheless, of the various work done in the period that JCB spent at Bose Institute (*i.e.*, from the starting of the institute till his death, 1918-1937), one that deserves special mention is his detailed work on photosynthesis. Of the many research endeavours of JCB in the plant sciences area, this topic is somewhat less known, but in terms of implications, ranks in the frontline of plant science research. Raghavendra and Govindjee have discussed in detail JCB's work on photosynthesis in a small review, "Sir Jagadish Chandra Bose (1858-1937): A Pioneer in Photosynthesis Research and Discoverer of Unique Carbon Assimilation in *Hydrilla*". In their words[1],

> "Bose had several out-of-box concepts and designed his own innovative instruments to facilitate his research. ... He fabricated and used a unique photosynthesis recorder to study extensively the carbon assimilation pattern, actually measured through oxygen evolution, in an aquatic plant, *Hydrilla verticillata.* Bose made a phenomenal discovery that a unique type of carbon fixation pathway operated in *Hydrilla.* The plants of *Hydrilla* during summer time were more efficient in utilizing CO_2 and light. The summer-type plants used malate as a source of CO_2 and appeared to be different from Crasulacean Acid Metabolism (CAM) plants. These findings of Bose appeared anomalous at his time but are now known

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to illustrate an instance of non-Kranz single cell type C_4-mechanism. In view of his major research contributions, we consider J.C. Bose as a pioneer of photosynthesis research not only in India but also in the world."

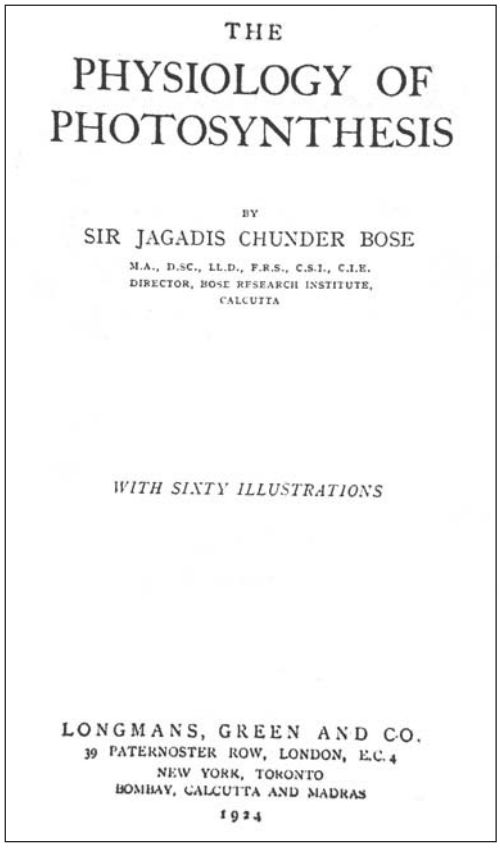
Additionally, JCB's extensive studies in this area (photosynthetic characteristics of *Hydrilla* and other contributions to photosynthesis research in India) have been highlighted in several publications: Bose[2] , Bose and Rao[3], Raghavendra *et al.*[4] and Mukherjee and Sen[5] . JCB himself has given a detailed account of this work in a book published in Great Britain in 1924 (Figure 1)[6]. This 328-page volume contains 28 chapters (see Table 1), the names of which reveal the extent and depth of JCB's research. In the preface to this book, after giving a historical account of the subject (where he has mentioned the serendipitious discovery by Priestley and research by Ingenhousz, Sénébier and de Saussure), JCB writes,

"It would appear, therefore, that almost everything that can be known about photosynthesis has now been ascertained. It may be admitted that this is approximately true in the *qualitative* sense, but certainly not in the *quantitative* sense. In spite of many laborious researches, it is not yet possible to attach definite numerical values to the efficiency of light of various wave-length and energy; nor to the effect of a rise of temperature, or of a variation in the amount of available carbon dioxide, upon the activity of photosynthesis. The present volume is essentially a record of quantitative research in these various directions...."

TABLE 1. Contents of the book 'The Physiology of Photosynthesis' by J.C. Bose (1924).

Chapter	Title
I	Introduction (Method of chemical analysis in photosynthetic investigation, Difficulties of the problem arising from complicating factors, The method of evolution of oxygen by water plants, ... New method for securing high accuracy in quantitative determinations, ...)
II	The Evolution of pure oxygen under light
III	Determination of rate of evolution of equal volumes of oxygen
IV	The automatic record of the rate of evolution of oxygen
V	Photosynthesis under increasing intensity of light
VI	Relation between the quantity of light and the amount of photosynthesis
VII	The physiological factor in photosynthesis
VIII	Change in photosynthetic activity under stimulus, anaesthetics and poisons
IX	Effect of infinitesimal traces of chemical substances on photosynthesis
X	The electric response to light
XI	Phenomenon of photosynthetic induction
XII	Effect of intermittent light on photosynthesis
XIII	The automatic radiograph
XIV	The electric photometer
XV	Relation between CO_2-supply and photosynthesis
XVI	Photosynthetic evolution of oxygen in the complete absence of carbon dioxide
XVII	Effect of variation of temperature on photosynthesis
XVIII	The tonic factor in photosynthesis
XIX	The daily variation in photosynthetic activity
XX	Determination of the photosynthetic effeciency of light of different colours
XXI	Determination of the energy of the different rays in the solar spectrum
XXII	Determination of photosynthetic effciency of the spectral rays
XXIII	Determination of the increase of weight due to photosynthesis
XXIV	Simultaneous determination of carbohydrate-formation by two independent methods
XXV	Efficiency of the photosynthetic organ in storage of solar energy
XXVI	The physiological scale and the law of photosynthesis for the different factors
XXVII	Photosynthesis under simultaneous variation of different factors
XXVIII	General Review

A proper appraisal of the book is beyond the scope of this article. JCB had chosen the aquatic plant *Hydrilla verticillata* as his system. The reasons for this choice are given in the third chapter of his book. This simple plant, lacking stomata and transpiration, could be readily maintained in a vessel of water, and the released oxygen during photosynthesis could easily escape through the water. JCB started this chapter with the comments, "The most important factor in the accurate estimation of photosynthetic activity is the measurement of the rate of evolution of oxygen. The usual method of estimation by counting the bubblets given out by the plant is, however, not trustworthy...". This is followed by enumerating



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PHYSIOLOGY OF PHOTOSYNTHESIS

BY

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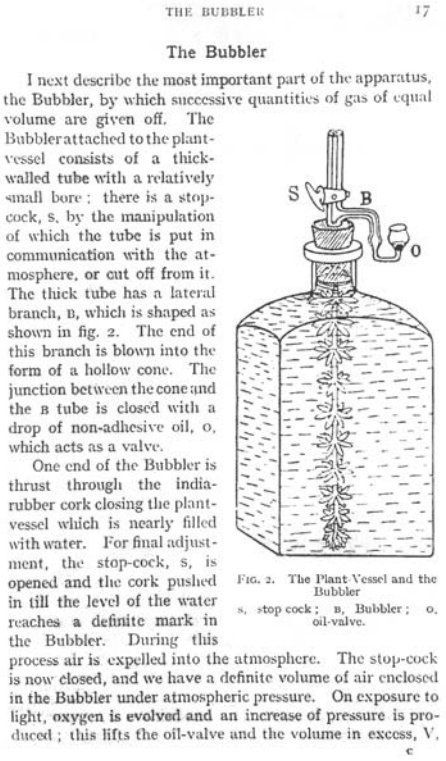
WITH SIXTY ILLUSTRATIONS

LONGMANS, GREEN AND CO.
39 PATERNOSTER ROW, LONDON, E.C.4
NEW YORK, TORONTO
BOMBAY, CALCUTTA AND MADRAS
1924

Figure 1. J.C. Bose's book on photosynthesis, published in 1924.

the reasons. He listed four such reasons, alluding to how another worker, Wilmott, had attempted to obviate one of the difficulties[7]. However, JCB thought it necessary to "measure the absolute rate of evolution of oxygen and its modification under definite external variations" in order to determine the photosynthetic activity. He was also concerned about personal errors which could influence his measurements, and in order to eliminate these, wanted to have a means for automatic record of the rate of evolution of oxygen. For this purpose, he constructed two equipments: the Photosynthetic Bubbler (Figure 2) and the Electromagnetic Recorder. Using these, he examined how the supply of carbon dioxide was related to photosynthesis and defined the coefficient for concentration of CO_2 as a measure of utilization of CO_2 . The average values of the calculated coefficients in summer were about twice of those obtained in winter[6,1], clearly pointing out the increase in the photosynthetic efficiency of carbon assimilation in *Hydrilla* during summer. Further, JCB noticed that "while the juice of the plants was practically neutral in winter and spring, it was very strongly acid in summer." He found that this acidity was caused by the presence of malic acid and small amounts of oxalic acid. A page of his notebook describing these observations is shown in Figure 3.



THE BUBBLER 17

The Bubbler

I next describe the most important part of the apparatus, the Bubbler, by which successive quantities of gas of equal volume are given off. The Bubbler attached to the plant-vessel consists of a thick-walled tube with a relatively small bore; there is a stop-cock, S, by the manipulation of which the tube is put in communication with the atmosphere, or cut off from it. The thick tube has a lateral branch, B, which is shaped as shown in fig. 2. The end of this branch is blown into the form of a hollow cone. The junction between the cone and the B tube is closed with a drop of non-adhesive oil, O, which acts as a valve.

FIG. 2. The Plant-Vessel and the Bubbler
S, stop cock; B, Bubbler; O, oil-valve.

One end of the Bubbler is thrust through the india-rubber cork closing the plant-vessel which is nearly filled with water. For final adjustment, the stop-cock, S, is opened and the cork pushed in till the level of the water reaches a definite mark in the Bubbler. During this process air is expelled into the atmosphere. The stop-cock is now closed, and we have a definite volume of air enclosed in the Bubbler under atmospheric pressure. On exposure to light, oxygen is evolved and an increase of pressure is produced; this lifts the oil-valve and the volume in excess, V, c

Figure 2. The photosynthetic bubbler (from the book shown in Figure 1).

JCB demonstrated that photosynthesis in *Hydrilla* (as measured by the evolution of oxygen) had some unique properties. Acids accumulated (mainly malate) and malic acid could substitute for CO_2 , and photosynthesis could occur even in the absence of externally added CO_2 . Very significantly, he showed that

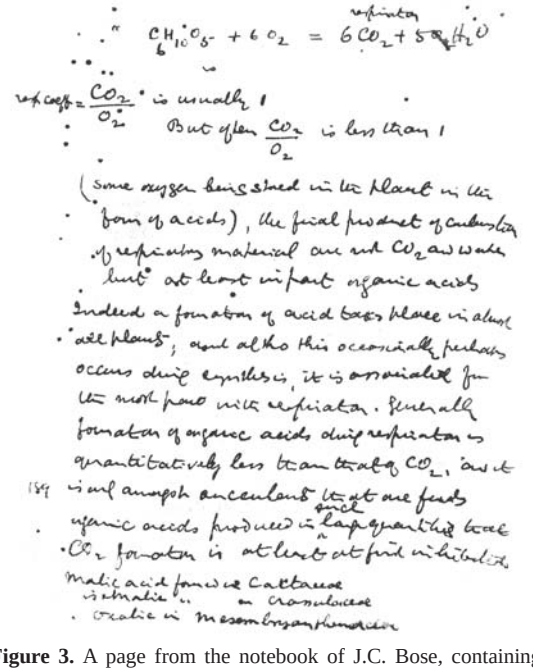


Figure 3. A page from the notebook of J.C. Bose, containing notes on photosynthesis experiments using the photosythetic bubbler.

i. *Hydrilla* utilised malate instead of CO_2 during photosynthesis, and

ii. Uptake of CO_2 in *Hydrilla* was less than normal.

These results had deep implications, as has been found years later. Raghavendra and Govindjee[1] commented that "Bose had visualized the idea of the operation, in *Hydrilla*, of a quite different photosynthetic pathway, which utilizes malate." The primary route of assimilation of carbon in plants takes place through the so-called C_3-pathway, established by Calvin, Benson and coworkers[8-11] and is known as the 'Calvin Cycle'. Most plants on the earth follow this Calvin cycle for assimilation of carbon. About 3% of all plants (mostly different types of grass including maize and sugarcane), on the other hand, assimilate carbon through photosynthesis using the C_4 pathway that is advantageous under conditions like drought, high temperature or limited carbon dioxide. The C_4 pathway is presently known as the 'Hatch-Slack' pathway, its discovery having been attributed to two Australian scientists[12] who provided a mechanism for this pathway. Much later, it was discovered that the first experiments indicating that some plants do not fix carbon using the more common C_3 cycle and instead produce malate and aspartate in the first step may have been done in the 1950s and early 1960s by Hugo Kortschak[13] and Yuri Karpilov[14] . There is a third type of carbon assimilation in the so-called CAM plants (that undergo Crassulacean Acid Metabolism) that also use malate and other acids for concentrating CO_2 inside cells during dark, and use these acids in the presence of light. Succulents follow this CAM pathway. JCB was apparently aware that what he observed with *Hydrilla* in summer was different from acid accumulation in the succulents (CAM plants). Indeed, he remarked that "The organic acids stored during the night (in succulent plants) provide *indirect* material for photosynthesis during the day in the form of CO_2 . The *Hydrilla* plant appeared to be most suitable for further investigation on the subject that the organic acid served *directly* for photosynthesis"[6].

It now appears that the first significant observation of malate accumulation during photosynthesis had been made by none other than J.C. Bose, almost four decades earlier than even Kortschak or Karpilov. However, JCB's observations on *Hydrilla* or the biochemical knowledge of that time lacked the detail to suggest a C_4 mechanism for these plants. Many of JCB's observations could be explained by detailed studies on the physiology, biochemistry and molecular biology of photosynthetic carbon assimilation in *Hydrilla* and other aquatic plants done more than five decades later. The unique pathway for assimilation of carbon in these plants is presently considered "an example of non-Kranz single cell C_4 -pathway in aquatic angiosperms".[1]

Shyamadas Chatterjee : First Observation of Spontaneous Fission

Shyamadas Chatterjee (SDC) excelled in building instruments. In 1938, D.M. Bose left Calcutta University to take up the directorship of Bose Institute, following the death of J.C. Bose. SDC, who had joined D.M. Bose for research in Calcutta University around 1932, moved with him to Bose Institute. It was here that he constructed a modified and better version of the Wilson cloud chamber. He also learnt the knowhow of making Geiger Muller and proportional counters from Radheshyam Ghosh who had been trained in Germany by none other than Geiger himself. His extreme devotion to his work led SDC to even spend from his personal funds for purchasing items for this purpose. With the help of such counters, he initiated experimental studies on radioactivity at Bose Institute. It has been reported that Shyamadas was at home with all the various techniques that are required in instrumentation, such as glass blowing, machine shop work, electronics, production of vacuum etc[15].

Nuclear fission was discovered in the end of December 1938, by Lise Meitner and Otto Frisch[16] . They had been trying to solve the perplexing results of experiments carried out by Meitner and Otto Hahn[17] . The news of this discovery startled the physics community, and also reached Kolkata. Soon thereafter, SDC set up experiments to study fission and carry out nuclear reactions by bombarding Uranium and other elements by neutrons, in 1939. Using the counters that he had made, he started observing fission events with an oscilloscope and showing them to people around. In the course of his experiments, SDC started observing strange pulses in the oscilloscope even when the Ra-Be neutron source was far away. He discussed these results with S.N. Bose, whom he must have known while at Calcutta University. On the latter's suggestion, SDC checked the possibility of cosmic rays causing the strange pulses. But even with thorough shielding of his chambers, large spurious pulses started appearing as before. This result was interpreted by S.N. Bose as arising from 'spontaneous fission', and Shyamadas worked out the lifetime of these events as observed by him. The value he obtained was about 10^{16} years, about a million-fold quicker than the theoretical value of 10^{22} years for spontaneous fission which had just been published, in September 1939[18].

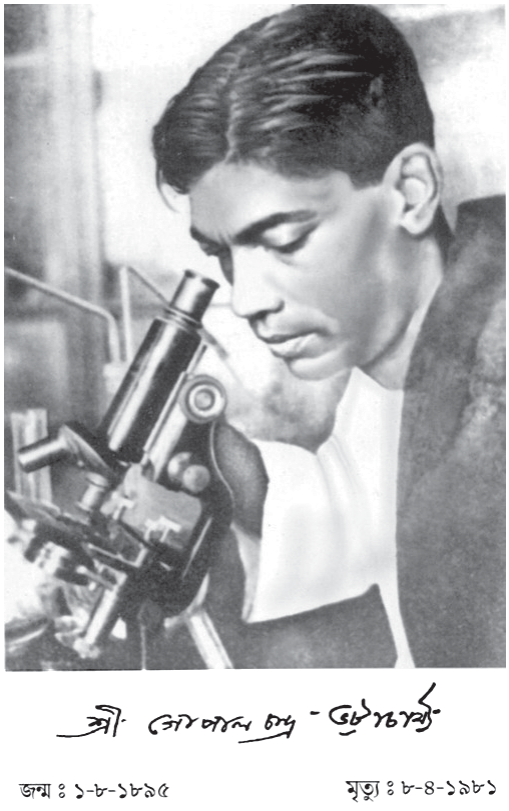
This observation of spontaneous fission by SDC was arguably the first ever worldwide. For an event like the

spontaneous fission of uranium with its wide implications even beyond the realms of physics, this was a very big achievement. At the insistence of S.N. Bose, SDC wrote a letter describing this result and submitted it to *Science and Culture.* His thesis supervisor D.M. Bose (who was also the editor of this journal) was away during the time that Shyamadas was doing all this. Upon his return, D.M. Bose made SDC withdraw the paper. It is not clear whether he was uncomfortable with the discrepancy of the measured mean life value with that calculated by Bohr and Wheeler[18], or did not believe SDC's results at all. Whatever be the case, SDC's observations remained unpublished at that point of time. About two months later (on July 1, 1940) the Russian scientists Flerov and Petrjak published similar results[19] and are on record the first to have observed spontaneous fission. Shyamadas did eventually publish his results much later, with more reliable measurements using proportional counters[20-22] , and continued to make important contributions in atomic and cosmic ray research. Nevertheless, the honour of being the first observer of spontaneous fission that rightfully belonged to him, had eluded him for circumstances beyond his control. It may be noted that the tradition of publishing in journals like *Science and Culture* or *Transactions of Bose Institute,* which did not have a circulation in the wider scientific community, could also have proved costly for him.

Shyamadas Chatterjee's passionate involvement with scientific research is reflected in his spending his own money for buying material for research during his student days, as well as setting up of a foundation to carry out research after his formal retirement. He was a master innovator who initiated research in many fields in India, including measurement of environmental radioactivity and radiocarbon dating, using equipments that he built himself. In this sense, he was a true follower of Acharya Jagadish Chandra[15].

Gopal Chandra Bhattacharya : Pioneering Work with Ants and Tadpoles

Gopal Chandra Bhattacharya (GCB) was a born naturalist, who has been compared to the likes of Charles Darwin, Jean Henri Fabre or Eugene Maris[23]. An extraordinary observer, he joined Bose Institute as an assistant to JCB in 1921 and worked there as a scientist till 1971, well after his formal retirement. It is during this period that he was engaged in various studies, mostly observational, on spiders, butterflies and ants in particular (Figure 4). In the later years, he made some novel observations on the effect of penicillin and vitamin B_{12} on the metamorphosis of tadpoles. GCB is very well known as a popularizer of science in Bengali, and was associated with S.N. Bose in founding the "Bangiya Bijnan Parishad", an organization devoted to making science accessible to the general public, mainly through communicating in the native language (Bengali). For many years, he also edited the Bengali journal 'Jnan O Bijnan', published by the Parishad.



জন্ম ঃ ১-৮-১৮৯৫ মৃত্যু ঃ ৮-৪-১৯৮১

Figure 4. Gopal Chandra Bhattacharya

Although most of GCB's publications have been non-technical and in Bengali (he published several in "Prabasi", a well known Bengali literary magazine), he did publish a few papers in journals like *Natural History*, *Journal of the Bombay Natural History Society*, *Science and Culture,* or in *Transactions of Bose Institute.* In 1943, he published a paper entitled 'Reproduction and Caste Determination in Aggressive Red Ants, *Oecophylla smaragdina,* FABR."[24]. In this paper, he addressed the issue of caste determination and polymorphism in ants (which is how workers, males and the queen would evolve from the eggs), then a somewhat controversial issue. Opinion was divided in the question of whether caste determination was blastogenic (determined genetically) or trophogenic (determined by nutrition). In the above paper, he has described the puzzling situation that led him to the problem:

> "...a small nest of red-ants, built on a potted mango plant, was placed along with the spiders' abode in a

place completely surrounded by water. There were no eggs or larvae inside the nest when it was placed in the secluded area. About six weeks later the nest appeared bigger than its original size. On opening the nest it was found that not only the number of ants had increased but also a large number of eggs and larvae were present in it. As there were only workers in the nest and no females, how could the eggs and larvae appear therein? It seemed very perplexing."

The novel experimental design that GCB adopted deserves special mention, and was by itself a clever innovation. The ants he worked with were arboreal. They built ball-like nests by stitching leaves. In order to observe them, GCB constructed transparent artificial formicaries with the help of thin cellophane paper (Figure 5), and was able to have the ants move to this transparent formicary which was placed on a miniature island completely surrounded by water. After several trial and errors, he was able to identify the right conditions and obtain populations of ants with larvae, moving over to reside in the artifically constructed transparent abodes. Now he could observe their actions inside their homes. Food was supplied on the platform of the surrounded area, where ants would come to feed and drink.

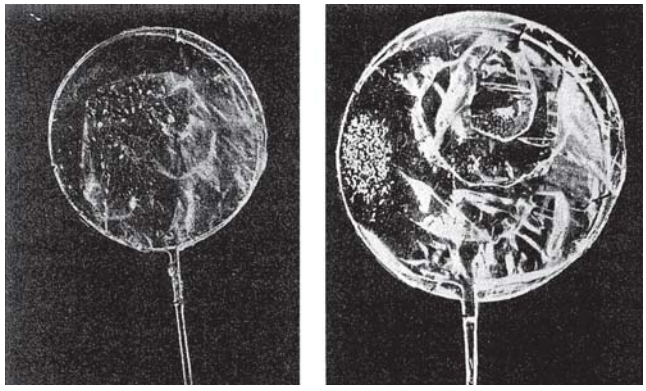


Figure 5. The artificial ant nest made by Gopal Chandra Bhattacharya with transparent cellophane paper, crucial to his experiments to determine the blastogenic/ trophogenic caste determination in ants (from *Transactions*, 1943: ref. 24). The caption on the left figure reads: "Ants are quite comfortable in the artificial cellophane nest", and the one on the right has the caption "Eggs and larvae are arranged on the inner surface of cellophane nest by the nursing workers."

In the 1943 paper[24] GCB described a series of breeding experiments that started in September 1939 and continued till October 1941, involving five batches of experiments and twenty two nests. He would make a rough count of the number of workers, and also count the numbers of males and females (queens) in each nest, before and after several months had passed. The food was varied across batches. He would also make a note of the average temperature for each nest. From these experiments, he concluded that the worker-ant lays eggs parthogenetically throughout the year from which workers are produced, while the fertilized queen lays eggs only at a certain time. Workers are produced from these eggs too. However, only males are produced from the unfertilized eggs of the queen, while "when.... larvae in the earlier stages are fed with some activating substances, such as vitamins or enzymes along with the regurgitated food, males and females are found to be produced in artificial nests, exclusively inhabited by worker-ants." He also observed polymorphism in workers, males and queens, and related the difference (which was only in body lengths) to the difference in nutrition. Externally, there was no difference among the eggs that developed into workers, males or queens.

It had been reported that workers and females were produced from the same kind of larva by nutritional variation, in the honey bee species *Apis mellifica*[25]. In these bees, queens emanated from larva fed with 'royal-jelly', originally supposed to be rich in vitamin E[26]. GCB proceeded to test this assertion in ants, and started feeding the young larvae regularly with pulverized wheat-germs and yeast, containing sufficient quantities of vitamin E. The results, however, showed that vitamin E was not the factor responsible for sex-differentiation in *O. smaragdina*. A similar result had also been reported for bees (*A. mellifica*) by Melumpy and Mason[27]. In 1939, Melumpy and Jones had pointed out the existence of vitamin B_1 and identified its concentration by studying the composition of royal jelly[28]. GCB tried to test this by adding different concentrations of thiamin (synthetic vitamin B_1) to the food of his ants, but failed to induce the change-over of the larvae from workers to females. Eventually, by experimenting with various feeds, he could conclude that " Some factor or factors in the nutritive element, of which vitamin B_1 may be one, must have brought about the physiological and morphological changes during the growing stages of larvae." He also found that the difference in quantities of this nutritive factor caused the production of females (sufficient quantities of the factor) or males (insufficient quantities of the factor). In the absence of this factor, only workers were produced. Gopal Chandra thus convincingly demonstrated that caste determination in his ants was trophogenic, and while vitamins E and B_1 were necessary for sex determination, they were not sufficient. This work of his on royal jelly, in ants using simple and indigenous experiments, painstakingly performed for many months, is worthy of remembering GCB as a Pioneer in this field at the international level.

The other achievement of GCB that stands out is his pioneering work on the inhibitory effect of drugs on the metamorphosis of tadpoles, whch could be reversed by administering vitamin B_{12} . Starting with 250 tadpoles in May 1951, he continued these experiments till March 1954. Treating different batches of tadpoles with increasing concentrations of penicillin, he compared the observed effects with a control set of untreated tadpoles. While all the tadpoles of the control set metamorphosed into froglets by six weeks, metamorphosis in all the penicillin-treated ones were delayed from two to four months. Subsequently, many died. The ones that survived were stuck at different stages of metamorphosis. None of them grew into froglets, and subsequently all died.

Upon administering two different doses of vitamin B_{12} to penicillin-treated tadpoles (in which metamorphosis had been arrested), different types of results were obtained. While for the lower dose of vitamin the tadpoles now underwent metamorphosis into froglets (though they did not survive for long), the higher vitamin dose had apparently no effect[29] . GCB also observed similar results when streptomycin rather than penicillin was used. Very significantly, he concluded that (i) "The metamorphosis retarding action of the antibiotic substances on the tadpole is not direct, but probably effected indirectly through some micro-organisms", and (ii) the effect of vitamin B_{12} and antibiotics appeared to neutralise each other's action on metamorphosis. Thus Gopal Chandra very correctly guessed the effect of microbiota on development, much ahead of times, when practically nothing was known about them.

Sambhu Nath De : Rabbit Ileal Loop Assay and the Discovery of Cholera Toxin

Eugene Garfield, the editor of *Current Contents*, a valuable bibliographic resource before the age of electronic databases, became interested in knowing about Dr. S.N. De (SND), whose 1953 paper, "An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane"[30] had received a very large number of citations. That was in April 1985. De was in coma. He passed away without knowing that his work had become a citation classic. Next year, Garfield wrote about the history of cholera research in *Current Contents*, paying tribute to S.N. De[31]. Immediately, everybody noticed that there was an Indian medical scientist who had quietly been making epoch-making discoveries in research involving *Vibrio cholerae*, the bacteria that causes the deadly cholera disease.

Unlike the three scientists mentioned earlier, Sambhu Nath De did not formally belong to Bose Institute. Nevertheless, his association with Bose Institute around 1958-59 resulted in a landmark discovery for which he is now revered worldwide. His identification of the cholera exotoxin as the causative agent of the disease introduced a new paradigm in cholera research[32].

The causative agent of cholera had been identified by the Italian anatomist Fillipo Pacini from microscopic observations, way back in 1854[33] . Decades later, Robert Koch for the first time cultured *V. cholerae* in 1884. However, due to the lack of an animal model, the pathogenesis of the disease could not be studied for many years. Following Koch, it was widely believed that the disease was caused by a 'poison' which is absorbed by the body. As a result, no efforts were made to pay attention to the intestine, and every preparation of *V. cholerae* was injected parenterally, rather than enterally. The effects of such experiments had nothing to do with cholera, and Koch opined that cholera cannot be reproduced in animals[34] . SND solved this long-standing problem and introduced an assay that is still used widely for many diarrheal diseases. De himself elegantly described this discovery in an unpublished oration lecture he delivered in 1980 (as quoted by Sen and Sarkar[34]) :

> "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. Se 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. ricewater fluid while the control loop receiving the sterile medium was collapsed. This represents cholera localized to a small segment of the intestine."

De's innovation was actually a re-invention of a much older method, which had fallen into obscurity and was unknown to him or anyone else[35,36] . SND's rabbit ileal

loop assay (RIL assay) (Figure 6) became extremely useful and popular with clinical biochemists and pathologists, and his 1953 paper[30] became a citation classic, as mentioned before. In the years that followed, he carried out pioneering studies on the enteropathogenic activity of *E. coli* cultures using the RIL assay[37] . Finally, in a paper in *Nature* in 1959 he identified the cholera-causing agent as an exotoxin[32] . At this time he was actively associated with Amitabha Sen at Bose Institute and was using facilities available in Sen's laboratory.

Figure 6. Rabbit ileal loop used by S.N. De for assay of cholera toxin. This type of ileal loops in rabbits and other animals are widely used to this day by researchers and pathologists.

Though SND died as an 'unsung hero', he had actually received recognition of some sorts at the international level. He was nominated twice for the Nobel prize by Joshua Lederberg, who had commented that "De's clinical observations led him to the bold thought that dehydration was a sufficient cause of pathology of cholera, that the cholera toxin can kill 'merely' by stimulating the secretion of water into the bowel"[38]. A man with little ambition, SND retired from his hospital service and continued research at his self-built laboratory at home and at Bose Institute. In 1978, the Nobel Foundation had to find him out from retirement to request him to participate in a symposium in Stockholm on cholera and related diarrhoeas. De delivered a talk in this meeting [39]. In spite of these, he remained in oblivion and was honoured in India only after 1986. His busts were placed in all the places he had worked in (including Bose Institute), and the journal *Current Science* published a special issue on SND in July 1990.

Birendra Bijoy Biswas: The Myo-inositol Phosphate Metabolic Cycle

B.B. Biswas (BBB) began his long association with Bose Institute as a doctoral student in 1954, leaving a lecturer's job in Banaras Hindu University where he had started as a research assistant in 1952. Of his many accomplishments in Bose Institute, he is remembered for pioneering research using molecular biology and starting the use of radioactive tracers in India to investigate metabolic pathways, as early as in 1954. He was also instrumental in establishing the Department of Biochemistry at Bose Institute in 1972 and building it up as a very active research centre in the country, and for obtaining large research grants that supported many scientists at the Bose Institute; for setting up an advanced centre for sophisticated equipments, and a centre for bioinformatics. He retired from Bose Institute in 1991 after serving as its director, and continued to teach and carry out research in the biophysics department of Calcutta University for many years. At the age of 89, he is the only surviving member in this list.

At the time that BBB started his research career in Bose Institute, the structure of DNA had just been discovered. In those early days of DNA research, when the isolation of DNA from plant-like material was not standardized, he ventured to work on a self-identified project of characterization of DNA from blue green algae. He used radioactive phosphorus (not available with high specific activity) and paper chromatography to work out the ratios of the different bases. During 1954-1958, using radioactive tracers, he worked on sulphur metabolism in plants[40] as well as on biochemical pathways for nucleic acid synthesis[41] and carbon assimilation[42] . He also went on to work on the effect of colchicine on the synthesis of nucleic acids and proteins[43]; on the effect of polyribonucleotides on chloroplast ribosomes[44]; on an enzyme system from spinach chloroplasts[45], and on indoleacetic acid[46] and polyguanylic acid[47]. In addition to all this, he is best remembered and revered for supporting and promoting a significant number of bright scientists in their early research careers at Bose Institute.

In the early sixties, BBB initiated work on *myo*-inositol phosphate metabolism in germinating mung bean seeds (*Phaseolus aureus*) that acts as a phosphate storage system in seeds and utilizes them during germination. Starting from the discovery of the enzyme inositol hexaphosphate ADP/GDP phosphotransferase[48] , BBB and his coworkers went on to work out various details of this system[49-55] for many years. His group identified the different enzymes associated in transferring phosphate groups in the system. These results culminated in his proposal of a new metabolic cycle for the synthesis and breakdown of inositol phosphates, that are active during maturation of seed and during germination. This cycle was linked with the pentose phosphate cycle[56,57] (Figure 7) and explained many aspects of germination not so well understood earlier.

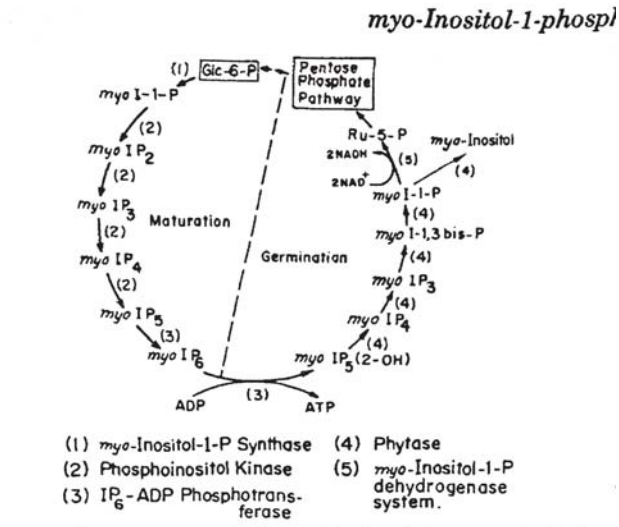


FIG. 3. **Proposed metabolic cycle involving *myo*-inositol phosphates during formation and germination of seeds.** *myo-I-1-P*, *myo*-inositol 1-phosphate; *myo-IP*$_2$, *myo-IP*$_3$, *myo-IP*$_4$, *myo-IP*$_5$, and *myo-IP*$_6$ correspond to di-, tri-, tetra-, penta-, and hexaphosphates of *myo*-inositol, respectively.

Figure 7. The myo-inositol phosphate metabolic cycle proposed by B.B. Biswas (from De and Biswas, 1979: ref. 56) .

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