Yellapragada SubbaRow

The Man of Miracle Drugs

Gopalpur Nagendrappa

G Nagendrappa was a Professor of Organic Chemistry at Bangalore University, and Head of the Department of Medicinal Chemistry, Sri Ramachandra (Medical) University, Chennai. He is currently in Jain University, Bangalore. He continues to teach and do research. His work is in the area of organosilicon chemistry, synthetic and mechanistic organic chemistry, and clay-catalysed organic reactions (Green Chemistry).

This is the story of a determined Indian who strived very hard and achieved greatness. He went to Harvard Medical School with very little money, braved many grave hardships and eventually fulfilled his ambitions to a large extent. Yellapragada SubbaRow did research in Harvard Medical School in biological chemistry and directed research at Lederle Laboratories, a pharmaceutical giant near New York. He played an important part in several historic and extraordinary discoveries made in biological chemistry and medicine during 1924–1948. Some of the important ones are phosphocreatine, adenosine triphosphate, vitamin B_9, antifolate cancer drugs, filaricides and antibiotics. He made many personal sacrifices to accomplish what he wanted to. He received little recognition during his lifetime, but is now hailed as one of the most eminent medical minds of the century.

Keywords
SubbaRow, phosphorus estimation, phosphocreatine, folic acid, antifolates, cancer, methotrexate, filariasis, hetrazan, tetracycline.

Introduction

As long as the medical fraternity uses chemotherapy to treat several cancers, needs to administer folic acid (vitamin B_9) to cure pernicious anaemia and tropical sprue patients, prescribes hetrazan to rid the pain and deformity of people afflicted with filariasis, needs to use tetracycline antibiotics against plague and bacterial diseases, both doctors and patients should be greatful to Yellapragada SubbaRow who made all this possible. To be fair, SubbaRow did not achieve so much all alone, but he played a major role and provided leadership in research efforts that culminated in drugs that have been in use for more than half a century now. Before making these extraordinary contributions to the human well-being, SubbaRow, with his guide Cyrus Fiske, had become a textbook name for developing a very practical colorimetric method to accurately estimate serum phosphorus. Even

today, very close to a century later, it is a procedure preferred by clinical biochemists, pathologists, analytical chemists and others for the determination of organic and inorganic phosphorus as phosphate. Using this simple procedure SubbaRow discovered adenosine triphosphate (ATP) and its role as a source of energy in muscles. He enriched chemistry, biochemistry and medicine with great discoveries which were not meant just to fill some pages of scientific journals or books, but to alleviate the sufferings of millions of people afflicted with deadly diseases.

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Early Life

Yellapragada SubbaRow was born on 12th January 1895, in Bhimavaram, a town in West Godavari district of Andhra Pradesh, then in Madras Presidency. He was one among four brothers and three sisters. His father Jagannatham was a revenue inspector and mother Venkamma, a traditional Brahmin housewife. The family income was low and the Yellapragadas led a life of hand-to-mouth existence. Deeply affected by poverty at home, SubbaRow at the age of thirteen planned to get rich by becoming a trader. For this he considered the famous pilgrimage centre Varanasi to be the right place where he could earn money by selling bananas to the large number of visiting pilgrims. He left home secretly in order to realise his plan. But his absence was soon noticed and it was not difficult to trace him and bring him back home. After this incident Venkamma kept a close watch on his activities and put pressure on him to study and do well in school.

SubbaRow had his pre-high school education in Narasapur and Rajahmundry, and high school education at Hindu High School, Madras (Chennai). His father passed away in 1913, when SubbaRow was 18 years. This probably affected his studies in the high school and he had to make three attempts to pass the matriculation examination, though he seems to have been very good in mathematics. He did his intermediate studies at Madras Presidency College. Around this time, SubbaRow seems to have lost interest in worldly life and wanted to become an ascetic in Ramakrishna Mission. Again Venkamma intervened and did not

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permit this deviation. Without Venkamma's permission, the Ramakrishna Mission did not admit SubbaRow into its fold. The Mission advised him to pursue medical studies; by becoming a doctor, he could achieve the same goal of serving society and people as he would by joining the Mission. He was an ambitious man and wanted to make a big name by whatever means that was possible for him, as he used to tell his mother. This desire to achieve fame defined his restless behaviour and attitude to do something that was not quite normal for a young boy. At about this time he studied Hindu scriptures, *Bible*, *Quran* and other religious literature.

Medical Education and a Stint with Ayurveda

After completing a two-year intermediate course, SubbaRow joined Madras Medical College in 1915. Financial support for his medical education came from his friends and Kasturi Suryanarayana Murthy. In 1919, two years before completing his medical studies, SubbaRow married Seshagiri, the 15-year-old daughter of Murthy. For SubbaRow it was a marriage of convenience, as Murthy supported his medical studies and later his passage to USA.

Under Gandhiji's influence, SubbaRow wore Khadi dress – he even abandoned the British surgical dress. This earned him the displeasure of his surgery professor, M C Bradfield who punished SubbaRow by undervaluing his surgery performance.

It was the time when the Indian Independence Movement had picked up great momentum. Most of the school and college students participated directly or indirectly. Under Gandhiji's influence, SubbaRow wore Khadi dress – he even abandoned the British surgical dress. This earned him the displeasure of his surgery professor, M C Bradfield who punished SubbaRow by undervaluing his surgery performance; as a consequence SubbaRow was given an LMS diploma instead of an MBBS degree, in 1921. He could not get into Madras State Medical Service. The next best he could do was to join an Ayurvedic College (Dr. Lakshmy Pathi's Ayurvedic College) in Madras as a lecturer to teach anatomy. He set out to make the best use of the opportunity that came his way. He started working on the ways and methods to make Ayurveda conform to modern scientific principles.

In the 1920s, allopathic medical practice was not much advanced.

There were very few drugs available to control a vast number of diseases. Though scientists like Pasteur, Ehrlich and others had discovered vaccines and chemicals to fight certain diseases, there were no antibiotics, no anticancer drugs, etc. Very few vitamins were known, very little was known about the formation or functions or constituents of biological molecules like proteins, DNA, RNA, etc. Compared to what we have today, the medical practice in the 1920s was quite primitive. In such a situation, an expert Ayurvedic practitioner possessed healing powers as good as, or probably even better than an allopathic medical practitioner. SubbaRow contemplated the possibility of obtaining potent drugs from Ayurvedic medicinal plants.

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In the same year, his two brothers were afflicted with Tropical Sprue, and both died of this fatal disease, the elder one in July and the younger in August[1]. His brothers' death had a profound effect on the doctor in SubbaRow; he made it his life's mission to find an effective remedy to cure tropical sprue. This sudden unforeseen sad incident cost him his admission to Harvard School of Tropical Medicine in 1921. His association with Ayurveda became a disqualification for readmission there. However, his dogged efforts eventually got him admission a second time in 1923.

[1] This information is based on SubbaRow's letter to the Dean of Harvard Medical School.

Journey to the Institution of his Dream

Before applying to Harvard, SubbaRow had the opportunity to meet Dr. Kenrick of Philadelphia University, who was in Madras on the Rockefeller Foundation's anti-hookworm campaign. On observing the enthusiasm and passion of SubbaRow to discover cures for diseases, the doctor suggested that he should go to Harvard Medical School in Boston in order to realise his ambitious dreams. This cue was enough to motivate the enthusiastic SubbaRow to work out a way to achieve it. His father-in-law was sufficiently well-off and generous enough to finance his passage to Boston and some of his initial expenses there. The generosity of Suryanarayana Murthy is commendable, not just because he was providing financial help, but he was willing to let his son-in-

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law go while Seshagiri was pregnant. To add to that, it was an era of very poor communication, and it was not so common for Indians to go to the United States[2].

[2] SubbaRow mentions in one of his letters to his father-in-law dated *circa* end of 1923, that there were only about 300 Indian students in the whole of the US, and only 23 of them in Boston. Compare this to today's number of more than 100,000 Indian students studying in various universities across the US.

SubbaRow travelled to the US via London by steamers. His finances were insufficient to purchase a ticket even for third class, which forced him to stay on deck during the journey. He was the only vegetarian on board, but he had no trouble finding meat-free meals. Luckily, he did not suffer from seasickness or any other illness during the voyage, which took about a month during September–October of 1923. He arrived in Boston on the 26th of October 1923, and enrolled in the Harvard School of Tropical Medicine for a diploma course. This was necessary for him to get admission to the Medical School as his Indian degree was considered inadequate for his admission there.

At the School of Tropical Medicine

SubbaRow had come to Boston with very little money, but managed to enrol for the course. Richard Strong, the Director of Harvard School of Tropical Medicine, was very helpful and provided him working facilities in the laboratory and a scholarship. SubbaRow would also request his father-in-law to send him money. But, to sustain himself he needed additional regular income, and for this he worked in Peter Brent Bright Hospital in Boston from 7 to 1 in the night after attending classes from 9 to 5 in the day. He had very little time left to sleep or do other things. He had no choice but to slog, in order to survive and move forward. Despite so much of work and a tight schedule, SubbaRow used to write nice, long letters very regularly to his relatives, particularly to his wife (in Telugu) and father-in-law (in English), reporting about his life in America and enquiring about his people in India. The letters mirror his writing skills and personality.

SubbaRow's letters mirror his writing skills and personality.

On April 12, 1924, Seshagiri gave birth to a baby boy in Anaparthi, her parents' place. SubbaRow was not very excited about it. He was a great believer in astrology, fate, the existence of the soul, etc. His astrological calculations predicted that his son would not survive long after birth. Unfortunately, the prediction came true,

and on the 24th of December, the boy succumbed to Erysipelas (Sarpi), less than nine months after birth.

Phosphorus, Phosphocreatine and ATP

SubbaRow obtained his Diploma in Tropical Medicine in June 1924, and joined Harvard Medical School for the PhD programme in Biochemistry in the Department of Biological Chemistry. Cyrus Hartwell Fiske (1890–1978), an Associate Professor, was his research guide. The department was headed by Otto Folin. At the time, there was much interest and activity in the chemistry of phosphorus in living organisms. SubbaRow was assigned to develop a reliable, rapid, analytical method for the determination of phosphorus in body fluids. The intelligence, experimental skills and hard work of SubbaRow produced a simple, accurate colorimetric method for the determination of inorganic as well as organic phosphorus, within a short period of time. The work was published soon in the *Journal of Biological Chemistry* [1]. The paper was instantly accepted by the scientific community and became a part of biological chemistry textbooks in 1925. Even today, the Fiske–SubbaRow procedure is one of the popular methods used widely for the determination of phosphorus [2]. The paper has become one of the most highly cited classics [3]. The principle of the method is as follows.

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The phosphate in biological extracts is converted into ammonium phosphomolybdate. Molybdenum(VI) in the latter is reduced to molybdenum(V) to get the intense blue species. See equations (1) and (2). Earlier, the reduction was effected by hydroquinone, which was not satisfactory. After trying several other reagents, SubbaRow succeeded in finding 1,2,4-aminonaphthosulphonic acid, to be the right one for getting accurate results. It was somewhat serendipitous, as SubbaRow tried several chemicals available in the laboratory.

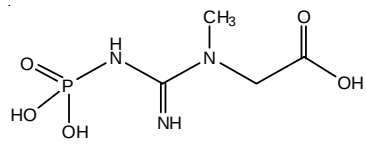
$$7\ H_3PO_4^{3-} + 12\ (NH_4)_6Mo^{VI}{}_7O_{24} + 51\ H^+ \longrightarrow$$

$$7\ (NH_4)_3PO_4.12Mo^{VI}O_3 + 51NH_4^+ + 36\ H_2O \qquad (1)$$

Along with the discovery of phosphocreatine, SubbaRow's method for analysis of phosphorus became the basis for isolation and identification of adenosine triphosphate (ATP, **2**).

$$[PMo^{VI}{}_{12}O_{40}]^{3-} + 4\,e^- \rightleftharpoons [PMo^{V}{}_{4}Mo^{VI}{}_{8}O_{40}]^{7-} \qquad (2)$$

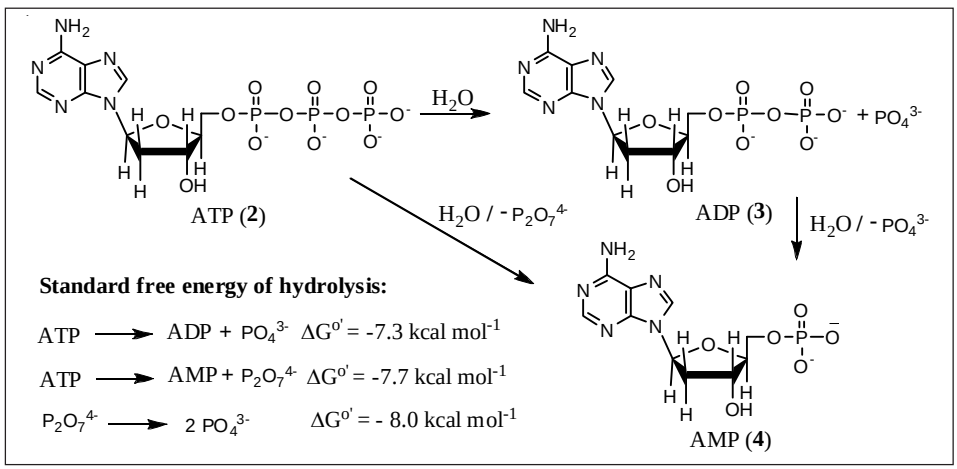
As the method was accurate and reliable, Fiske and SubbaRow used it to determine phosphorus content in various biological samples. They were able to show that muscle filtrate was phosphocreatine (**1**) having a phosphate group bound to a creatine molecule and not just inorganic phosphate as was believed earlier. They made a thorough investigation of its chemical and physical properties, and showed that it hydrolyzed when muscles contracted and reformed when they relaxed.



Phosphocreatine (**1**)

Along with the discovery of phosphocreatine, SubbaRow's method for analysis of phosphorus became the basis for isolation and identification of adenosine triphosphate (ATP, **2**). He demonstrated, as in the case of phosphocreatine, that one or two phosphate groups break away from ATP by hydrolysis during muscle contraction, which releases energy and results in the formation of adenosine diphosphate (ADP, **3**), or adenosine monophosphate (AMP, **4**). This is the energy that is used for driving biochemical reactions in living organisms (*Scheme* 1).

Scheme 1. Hydrolysis of ATP to ADP and AMP and the energy produced thereby.



Ironically, Fiske and SubbaRow did not get full credit for the ATP discovery. Instead, Karl Lohmann, a scientist working in the laboratory of Otto Meyerhof (1922 Nobel Laureate) in Germany, announced his similar findings at the 13th International Physiological Congress held in Boston, where Fiske and SubbaRow were also present. Lohmann had used Fiske–SubbaRow method for phosphate analysis. It seems Lohmann had some discussions earlier with Fiske on this, from which Lohmann gained much. In the light of this background, Lohmann's announcement of his characterisation of 'Adenylphosphate', as he had called ATP, upset Fiske. But it was a bit too late and the damage had been done. However, in order to retrieve the situation, Fiske saw to it that SubbaRow presented their work also in the same conference. Presently the credit is given jointly to Lohmann as well as Fiske and SubbaRow for independently discovering ATP, though most books mention only Lohmann's name. Both published their work at about the same time in 1929 [5].

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With so much of path-breaking novel work in phosphorus biochemistry published in six historic papers, SubbaRow obtained a PhD degree in biochemistry from Harvard Medical School in 1930.

Faculty Position Eludes at HMS

Soon after, he was appointed as Teaching Fellow, a junior teaching position at HMS which enabled SubbaRow to do independent research. However, he was denied any PhD students, separate laboratory and analytical facilities, possibility of research collaboration with other scientists, etc., usually enjoyed by higher faculty members. This severely restricted his creative genius. He had to be satisfied with working alone and hard at a time when it was common for a scientist of his calibre to have research projects, research assistants and collaborators.

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During the early nineteen thirties, SubbaRow continued to work on phosphorus compounds extracted from spleen, liver, pancreas and kidney and isolated 7–8 compounds. As he was still formally under the supervision of Fiske, the latter had taken the responsibility to write up the work for publication. But Fiske,

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inexplicably, never did it. In this context, George Hitchings, (1988 Nobel Prize in Medicine), who was SubbaRow's colleague in Fiske's laboratory, is believed to have remarked in 1965, that "SubbaRow had isolated several phosphorus compounds which were most likely nucleotides associated with RNA synthesis, and they had to be rediscovered by other scientists, because he was not allowed to publish that work". This shows how SubbaRow's efforts were in vain, and this perhaps delayed considerably the understanding of RNA.

In 1935, SubbaRow had to face a very unusual situation. Fiske was being considered for promotion from Associate Professor to full Professor. For this, the work on phosphorus, phosphocreatine and ATP was being taken as the basis, but the entire planning of the research and the imaginative ideas leading to it had to be Fiske's. It was known then that SubbaRow's share of credit for these discoveries was probably more or at least equal to Fiske's. But to save the day for Fiske, SubbaRow declared that he did not make any contribution to the ideas, but just provided a pair of hands to realise Fiske's ideas in the laboratory. It was a terrible sacrifice, because of which SubbaRow could never fulfil his dream of becoming a full faculty member, and have his own research team at HMS. He was appointed as an Instructor in 1936 and an Associate in 1938, which were still not completely independent teaching positions.

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Moves to Lederle Laboratories

The liver extract had caught the attention of the medical world then, as it was known to be curative for several deficiency diseases, particularly some forms of anaemia. Extraction, concentration and purification of organ constituents were jobs that required great skill and a lot of experience. SubbaRow had both in abundance. William Bell, the President of American Cyanamid, the parent company of Lederle Laboratories at Pearl River, New York State, had noticed the unequalled expertise of SubbaRow and had enlisted his services to guide them in making liver and other organ preparations. For this SubbaRow used to spend his

weekends at Lederle Laboratories (Pearl River is about 340 km from Boston). Lederle had a rabbit farm to make sera and vaccines, and to use the animals for testing new medicinal preparations.

The Lederle people recognised SubbaRow's intellectual capabilities, commitment, hard work and honesty, which were not adequately rewarded in the HMS. William Bell used the opportunity and offered him a job in Lederle Laboratories. This eventually culminated in SubbaRow moving there in May 1940, as the Associate Director of Research. A new chapter in SubbaRow's life had begun, which was highly fruitful in terms of his contributions to discovery of vitamins, antibiotics and other drugs which have helped millions of people to live longer and healthier. He was soon elevated to the position of Director of Research.

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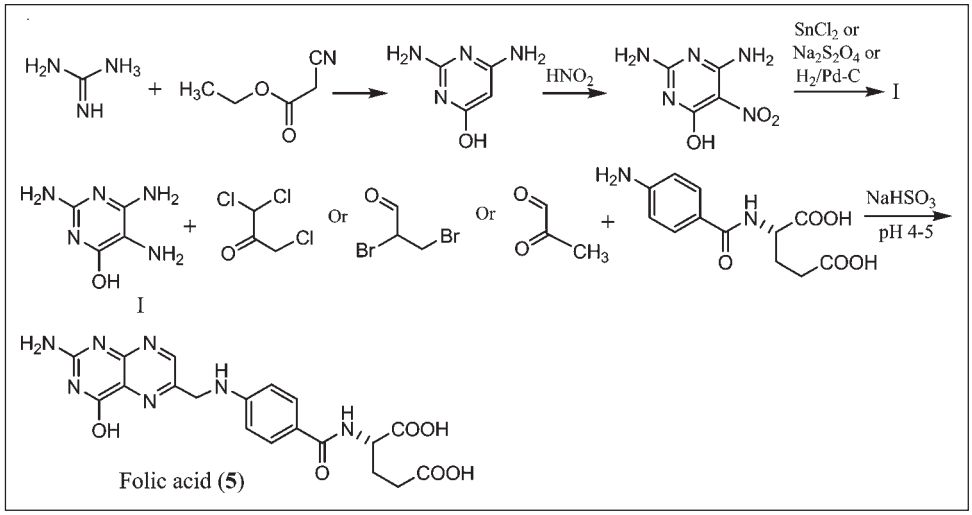
The Department of Biological Chemistry at HMS gave SubbaRow a grand farewell party with speeches of plaudits and dinner befitting a well-established faculty member.

Anaemia and Folic Acid

Many diseases result from dietary dificiencies, and some of them used to be treated by feeding the patients with specific organs. Pernicious anaemia used to be cured in this manner, particularly by including 400 g of liver in a patient's daily diet. Since this was not practical, scientists had focused on using organ extracts instead. Lederle Laboratories was playing a leading role in this research, and collaborated with doctors and scientists working in hospitals and research institutions.

It was supplying spleen extract to Richard Lewisohn, a surgeon at the Mount Sinai Hospital, New York, who was investigating the extract's effectiveness in the treatment of cancer. He also used yeast and barley extracts in treating breast cancer patients. The results were promising, but not satisfactory. While SubbaRow was still at HMS, Lederle used to supply livers or liver extract to him in return for his technical help in their organ extraction work. In 1943, SubbaRow and his group isolated folic acid from liver



Scheme 2. Synthesis of folic acid (pteroylglutamic acid).

extract as a part of the investigation to find the active principle in it, which was found to be effective in curing pernicious anaemia. The work on pernicious anaemia was conducted in the 1930s by Lucy Wills in Haffkine Institute, Bombay (Mumbai). Wills had noticed dietary deficiency that caused this disease among wheat-eating pregnant women of poorer sections in India. She also found that liver extract was a cure for this. The chemical that might be responsible for the cure was called Wills' factor.

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Because of its importance in curing certain dietary deficiencies in humans, chicken, and other animals, there was considerable competition in unravelling its chemistry. Eventually, vitamin B_9, or folic acid, was isolated, its chemical identity was established by chemical degradation methods and then it was synthesized by SubbaRow and his colleagues (*Scheme* 2) [6]. It was named folic acid because it was first isolated by Mitchell *et al*. in 1941 from spinach leaves, (Latin, *folium* = leaf) [6]. Some of the names among many for folic acid are folicin, vitamin Bc, *Lactobacillus casei* factor, pteroylglutamic acid, etc. SubbaRow continued to work on other constituents of the liver extract and came very close to isolating vitamin B_{12}.

Folic Acid Antagonists and Cancer Chemotherapy

Folic acid cures pernicious anaemia by promoting the growth of

white blood cells in bone marrow. From this observation, the idea to treat leukaemia emerged. If folic acid helps blood cell growth, then a compound that hinders its function must be able to reduce cell proliferation. Based on this line of thought, SubbaRow and colleagues started work on synthesizing folic acid conjugates and antagonists to test as antileukaemia drugs by changing 2-amino and 4-hydroxy groups on triaminopyrimidine (I) and substituents on amino and benzoyl amide groups in *Scheme* 2. For clinical tests, SubbaRow collaborated with Sidney Farber, his former colleague at Harvard, working at the Children's Medical Centre in Boston [7]. Farber's initial treatment of cancer patients with folates[3], diopterin, and teropterin showed acceleration of the leukaemia process, suggesting the use of a drug that should function as antifolate.

Though the yields of folic acid were moderate, the success of its synthesis paved the way for the synthesis of its analogues and derivatives needed to carry out clinical tests, by modifying pterin, *p*-aminobenzoic acid or glutamic acid parts. Lewisohn's work had shown that pteroyltriglutamic acid (three glutamic acid moieties, instead of one in folic acid), called teropterin, inhibited cancer in mice, but not folic acid. Therefore, when Farber started chemical trials, he started with diopterin (two glutamic acid groups) and teropterin. Both were found to accelerate cell multiplication instead of inhibiting it. Therefore, pteroylaspartic acid (**6**) was made and supplied to Farber in the spring of 1947. It gave promising results and this turned out to be the first folic acid antagonist. Then, aminopterin (**7**) was used which produced remissions in acute leukaemia and the treatment has been hailed as the "landmark in cancer therapy as it showed that an antimetabolite could be an antineoplastic agent..."

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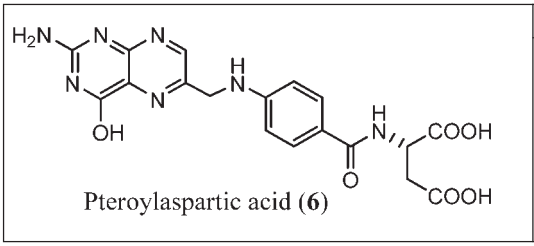
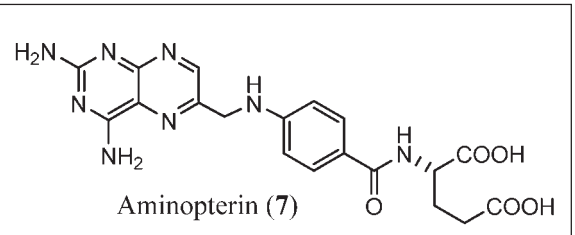
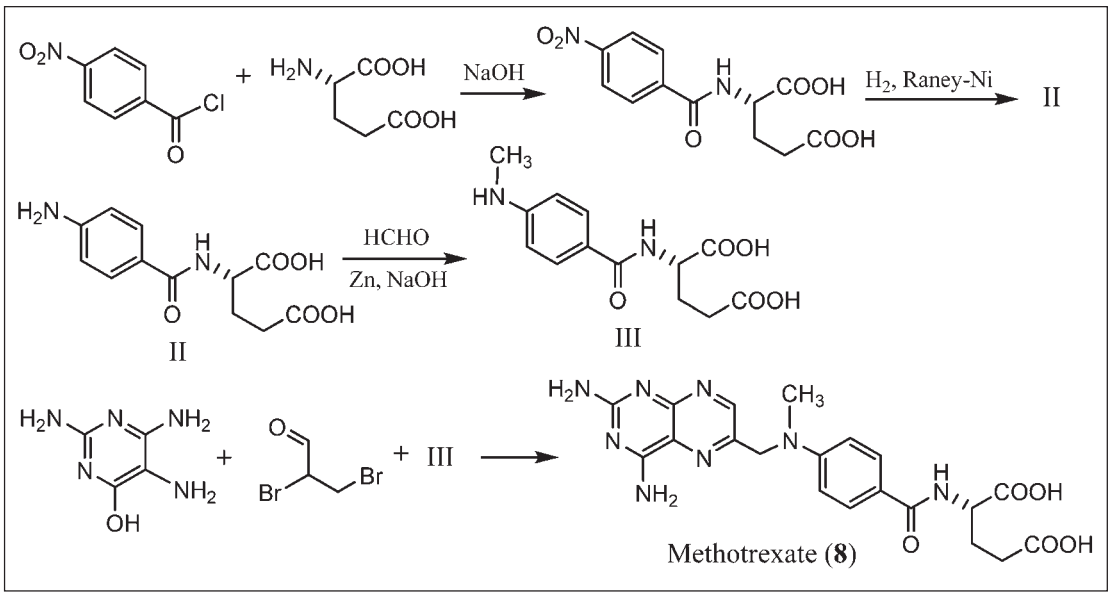
[3] Folates are B group vitamins including folic acid and related naturally occurring compounds. Synthetic compounds with similar medicinal properties are also called folates.



Pteroylaspartic acid (**6**)



Aminopterin (**7**)



Scheme 3. Synthesis of methotrexate or A-methopterin.

Farber gave full credit to SubbaRow, "The present plan of study concerning the action of folic acid antagonists was along the lines decided with Dr. SubbaRow in the spring of 1947..." He further says, "Through him and through his able colleagues who synthesized and made available a large series of folic acid antagonists, we were able to apply... to the treatment of children who were dying of acute leukaemia."

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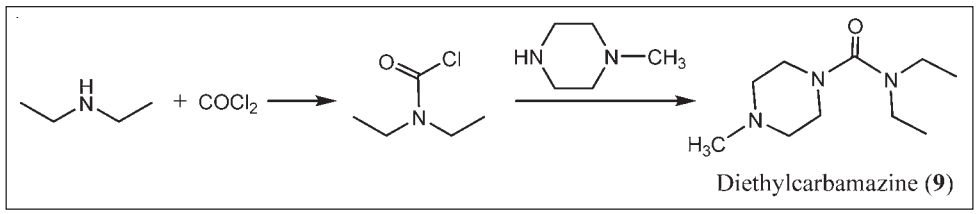
– Farber

The search for other antifolate drugs continued, which were more potent and at the same time less toxic, or in other words, the compounds that showed better therapeutic index. The quest yielded methotrexate (**8**), which is called the 'miracle drug' in the treatment of a variety of cancers and several other diseases (*Scheme* 3). In recent years methotrexate is being used to treat several other diseases like immune deficiency, psoriasis, rheumatoid arthritis, to name a few.

Diethylcarbamazine (Hetrazan)

During the same period when SubbaRow was supervising the historical discoveries in the treatment of anaemia, tropical sprue and cancers with folic acid and its modified versions, he was also



Diethylcarbamazine (**9**)

Scheme 4. Synthesis of diethylcarbamazine (DEC).

guiding research in tackling filariasis[4] which afflicts a large population of many countries in Asia (including India), Africa and South America. It is endemic to even some of the advanced countries. They synthesized and screened a large number of organic compounds. The search had a happy ending when they discovered diethylcarbamazine (DEC) (**9**) in 1946 [8], which was found to be effective against filariasis. It was synthesized as shown in *Scheme* 4. It is still the most widely used drug in the treatment of filariasis and is recommended by the WHO. It is effective against both macrofilarial and microfilarial parasites and is the drug of choice prescribed for most cases of filariasis. DEC came from Lederle Laboratories as a result of hard work of the researchers guided by SubbaRow.

[4] We are familiar with an extreme form of this disease, namely, elephantiasis or elephant leg disease. It is caused by nematodes (round worms) of filariodea family and spread by mosquitoes and black flies. If not treated, filariasis can be fatal.

Tetracycline

The discovery of penicillin in the late 1930s and streptomycin in the early 1940s ushered in the era of antibiotics. It was clear that two antibiotic drugs were just a small fraction of a large number of such drugs waiting to be discovered. This made several pharmaceutical industries to rush into this area. Lederle could not be left behind. In the mid 1940s, SubbaRow engaged Benjamin Duggar, who was Professor of Botany at University of Missouri and Chair of Plant Physiology at Cornell, Acting Professor of Biological Chemistry, Washington University Medical School, and Professor of Plant Physiology at the University of Wisconsin, and had just retired from university service at 70. As a plant pathologist he had over fifty years of experience in the physiology of fungi. He was appointed by Lederle as a consultant in mycological research and production. As the Second World War was coming to a close, the American soldiers from all over the world were requisitioned by Lederle to bring, while returning

The search for an effective drug against filariasis had a happy ending when they discovered diethyl carbamazine (DEC) (**9**) in 1946.

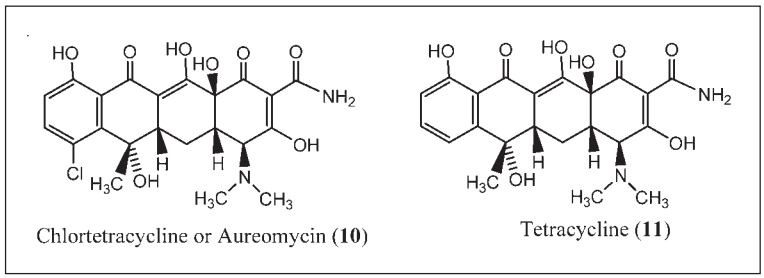
At the end of a three-year collaborative effort involving scores of researchers and excellent Lederle facilities, a golden yellow compound was extracted from *Streptomyces aureofaciens*. It was named as aureomycin (**10**).

home, soil samples from places where they served. Thousands of samples were collected and from many of them antibiotic producing organisms were isolated. Further work on isolation of the antibiotic molecules, their physiological tests, etc., for human disease control was carried out under the supervision of SubbaRow. At the end of a three-year collaborative effort involving scores of researchers and excellent Lederle facilities, a golden yellow compound was extracted from *Streptomyces aureofaciens* [9]. It was named as aureomycin (**10**) because of its golden colour (Latin, *aurum* = gold). It is the first broad-spectrum antibiotic effective against both Gram positive and Gram negative bacteria. In contrast, the wonder drug penicillin, introduced a few years earlier (1943), was useful in treating diseases caused by only Gram negative organisms, and streptomycin, which had come into use just then (1947), was effective only against Gram positive organisms. Soon after, tetracycline (**11**) was identified.

There are several other antibiotics, both natural and synthetic, closely related in structure to tetracycline and they are all collectively called tetracycline antibiotics. When pneumonic plague epidemic broke out in 1994 in Surat city of Gujarat and a few other cities elsewhere in India, tetracycline/tetracycline analogue was used to treat the affected patients. This brought the epidemic quickly under control, and the devastating impact that was expected was prevented. This reminded India of the services of SubbaRow and the next year (1995) his birth centenary was celebrated in medical institutions in many parts of the country. The Government of India released a commemorative stamp.

When pneumonic plague epidemic broke out in 1994 in Surat city of Gujarat and a few other cities elsewhere in India, tetracycline was used to treat the affected patients. This brought the epidemic quickly under control.

Besides his immensely important part in the discovery of



Chlortetracycline or Aureomycin (**10**)

Tetracycline (**11**)

phosphorus assay, phosphocreatine, ATP, folic acid, cancer drugs, antifilarial drug and antibiotics, SubbaRow significantly contributed to research on pantothenic acid (vitamin B_5), vitaminB_{12}, steroids, tuberculosis and blood pressure drugs and several others. Most of this work has been published in more than one hundred papers, but some of it has remained unpublished. He has a few papers on his Ayurveda research which he did while he was at Ayurveda College.

SubbaRow significantly contributed to research on pantothenic acid (vitamin B_5), vitamin B_{12}, steroids, tuberculosis and blood pressure drugs and several others.

Personality

Right from a young age SubbaRow was driven by an urge to become an important and famous person. In order to achieve this he did not mind to get away from the poverty ridden family to the distant unfamiliar Varanasi city or to become an ascetic in Ramakrishna Mission. When he failed in both, he took the education route to fulfil his ambition. In this pursuit, he had to face hurdles at every step, but he overcame each one of them with grit and determination. He borrowed money from friends, charities and well-wishers to finance his medical education. He married while still in the middle of his medical course in order to gain financial support from his well-off father-in-law. When he was given a choice to marry one of the two girls of the same family (not sisters), he chose the girl who was 13 years younger than him, but not the other who was just about a year younger. His calculation was that he need not start a family immediately, which would be an obstacle in his studies. After taking the job in Ayurveda College, which fetched him a very modest salary, he rented a small house where he led a frugal life with his wife and mother.

Once he decided that he would go to Harvard Medical School for higher studies, nothing could stop him. The first time he applied to HMS, he got admitted, but the family tragedy due to his brothers' death robbed him of the wonderful opportunity. When he tried the second time, the admission was denied. But he would not take 'No' for an answer; his persistent request made HMS to relent. He had no money to pay for his passage to Harvard and to bear at least the initial costs there. He requested his father-in-law

SubbaRow did not mind to get away from the poverty-ridden family to the distant unfamiliar Varanasi city or to become an ascetic in Ramakrishna Mission.

and a landlord, whom he had treated, to support him. Seshagiri, her father and others in the family were not willing to allow him to go to an alien land, despite his promise of returning in 2–3 years, with glory. So Murthy was initially reluctant to oblige his son-in-law by giving money, but yielded when SubbaRow made it clear that he would undertake his mission even without his help. These instances point to SubbaRow's unshakable resoluteness.

At the same time he was kind hearted. He would help his friends and colleagues in any possible manner. He gave up the intellectual right of his contribution to the work on phosphorus when Fiske's promotion was hanging in the air. His publications, even from Lederle Laboratories, have his name in the middle or the end of the authors' list, but few in the beginning; he gave more credit to the efforts of his co-workers. He would give away biological or chemical samples to any friend who needed them. He was so well known for his biochemical analytical skills that hospitals and individual researchers referred analytical procedures to him for his opinion and approval with regard to their accuracy and desirability to use them in clinical analysis. He made donations to a church and charities.

He was considerate to his family, relatives and friends. He used to write to Seshagiri and his father-in-law very regularly and frequently, and often to others right from the time he went to Harvard, despite great constraints of time and cost. In the initial years he wrote in some detail giving a lot of information about his life in America and enquiring about his people in Anaparthi and Bheemavaram. Very often he used to express his interest to return to India, which slowly changed. He knew quite well that in India he could not get the kind of research facilities which were available to him in the US. When he realised that his return was being postponed, he made some attempt to get his wife to the US sometime between 1928 and 1930, but for reasons not quite clear, backed out; the two never met again. The exchange of letters also stopped sometime in 1930s. Each passing day Seshagiri hoped and expected that her husband would join her one day, which never happened. In 1941, SubbaRow cabled to Anaparthi that he

SubbaRow was so well known for his biochemical analytical skills that hospitals and individual researchers referred analytical procedures to him for his opinion and approval.

had annulled his marriage to Seshagiri and had remarried in America. It is simply not true, as there is no record of either. He was totally devoted to his research in finding remedies to major diseases. He seems to have said sometime in his last days, "If God will spare me another couple of years, maybe we can cure another disease."

SubbaRow was totally devoted to his research. "If God will spare me another couple of years, maybe we can cure another disease."

SubbaRow paid back the debt with interest and more he owed to his father-in-law sometime in 1946–1947. It was a timely help, as Murthy's family was going through very difficult times then. After SubbaRow's death in 1948 his belongings, his cash savings, which was not much, and other useful things were given to his widow, his mother and father-in-law. In the background, there is the poignant story of Seshagiri and her sacrifice. She told in an interview to S P K Gupta, who wrote SubbaRow's biography, "It is my misfortune that he did not come back. But our marriage served him to get the mission of his life fulfilled because it gave him the opportunity to get his medical degree and to go to USA for research."

Despite making important contributions to American science, medicine and pharmaceutical industry, SubbaRow could not get a permanent resident visa, even after staying for more than twenty years, until the end of the war. His visa used to be renewed every two years and he was treated as an alien. Most part of the period during which SubbaRow lived in America, he had to face, like other non-white aliens, the consequences of the Great Depression and then the World War II. Racial prejudices also prevailed (Martin Luther King, Jr., had not yet come to prominence). Sometime after the end of the war, he submitted a declaration of his intention to change his residence status. He was granted permission to do so, but was hesitant to take it. Perhaps some lurking desire to come back to his motherland could have been a good reason for him to postpone changing his visa status and he breathed his last as an Indian.

"It is my misfortune that he did not come back. But our marriage served him to get the mission of his life fulfilled..."

– Seshagiri

SubbaRow seems to have had very little interest outside of his research. However, he liked discussing politics, "matter of heart",

SubbaRow seems to have had not much faith in God or religion, though he wanted to be a Hindu monk and had interest in religious scripts in his youth.

and football to the extent that he would bet on the game with friends. He learnt flying and used to fly now and then.

SubbaRow while in India believed in and practiced astrology. The belief remained for 2–3 years after he went to America. However, he seems to have had not much faith in God or religion, though he wanted to be a Hindu monk and had interest in religious scripts in his youth. It is unlikely that he remained a vegetarian in America. He was a heavy smoker, which perhaps was responsible for his early death on the night of August 8, 1948.

Awards and Recognition

It is very surprising that SubbaRow did not get any award or recognition during his lifetime. Whatever be the reason, the scientific world of his time failed to take note of his yeoman service. Was he too shy to project himself as an inventor or too generous not to claim any major credit for his contributions to important discoveries? We would perhaps not know the whole truth. He made hardly any lecture circuits and rarely presented his work in conferences; instead he allowed his co-workers to do that, while he would sit back and listen. We should not, however, forget that his co-workers in his major discoveries were well-known scientists like Fiske, Farber, Hewitt, Angier, Kushner, Duggar and several others, who were more visible.

SubbaRow's co-workers in his major discoveries were well-known scientists like Fiske, Farber, Hewitt, Angier, Kushner, Duggar and several others, who were more visible.

Even Lederle Laboratories or its parent company, American Cyanamid, which derived considerable benefit from SubbaRow as its Director of Research, did not seem to have given much in return while he was alive. However, after his death a library block in Lederle campus was named after him and a fungus was named in his honour as *Subbaromyces splendens*. A tetracycline antibiotic was called subamycin. Scientific journals and newspapers paid tributes. *The New York Herald–Tribune* considered him as one of the most eminent medical minds of the century. In 1995, his birth centenary was celebrated in India by arranging conferences and lectures; the Government of India issued a commemorative stamp. Several medical institutions in India now

conduct annual SubbaRow commemorative orations. There is a SubbaRow Club which organizes seminars, memorial meetings and photo exhibitions, and has created the website 'Yellapragada SubbaRow Archives Online'. Doron Antrim (a news reporter) wrote in 1950 in *Argosy* (a magazine which was published from New York till 1978 and carried stories by well-known authors), "You've probably never heard of Dr. Yellapragada SubbaRow. Yet, because he lived, you may be alive and are well today. Because he lived you may live longer".

"You've probably never heard of Dr. Yellapragada SubbaRow. Yet, because he lived, you may be alive and are well today. Because he lived you may live longer".

– Doron Antrim

Suggested Reading

[1] **C H Fiske and Y SubbaRow, *J. Biol. Chem*., Vol.66, pp.375–400, 1925.**

[2] **G D Christian, *Analytical Chemistry*, 6th Ed., John-Wiley, pp.769-770, 2004; R Sood, *Medical Laboratory Technology-Methods and Interpretations*, 5th Ed., Jaypee, New Delhi, pp.450–452, 2003**

[3] **R D Simoni, R L Hill and M Vaughn, *J. Biol. Chem*., Vol.277(32), e21, 2002.**

[4] **C H Fiske and Y SubbaRow, *J. Biol. Chem*., Vol.74, pp.22, 1927; C H Fiske and Y SubbaRow, *J. Biol. Chem*., Vol.81, pp.629–679, 1929.**

[5] **C H Fiske and Y SubbaRow, *Science*, Vol.70, pp.381–382, 1929; K Lohmann, *Naturwissenschaften*, Vol.17, pp.624–625, 1929.**

[6] **C W Waller, B L Hutchings, J H Mowat, E L R Stokstad, J H Boothe, R B Angier, J Semb and Y SubbaRow (Lederle Laboratories), D B Cosulich, M J Fahrenbach, M E Hultquist, E Kuh, E H Northey, D R Seeger, J P Sickels and J M Smith, Jr. (Calco Chemical Division of American Cyanamid Company), *Ann. New York Acad. Sc*., Vol.485(5), pp.283–287, 1946; H K Mitchell, E E Snell, and R J Williams, *J. Am. Chem. Soc*., Vol.63, pp.2284, 1941.**

[7] **S Farber, L K Diamond, R D Mercer, R F Sylvester, Jr., and J A Wolff, *New Engl. J. Med*., Vol.238, pp.787–793, 1948; S Jolivet, K H Cowan, G A Curt, N J Cleudenin and B A Chabner, *N. Engl. J. Med*., Vol.309, pp.1094–1104, 1983; T H Jukes, *Cancer Res*., Vol.47, pp.5528–5536, 1987.**

[8] **R I Hewitt, S Kushner, H W Stewart, E White, W S Wallace and Y SubbaRow, *J. Lab. Clin. Med*., Vol.32, pp.1314–1329, 1947; H W Hewitt, R J Turner, J J Denton, S Kushner, L M Brancone, W L McEven, R I Hewitt and Y SubbaRow, *J. Org. Chem*., Vol.13, pp.134–143, 1948; S Kushner, L M Brancone, R I Hewitt, W L McEwen, H W Stewart, R J Turner, J J Denton and Y SubbaRow, *J. Org. Chem*., Vol.13, pp.144–153, 1948.**

[9] **B K Harned, R W Cunningham, M C Clark, R Cosgrove, C H Hine, W J McCauley, E Stokey, R E Vessey, N N Yuda and Y SubbaRow, Presentation at New York Academy of Science, New York, July 21, 1948; BM Duggar, *Ann. N. Y. Acad. Sci*., Vol.51, pp.177–181, 1948.**

[10] **S P K Gupta and E L Milford, *In Quest of Panacea – Successes and Failures of Yallaprgada SubbaRow*, Evelyn Publishers, New Delhi, 1987 (Biography).**

[11] **Yellapragada SubbaRow Archives Online (website).**

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