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Dmitri Iosifovich Ivanovski (1864-1920)

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FOREWORD

Ivanovski is one of the discoverers of the filterable nature of viruses and thus one of the founders of virology (3), yet very little is known about his life by persons outside of the Soviet Union. An example of this lack of knowledge is the article published in Phytopathological Classics whose author was unable to find the dates of birth and death of this important Russian scientist (2). All he could tell us was that Ivanovski died during or before 1924. When I drew this matter to the attention of Dr. L. V. Kalakoutskii of the Institute of Microbiology of the Academy of Sciences of the U.S.S.R., he kindly furnished me with a few articles and a book on Ivanovski.

The following sketch is based mainly on Ovcharov's book (4), with a few comments taken from other articles or suggested by the examination of those papers of Ivanovski that we were able to secure. A list of his most important publications will be found at the end of this paper. One will note that Ivanovski's scientific interests centered around four main topics: diseases of tobacco plants, yeast fermentation, soil microbiology, and photosynthesis. In addition, he enriched the Russian scientific literature with numerous publications, both critical and encyclopedic. Ivanovski's name is spelled here in the simplest form that will permit the reader to approximate the Russian pronounciation of his name. Germans use "w" to render the "v" sound.

In looking at the list of his publications, it will be useful to remember that a Russian scholar has to present and defend a number of dissertations during the course of his career. After the "candidate thesis," which is about the equivalent of our Ph.D., comes the "habilitation thesis." At this level, the candidate is considered capable of college-level teaching and receives the title of Docent, which might be compared to that of Assistant Professor. Finally, after several years' experience in teaching and research, a rather mature work is produced, the doctoral thesis.

The reader might find useful to keep in mind that the ruble of 1890 to 1910 was worth $0.50 of the period. The author is guessing that the value of the dollar went down about fourfold between that period and 1972.

THE FORMATIVE YEARS

Dmitri Iosifovich Ivanovski (Plate I) was born in Russia, in 1864, as his country was undergoing many changes under the leadership of Alexander the Second, who was attempting a "revolution from above" that had resulted in the abolition of serfdom in 1861 and was changing the whole social structure of the country.

The exact date and location of the birth of Dmitri Iosifovich Ivanovski seem to be in doubt. Ovcharov (4) places the happy event on the 28th of October 1864, in the village of Niz, district of Gdov, in the region of St. Petersburg; according to Gutina (1), Dmitri was born on the 9th of November 1864, in Gdov itself. The difference in dates may be no more than the usual confusion caused by the Russians' changing from the Julian to the Gregorian calendar in 1918.

In any event, Dmitri's father was Iosip (Jo-

PLATE I. *Dmitri Ivanovski as a young man. From Izvestiia Akademii Nauk SSSR, 1950 (no. 6).*

seph) Antonovich Ivanovski, descendant of an impoverished noble family from the district of Kherson.

Dmitri had two brothers, Nikolai and Aleksei, the latter to become a famous sinologist, and two sisters, Lydia and Olga. After the death of the father, the family moved to St. Petersburg, where they lived modestly on a small pension given to the widow.

Dmitri attended the Larinski secondary school and graduated with high honors in spite of much time and energy spent tutoring other students in order to enable his mother to make ends meet.

Dmitri's school years witnessed many important events. In 1881, Alexander II was murdered to be succeeded by Alexander III who, having seen his father's violent end, took a reactionary course, and Russia again became the most despotic country in Europe to be slowly undermined by revolutionaries.

In the field of biology, Pasteur and his assistants were laying the foundation of a new science, the study of the infinitely small beings, and were showing the infinitely large role that they were playing in men's lives. Not only were microbes responsible for fermentations and the cause of some diseases, but it was also demonstrated that, through the use of attenuated strains, immunity could be conferred. The doctrine of spontaneous generation was abandoned and aseptic techniques were developed.

Shortly after Ivanovski entered the Department of Natural History of the Faculty of Physics and Mathematics of the University of St. Petersburg, in 1883, Robert Koch announced the discovery of tubercle bacilli, and Friedrich Löffler isolated the causative agent of diphtheria. Elie Metchnikoff, an embryologist attracted by the glitter of the new science of microbiology, proposed his famous theory of cellular immunity. The year was 1884, and an ingenious disciple of Pasteur, Charles Chamberland, discovered the bacteriological filter, the indispensable tool for Ivanovski's work to come.

Following the potato blight of the 1840's in Ireland and the brilliant mycological investigations of Anton de Bary, the concept that fungi might cause plant diseases had been largely accepted and, in 1885, Pierre Millardet introduced the Bordeaux mixture which was to save so many crops. The concept that bacteria could also cause plant diseases was not accepted, and the studies of the Thomas J. Burrill in the U.S.A. and of J. H. Wakker in Holland were essentially ignored (3).

Ivanovski was a good student of meager resources. When he entered the University, he asked to be exempted from paying tuition, and he applied for a scholarship since he had no property other than belongings which were absolutely necessary (4).

At that time, the Department of Natural History of the University of St. Petersburg was staffed with a number of famous scholars representing the best that Russian science could offer at the time, including D. I. Mendeleev. The professor of Botany, A. N. Beketov (1825–1902), was a dedicated teacher who wanted to give "light, and more light" to the whole Russian nation.

Beketov was both a phytogeographer and a plant morphologist. He claimed that the study of morphology should enable one to investigate the factors controlling plant-form development and come to an understanding of the laws governing evolution. The University of St. Petersburg was a leader in the teaching of Plant Physiology. This was due not only to the efforts of Beketov but also to those of another teacher of Ivanovski, A. S. Famintsin (1835–1918), well known to general bacteriologists for his discovery of *Nevskia ramosa*.

The influence of these outstanding scholars had a considerable impact on young Ivanovski, who had already spent much time studying the great classics of natural sciences and philosophy. An entry in his student-days diary, related by Ovcharov (4), revealed his maturity: "I cannot understand how one can sit with a friend for the whole evening and do nothing but talk nonsense and enjoy it. I am bored if conversation does not—at least to a modest degree—nourish the mind. An evening spent in useless talk tires me."

At the University of St. Petersburg, Ivanovski served as an instructor in the laboratory of plant physiology and anatomy while he carried out some undergraduate research projects. He attracted the attention of both Beketov, then the dean of the natural scientists of St. Petersburg, and Famintsin, who decided to send him to study a tobacco disease which was then causing great damage.

Ivanovski was very happy to be given such an assignment and, in 1887, he departed in the company of another student, V. V. Polovtsev, for Ukraine and Bessarabia. The results of this expedition were published in a series of papers giving a detailed description of the pox disease (Russian = *Riabukha*) of tobacco as well as suggestions on how to curb it. Based on their own observations and on interviews with farmers, the two students concluded that the disease was most damaging in fields where tobacco had been grown for long periods of time, frequently for more than forty years. In addition, the fields planted with tobacco were often very small, nothing more than a few square yards where the peasants were growing the precious plant "for their own pipes." Thus, the most dramatic but realistic recommendation of the two students was that the farmers introduce the practice of crop rotation.

Returning to St. Petersburg, Ivanovski successfully passed his preliminary examination towards the end of 1887 and, on the first of March of the next year, he presented his Ph.D. thesis "*On Two Diseases of Tobacco.*" The Faculty felt that he was an outstanding candidate and encouraged him to follow an academic career. After receiving his Ph.D. degree, Ivanovski was offered a post-doctoral fellowship at the University that he accepted reluctantly, being afraid of not fulfilling the high hopes of his teachers. In 1891, he resigned to accept a position as technician in the laboratory of botany of the Academy of Sciences. He then married the daughter of a political exile who, after having spent two years in prison, had been banished from St. Petersburg.

Those were difficult times, and the leading Russian scientists often had to resort to heroic measures in order to carry out their investigations. Mendeleev had a yearly laboratory budget of 300 rubles, which put him in a favored position, since another chemist of the University of St. Petersburg, N. N. Sokolov, had no support at all and was running his laboratory out of his own pocket, with the assistance of A. N. Engelgart, the agricultural chemist and publicist. Famintsin, tired of asking in vain for the creation of a laboratory of plant physiology at the Academy of Sciences, finally set one up in his own apartment.

The lack of space, greenhouses, experimental fields, and other facilities drove Russian botanists to turn to microorganisms for their physiological investigations. Such, indeed, had been the case of M. S. Voronin who brilliantly investigated cabbage hernia and discovered the myxomycete-like pathogen, *Plasmodiophora brassicae*, in 1878.

STUDIES ON ALCOHOLIC FERMENTATION

Towards the end of the nineteenth century, few people worked on alcoholic fermentations since all the questions one might ask seemed to have been answered by the studies of Pasteur. However, Ivanovski felt that the exact role of oxygen and of nitrogen-containing substances still had to be elucidated. His investigations proceeded at a rather slow pace, because he built most of his instruments himself and purified or checked by analysis all his media and reagent constituents. In addition, he was satisfied by the validity of his findings only after having obtained the same results again and again. Among the pieces of equipment built by Ivanovski was one which permitted him to carry out the continuous culture of yeasts.

Soon Ivanovski found that his results contradicted some of those published by others. Duclaux was of the opinion that fermentation by yeasts starts only after they have exhausted the oxygen present in the medium. This point of view was not confirmed by Ivanovski. Nägeli had claimed that an increase in the growth rate of yeasts resulted in an increase in fermentation capability. This was disputed by Ivanovski who found that, at equal sugar concentrations, increases in the quantity of the nitrogen-containing substances in the medium (peptone) resulted in an increase in growth and a decrease in fermentation. He reasoned that, for the formation of yeast protoplasm, nitrogen-containing substances were essential. The more you give the yeast, the more it forms protoplasm and the less it forms secondary metabolites such as alcohol.

In general, Ivanovski felt that if the yeasts are given ideal conditions for growth they will produce many cells and few by-products, such as alcohol. In addition, alcohol, if produced in small quantities, was not toxic to the yeast and could, under some conditions, even be metabolized by it.

Ivanovski refused to equate the anaerobic respiration of higher forms of life with the alcoholic fermentation of yeasts because he felt that all presumptions of this kind are more harmful than useful, for imaginary explanations tend to conceal existing gaps in knowledge and are thereby detrimental to science. However, Ivanovski had appreciated the value of comparative biochemistry since he stated that obviously the same physicochemical processes take place in both the cells of highly organized plants and in unicellular organisms. He also noted that, because of their ease of handling, microorganisms were ideal research tools (4).

Ivanovski had selected alcoholic fermentation as the subject of his habilitation dissertation. As he was completing his work in 1894, Nicolas the Second was crowned the Tsar of all Russians. He continued the despotic course traced by his father; he was to be the last Tsar. Since 1891, in St. Petersburg, Ivanovski had a most distinguished colleague, Sergei Vinogradski (1856–1953), who, having isolated autotrophic nitrifying bacteria, had been appointed head of the laboratories of general microbiology of the Imperial Institute of Experimental Medicine. In 1894, Vinogradski distinguished himself again by discovering that certain anaerobic bacteria were able to fix nitrogen.

In January 1895, Ivanovski defended his habilitation dissertation entitled "*Studies on Alcoholic Fermentation.*" The appointed reviewers were Fanintsin, who was by then Academician, and D. P. Konovalov. Their report was very favorable, and Ivanovski was appointed Privatdocent. His first task was to teach an elective course on the physiology of the lower plants. Within a year, due to the resignation of Famintsin, he was entrusted with the compulsory course on plant physiology and anatomy.

The laboratories of the University of St. Petersburg were too poorly equipped to permit the continuation of Ivanovski's studies on alcoholic fermentation. He solved the problem by starting a cooperative program with the Institute of Technology where, in 1896, he initiated a course on fermentation designed to train experts for the industry. This venture was quite successful, even attracting students from abroad.

Ivanovski's ideas on yeast fermentation can be summed up as follows: (i) Fermentation is not solely controlled by the lack of oxygen, the nutrients in the medium are of considerable importance; (ii) the fermentation products and the intensity of the fermentation depend not only on the cultural conditions employed but also on the strain of yeast used.

SOIL MICROBIOLOGY

Ivanovski was not a soil microbiologist nor was he an agronomist; still, his teaching in these fields had a tremendous influence in Russia. His main contribution was in the form of lectures and discussions at the meetings of the Commission for Soil Sciences of the Free Economic Society. His expertise was such that he was asked to join the editorial board of Pochvovedenie (Soil Science) founded in 1899, seventeen years before its American counterpart.

On the occasion of a meeting of the Commission on Soil Sciences in 1891, Ivanovski stated that the study of low forms of life had changed physiology and medicine and that there was no doubt that the same was to happen in soil sciences and agriculture (4).

As a plant physiologist and a microbiologist, Ivanovski recognized that there was great promise in the investigations of P. A. Kostychev who was elucidating the role of higher and lower plant forms in the formation of the fertile black topsoil called chernozem. In his studies, Kostychev pointed out that soil microorganisms were not only degraders of organic matter but that they also synthesized new compounds. Ivanovski supported this point of view since he felt that, no matter how little was known about the role of microorganisms in soil. it was already enough to claim that microorganisms play an obvious role in the genesis and life of the soil (4).

"There is no such thing as a dead soil," claimed Ivanovski. "The decomposition of organic matter in soil is a physiological process associated with the life activities of innumerable microscopic soil inhabitants" (4). Thus, Ivanovski was strongly opposed to the then prevailing concept that the decomposition of organic matter in soil was a strictly chemical process dependent on oxygen and controlled by light, temperature, and the moisture content of the soil.

With his considerable flair for recognizing competence, Ivanovski gave his backing to all those who, like Sergei Vinogradski, developed the science of soil microbiology. He attacked all those who performed faulty experiments or who tried to peddle miraculous agronomic concoctions.

At a time when many scientists doubted that microorganisms could fix atmospheric nitrogen, Ivanovski joined his teacher Famintsin in expressing the conviction that, in the future, good crops and rich harvests could possibly be induced by cultivation methods assuring an abundant growth of soil microorganisms (4).

Ivanovski promoted the use of bacterial fertilizers and, in particular, he warmly recommended the application of a preparation called Nitragin which was rich in bacteria which form nodules on the roots of legume plants. He recommended the use of specific bacterial fertilizers only after thorough testing had convinced him of the value of the product. He stood firmly against the premature acceptance of any product and, after having reviewed the experimental data collected in many countries, he discouraged the use of a preparation of this type called Alnit.

Always one step ahead, Ivanovski reminded his contemporaries that it was easy to dip the legume seeds in a suspension of Nitragin, but he added that they should try to determine the conditions which would favor the development of the bacteria and to understand the nature of the processes taking place in the soil.

Towards the end of his career in Warsaw, Ivanovski returned to the study of the legume bacteria. Because he published nothing of this work, little is known about this part of his research career.

DISCOVERY OF A FILTERABLE VIRUS

Although deeply involved in the study of alcoholic fermentations, Ivanovski did not forget the diseases of tobacco. At first, he paid special attention to the powdery mildew of tobacco. The Russian name for this disease, which is caused by an *Erysiphaceae*, is *pepelitsa*, which means "ashening."

His observations led him to the conclusion that the pathogen can grow only if enough humidity is available. He also noted that the conidial stage of the fungus (*Oidium* stage) attacks plants of the thistle family where it can over-winter and serve as a source of infection the following season.

As a preventive measure, Ivanovski recommended that farmers grow tobacco plants well spaced, in properly drained fields. If infection had started, he showed that the removal of diseased leaves and the nicking of the top of the plants often permitted one to curb the disease through increased proliferation of healthy leaves. He advocated the destruction of all excised parts by burning. Having solved this problem to his satisfaction, he then turned his attention to tobacco mosaic.

In 1887, when Ivanovski was still a student, he started his work on diseases of tobacco. He called the disease of tobacco that he was investigating *riabukha*, in Russian, and *Pockenkrankheit* in German (literally, pox disease). Later, in 1892, Ivanovski distinguished between the pox disease, *stricto sensu*, characterized by the formation of brown spots on the leaves, and the mosaic disease, characteristically manifested by a light- and dark-green mottling of the leaves.

The name mosaic disease of tobacco had been introduced in 1886 by Adolf Mayer, who believed that the disease was almost exclusively limited to Holland (2). Mayer rightly felt that names previously used in Holland, such as "bunt," "rust," and "smut," were not very appropriate.

Referring to the mosaic disease *stricto sensu*, Ivanovski noted that the disease always started in very young leaves just emerging from the buds and was subsequently transmitted to the whole plant. The main symptoms of the disease were manifestly the changes in color which led Mayer to coin its name: a mosaic of dark- and light-green spots later turning yellow.

Ivanovski repeated what were essentially the experiments of Mayer. Diseased plants, crushed in a porcelain dish, were filtered through linen, and the expressed sap was inoculated by means of capillary tubes into the leaf veins of healthy plants. This resulted in the production of the disease within two weeks in 80% of the plants inoculated.

Having convinced himself that the mosaic disease could be passed from plant to plant, Ivanovski autopollinated diseased plants. Not one out of the one hundred planted, ripened seeds produced diseased progeny, thus proving that the mosaic disease is not hereditary but contracted during the vegetative period of plant growth.

Ivanovski was able to transmit the mosaic disease by crushing infected leaves around the roots of healthy plants, but he was unable to transmit the disease from plant to plant simply by growing an infected plant next to a healthy one.

Based on these observations, Ivanovski was in a position to stress practical control measures such as the removal and the burning of all parts of the diseased plants, and, if all else failed, to have recourse to crop rotation.

After having established that, in fields where the disease had been prevalent for some time, the infection is transmitted through both leaves and roots, whereas in new fields the disease was transmitted only through the leaves, Ivanovski investigated environmental conditions that favored the contagion. In general, he found that the more favorable the conditions for the speedy growth of the vegetative part of the plants, the more susceptible they were to the disease. Thus, moist soil, humid air, and balmy temperatures were found to favor the spread of the mosaic disease.

Ivanovski had no confidence in some of the experiments of Mayer. The latter had reported a loss of infectivity when the sap was passed through a double layer of filter paper. Ivanovski went one step further and found, in 1892, that, even after passage through the filtering candle of Chamberland, infectivity was not lost.

Ivanovski concluded his communication of 1892 (2) with the hypothesis that the mosaic disease was of bacterial origin and that a filtrate passed through a Chamberland candle contained either bacteria or a soluble toxin which might be capable of provoking the appearance of the external manifestation of the disease. The latter hypothesis was particularly appealing at the time, since Roux and Yersin had demonstrated in 1888 that diphtheria was a disease caused by a bacterial toxin. In addition, Ivanovski was unable to cultivate bacteria from his candle filtrates.

Unaware of Ivanovski's work, one of the greatest figures of general microbiology, Martinus Beijerinck working at the Polytechnical School of Delft took advantage of the heated greenhouse of his school to continue, in 1897, some studies on tobacco mosaic disease that he had started in 1885, in order to help Adolf Mayer, when they were both at the School of Agriculture of Wageningen.

After one year of study, in 1898, Beijerinck published the following general conclusions: (i) The tobacco mosaic infection is not caused by microbes, but by a living liquid virus. (ii) The virus multiplies only in growing plant organs where cellular division takes place. (iii) The virus is inactivated by boiling but can be dried without losing its infectious properties.

In his doctoral dissertation, published in 1902 in Warsaw (*see* List of Selected Publications), Ivanovski was somewhat chagrined to remark that Beijerinck "filtered the sap of diseased plants through a porcelain filter and stated that the sap, sterilized in this fashion, retained its infectivity. The author does not know that I had already established this fact a long time ago."

Ivanovski repeated the diffusion-in-agar experiments of Beijerinck and concluded that the agar is indeed capable of transferring the infection. However, he was not ready to accept the idea that this experiment proved that the causative agent of the disease was a fluid. He ran some experiments of diffusion in agar containing India ink and concluded that small particles are capable of motion through a gel. However, he noted that such motion was possible only in old agar. He tried to use this last observation to determine whether the causative agent of tobacco mosaic disease was particulate or not.

The sap of diseased plants was simultaneously applied to the surface of old and fresh agar plates. After ten days, the upper layer of both agars was removed, and the lower layer was used for infecting healthy plants. Ten plants were inoculated with fresh agar and five with old agar. After 11 days, all the plants treated with fresh agar were still healthy, but two of the five plants inoculated with old agar were already sick. The fresh agar-treated plants remained healthy even after one month, and Ivanovski concluded that the cause of infection was particulate, since soluble substances could easily penetrate through fresh agar.

In another experiment, Ivanovski observed that the sap of diseased plants, dialyzed through a bovine diaphragm, was still infectious. India ink particles could also pass through the same membrane. It could, however, be made impervious to the passage of both types of substances with a coating of agar. In addition, neither substance could pass through a layer of parchment.

Using a Chamberland candle, Ivanovski filtered infected sap for a period of 36 hours. He collected the first few drops of filtrate and then further fractions at 12 and 36 hours. Healthy plants were inoculated with each fraction, but only the first cut was able to transmit the disease. This, Ivanovski felt, supported the particulate theory of the causative agent. Particles first passed through the pores of the filter; then, after a certain time, the pores became smaller and smaller as they became clogged, and the particles could no longer pass.

Summarizing the results of these experiments, Ivanovski stated in the Zeitschrift paper of 1903 (see List of Selected Publications): "We see that there is not a single fact which supports the hypothesis on the soluble character of the infectious agent of mosaic disease. On the contrary, the experiment with the diffusion into agar and especially the fractionated filtration clearly indicates that we are dealing with a *contagium fixum*."

Certainly, the infectious agent was particulate, but what was its nature? At first Ivanovski was inclined to believe that it was an elusive bacterium.

In 1901 Ivanovski concluded a short note in the Zentralblatt (see List of Selected Publications) by stating: "Thus there is a specific bacterium which causes Tobacco Mosaic disease and we have found that it is unnecessary to seek refuge in the completely untenable hypothesis of *contagium vivum fluidum*. A more complete paper will appear in 2 to 3 months."

Further experimentation was not to bring confirmation of the discovery of the tobacco mosaic disease bacterium, and the 1903 memoir in Zeitschrift (see List of Selected Publications) ended on a more cautious note: "On the whole, the question of the artificial cultivation of the mosaic disease microbe remains to be solved by future investigations."

Ivanovski's microscopic observations of diseased cells were illustrated in Plate III of his doctoral thesis which was Plate II of the 1903 Zeitschrift memoir (see List of Selected Publications). It is reproduced here as Plate II. Most interesting is the illustration of viral crystals.

Some of the diseased cells were fixed with alcohol, stained with methylene blue, washed with alcohol and aniline, and counterstained with eosin. The nuclei were stained blue on a pinkish background. On this rosy background, Ivanovski throught that he saw the rod-shaped bacteria of the mosaic disease stained blue (Plate II, Fig. 9 to 16). Attempts to stain these bacteria in living cells were unsuccessful.

In 1896, Ivanovski, then only a Privatdocent, had been appointed head of a department at the University of St. Petersburg with the understanding that, within a five-year period, he was to successfully defend a doctoral thesis. Research on tobacco mosaic was difficult and long, and, as we have noted, Ivanovski was not a man to rush into bold conclusions. In 1901, the five years had passed and the thesis had not been completed. He was relieved of his duties and replaced by V. I. Palladin. Ivanovski was a popular teacher and, upon hearing of his discharge, the students boycotted Palladin's lectures until they realized that he had nothing to do with Ivanovski's demotion.

Ivanovski presented his doctoral dissertation at the University of Kiev. The two official reviewers, S. G. Navashin and K. A. Purievich, were favorably impressed and rightly so. However, Purievich went far when he wrote in his report that he had noted with considerable satisfaction that Ivanovski had refuted the hypothesis of Beijerinck on the existence of an infectious agent having character of unorganized matter. The hypothesis of *contagium vivum fluidum*, he said, was a sad chapter in the annals of contemporary science (4).

THE YEARS AT THE UNIVERSITY OF WARSAW

In 1901, Ivanovski was appointed Associate Professor at the University of Warsaw. After having successfully defended his doctoral thesis in Kiev, in 1903, he concentrated most of his research time to the study of photosynthesis and associated pigments. In the course of his investigations on tobacco mosaic diseases, Ivanovski noted that the yellow parts of the leaves not only contained little chlorophyll but also little starch. This observation might have been the origin of his interest in photosynthesis, or the impetus might have come from his admiration for the work of one of his colleagues, Michael S. Tsvett.

The latter was born in Italy in 1872, of a Russian father and an Italian mother. He studied in Switzerland and received his Ph.D. in Botany from the University of Geneva. Tsvett, whose name means "color" in Russian, was interested in plant pigments. In 1897 he was teaching plant anatomy and physiology at St. Petersburg. His research on the chemistry of chloroplasts attracted the attention of I. P. Borodin, M. S. Voronin, and Ivanovski, who saw to it that he was elected a member of the Society of Natural Scientists of St. Petersburg. In 1901 he was appointed Assistant at the University of Warsaw; six years later he became Professor of Botany and Agronomy. In 1908 he was appointed Professor of Botany and Microbiology at the Polytechnic Institute of Warsaw. In 1906 he published a paper which is the basis of chromatography, a term which he coined (7).

In 1910, he received a Doctorate in Botany from the University of Warsaw. Like millions of others, his life was perturbed by the war and the revolution. In 1915 he had to flee Warsaw and, after some tribulations, he became Director of the Botanical Garden of the University of Dorpat, in Estonia. Again, German occupation forced him to flee. These trials must have been especially hard for him since he never felt that he really belonged to Russia.

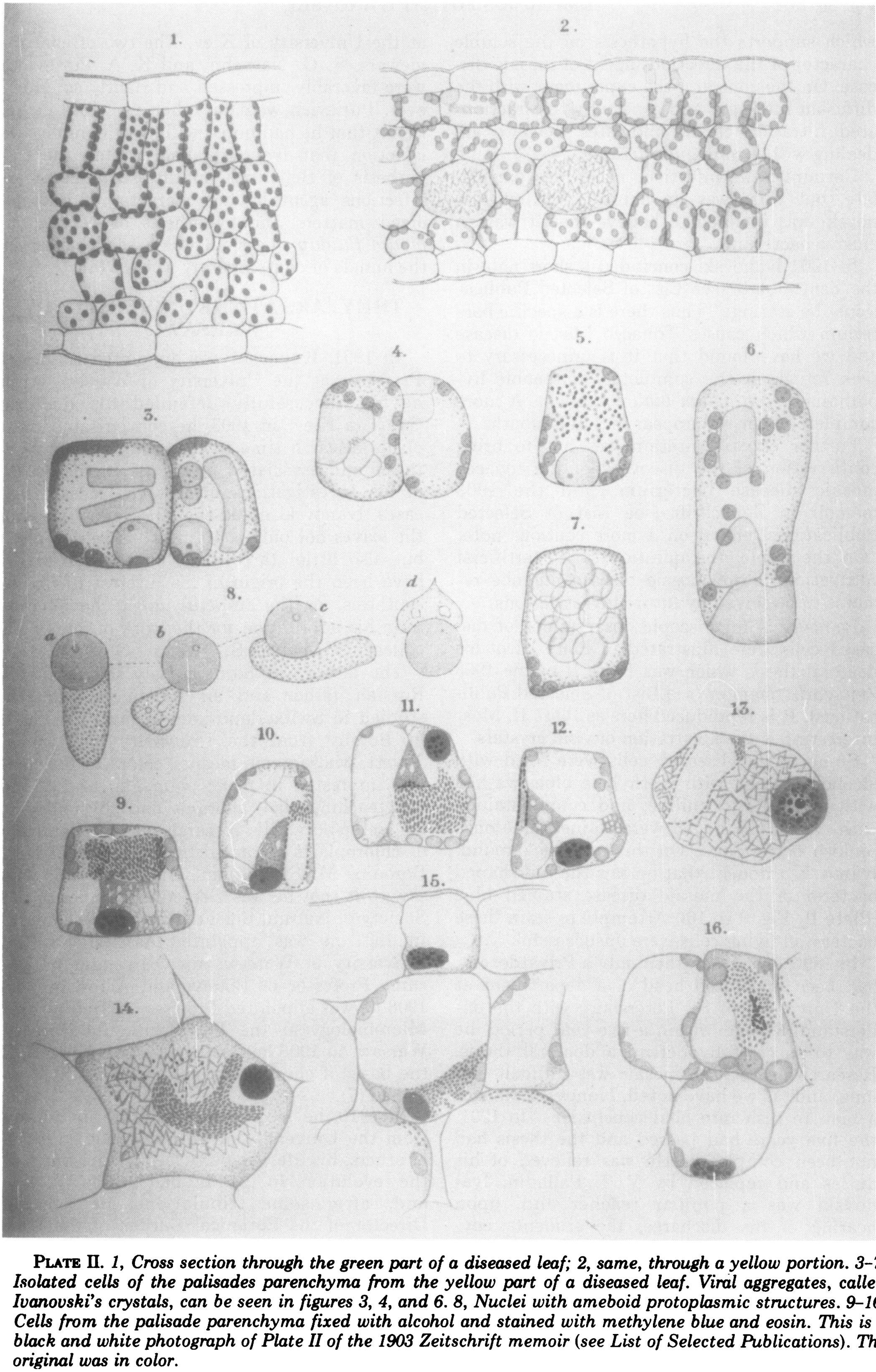

Plate II. *1, Cross section through the green part of a diseased leaf; 2, same, through a yellow portion. 3–7, Isolated cells of the palisades parenchyma from the yellow part of a diseased leaf. Viral aggregates, called Ivanovski's crystals, can be seen in figures 3, 4, and 6. 8, Nuclei with ameboid protoplasmic structures. 9–16, Cells from the palisade parenchyma fixed with alcohol and stained with methylene blue and eosin. This is a black and white photograph of Plate II of the 1903 Zeitschrift memoir (see List of Selected Publications). The original was in color.*

All his life he was a Swiss by heart. He died in 1919, in Voronezh. Tsvett's method, which has found its way into all laboratories where any interest in biochemistry exists, was ignored for many years. Its universal acceptance probably dates back to 1931, when Kuhn and Lederer separated carotenes and xanthophylls on columns of alumina and calcium carbonate (6).

In Warsaw, both Ivanovski and Tsvett studied problems connected with the action of light on plant pigments. Ivanovski concentrated on the physical aspects of the problem, whereas Tsvett studied the chemistry of the pigments.

It has been observed that chlorophyll extracted from plants and in laboratory solutions was less stable to light than native chlorophyll in the plant leaves. Ivanovski found that, when water was added to an alcoholic solution of chlorophyll, the chlorophyll turned from a clear solution to a colloidal one with a marked increase in photostability. He concluded, in 1909, that a colloidal solution of chlorophyll was 15 to 30 times more stable than a molecular solution. He thus felt that the stability of colloidal chlorophyll is comparable to that present in living leaves. He also observed that the light-absorption spectra of chlorophyll in living leaves suggested that the chlorophyll there was in a colloidal state. He reasoned furthermore, that, in the plants, chlorophyll is much more concentrated than in laboratory solutions and was presumably more stable in a concentrated form than when diluted.

It was also Ivanovski's opinion that the yellow pigments associated with chlorophyll in the leaves played no direct role in photosynthesis but protected the vital chlorophyll from the detrimental effect of blue and violet light. He carried out light-stability experiments on crude mixtures of leaf pigments, on pure chlorophyll and on chlorophyll to which the yellow pigments had been added. These experiments, made possible by the chromatographic method of Tsvett, yielded results that clearly supported his theory.

In Warsaw, Ivanovski was appointed the head of a department and Professor of Plant Physiology. This was at a time when Poles were strongly oppressed. In line with his general liberal philosophy, Ivanovski did not hesitate to appoint Poles to his staff. He also often intervened in favor of Polish students who were being refused admission to the University because their enrollment was subject to a quota.

Ivanovski tried to modernize the teaching of Botany at the University of Warsaw by combining teaching with research. His efforts might best be termed heroic if one considers the limitations of his budget: in the year 1907–8, for example, he had to make do with 24 rubles and 59 kopecks.

Ivanovski was a great teacher, popular with his students, not only because of his liberal views, which included fostering education of women, but also because of the quality of his presentation. One of his former students, Academician N. A. Maksimov, recalled that Ivanovski was not content merely to outline the established facts but, besides giving the historical background of any topic, tried to present various points of view before offering his own opinion.

In 1915, the University of Warsaw was hastily evacuated to Rostov-on-Don. In the confusion, Ivanovski lost all his equipment, his rich library, and many of his notebooks. These hardships, as well as the death of his only son, weighed heavily on him, faced as he was with the task of organizing a laboratory under wartime conditions in unfamiliar surroundings. Still, it is under these conditions that he prepared his most important pedagogical contribution, his textbook of Plant Physiology.

Dmitri Ivanovski died in Rostov-on-Don on 20 June 1920.

CONCLUSION

The discovery of the filterable nature of the causative agent of a disease, in 1892, by Ivanovski, was a most important contribution to knowledge. However, we find that Ivanovski had no influence on those who made similar observations at later dates, namely M. Beijerinck, also working on tobacco mosaic disease, F. Löffler and P. Frosch who investigated foot-and-mouth disease, both in 1898, and W. Reed and J. Carroll studying yellow fever, in 1901 (3). In addition, Ivanovski's work had less influence on the development of virology than the studies of the above mentioned pioneers.

One cannot say that Ivanovski was ignored because he published in Russian, a language not often understood by scientists. He was always careful to publish all his important contributions in an "accepted" language, and his German publications were in first-class journals. We thus have to find elsewhere an explanation for his minor position in the history of microbiology.

It is possible that Ivanovski had little impact because he did not travel abroad. Vinogradski and Metchnikoff studied in and traveled through many of the centers of western cultures. They were thus better known than

Ivanovski. Ivanovski may thus have been a victim of his poverty and lack of aggressiveness at obtaining travel grants.

However, in Ivanovski's own publications can certainly be found a cause for his lack of success. He did not take a strong stand in his paper of 1892 (2); then be kept faith in the existence of an elusive bacterium, cause of tobacco mosaic disease, which might be cultured one day. In his choice of a bacterium as the potential causative agent of tobacco mosaic disease, Ivanovski was showing some originality, since only after the studies of Erwin Smith at the turn of the century was it universally accepted that some bacteria were indeed etiological agents of some plant diseases (3).

LIST OF SELECTED PUBLICATIONS OF IVANOVSKI

A presumably complete list of Ivanovski's publications was compiled by Rizhkov in 1953 (5). A large number of his publications were critical appraisals of theses and books. He also contributed generously to an encyclopedia. The following is a list of some of his publications which seem to be the most important scientifically. Often, apparently the same paper was published in Russian and in German. The German reference is given here preferentially.

Ivanovski, D., and V. V. Polovtsev. 1890. Die Pockenkrankheit der Tabakspflanze. (Pox disease of the tobacco plant.) Mém. Acad. Sci. St. Petersbourg, Ser. 7, **37** (7):1-24.

Ivanovski, D. 1892. O dvukh bolezniakh tabaka. Tabachnaia pepelitsa. Mozaichnaia bolezn tabaka. (On two diseases of tobacco. Tobacco mildew. Mosaic disease of tobacco.) 19 pages. St. Petersbourg. This paper was published both as a booklet and in Selskoe khazaistvo i lesovodstvo, St. Petersbourg, vol. 189 (2), p. 104-121. It was probably Ivanovski's Ph.D. thesis.

Ivanovski, D. 1892. Über die Mosaikkrankheit der Tabakspflanze. (On the mosaic disease of tobacco plant.) Bull. Acad. Imp. Sci. St. Petersbourg, Nouv. Sér. 3 **35**:67-70. This paper was translated by J. Johnson (2).

Ivanovski, D. 1893. Über die Wirkung des Sauerstoffes auf die alkoholische Gärung. (On the effect of oxygen on alcoholic fermentation.) Bull. l'Acad. Imp. Sci. St. Petersbourg, Nouv. Sér. 4 **36**:391-413.

Ivanovski, D. 1894. Issledovaniia nad spirtovym brojeniem. (Investigations on alcoholic fermentation.) 76 pages. Acad. Sci. St. Petersbourg. This is Ivanovski's habilitation thesis.

Ivanovski, D. 1896. L'Influence de l'oxygène sur la fermentation alcoolique. (Influence of oxygen on alcoholic fermentation.) Monit. Sci. Paris, Sér. 4 **10**:148-149.

Ivanovski, D. 1899. Nitragin i alinit. K boprocu ob udobrenii pocho bakteriiami. (Nitragin and Alnit. The question of fertilizing soil with bacteria.) Pochvovednie **1** (4):223-238.

Ivanovski, D. 1899. Über die Mosaikkrankheit der Tabakspflanze. (On the mosaic disease of tobacco plant.) Zentralbl. Bakteriol. Parasitenk. Infektionskr. Abt. II **5** (8):250-254.

Ivanovski, D. 1901. Über die Mosaikkrankheit der Tabakspflanze. (On the mosaic disease of tobacco plant.) Zentralbl. Bakteriol. Parasitenk. Infektionskr. **7** (4):148.

Ivanovski, D., and S. Obraztsov. 1901. Über die Wirkung des Sauerstoffes auf die Gärung Verschiedener Hefearten. (On the effect of oxygen in the fermentation of various types of yeasts.) Zentralbl. Bakteriol. Parasitenk. Infektionskr. **7** (9/10):305-312.

Ivanovski, D. 1902. Mozaichnaia bolezn tabaka. (The mosaic disease of tobacco.) 72 pages. Warsaw. This is Ivanovski's doctoral dissertation which was also published the same year, in two parts, in volumes 5 and 6 of Izvestiia of the University of Warsaw.

Ivanovski, D. 1902. Die Mosaik und Pockenkrankheit der Tabakspflanze. (The mosaic and pox disease of tobacco plants.) Z. Pflanzenkr. **12**:202-203.

Ivanovski, D. 1903. Über die Mosaikkrankheit der Tabakspflanze. (On the mosaic disease of tobacco plant.) Z. Pflanzenkr. **13**:1-41.

Ivanovski, D. 1907. Über die Ursachen der Verschiebung der Absorbtionsbänder in Blatt. (On the cause of the shift of the absorption bands in the leaf.) Beri. Deut. Bot. Ges. **25** (8):416-424.

Ivanovski, D. 1913. Kolloidales Chlorophyll und die Verschiebung der Absorptionsbänder in lebenden Pflanzenblättern. (Colloidal chlorophyll and the shift of the absorption bands in living plant leaves.) Biochem. Z. **48**:328-331.

Ivanovski, D. 1913. Über das Verhalten des lebenden Chlorophylls zum Lichte. (On the relationship of living chlorophyll to light.) Ber. Deut. Bot. Ges. **31**:600-612.

Ivanovski, D. 1913. Über die Rolle der gelben Pigmente in den Chloroplasten. (On the role of yellow pigments in chloroplasts.) Ber. Deut. Bot. Ges. **31**: 613-617.

Ivanovski, D. 1914. Ein Beitrag zur physiologischen Theorie des Chlorophylls. (Über das zweite Assimilations-Maximum). (A contribution to the physiological theory of chlorophyll. On the second assimilation-maximum.) Ber. Deut. Bot. Ges. **32**:433-447.

Ivanovski, D. 1917-1924. Fiziologiia rastenii. (Plant physiology) 618 pages. Kharkov and Rostov-on-Don, 1917-1919. The first volume was published in 1917, the second in 1919. A second edition was published in Moscow, by The State Publishing House, in 1924.

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