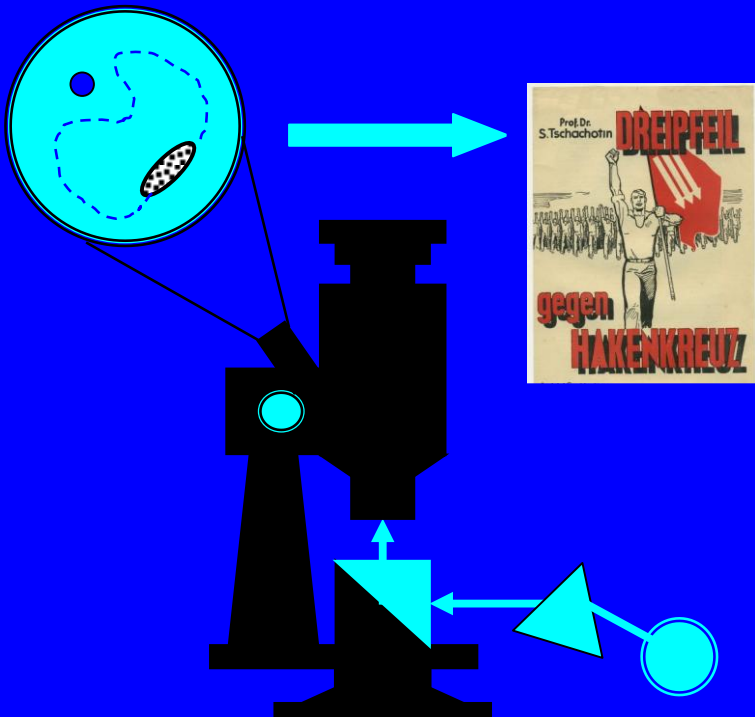


Yuriy POSUDIN

SERGEI CHAKHOTIN

- HIS CONTRIBUTIONS TO
SOCIAL PSYCHOLOGY AND
BIOPHYSICS



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Posudin, Yuriy. Sergei Chakhotin – His Contributions to Social Psychology and Biophysics. Kiev, 2015. Artmedia print.–115 p.

This book is a biographical critique of the life and the scientific and public contributions of Sergei Chakhotin, an outstanding biophysicist, tireless innovator, an expert in crowd psychology and political propaganda.

He was the first to use focused ultraviolet radiation to alter a living cell and developed a series of unique methods and tools for microscopic operations on test objects.

Chakhotin found himself in the midst of political, military and social events in Europe during his life. While watching the emergence of totalitarian regimes, Chakhotin studied the problems of social psychology focusing upon human instinct and conditioned reflexes, particularly with regard to the behaviour of masses, and to elucidate the mechanism of transforming a large segment of the population such that it could be governed by leaders by means of political propaganda.

Sergei Chakhotin concluded that freedom, peace, and clemency should be an integral part of human nature, those responses are fixed deeply in every human being. The achievement of this goal is possible in accordance with the Pavlov's theory through the formation of a reasonable form of corresponding conditioned reflexes, propaganda, and especially education.

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*Леонид Брежнев
Ленинград.*

(1883-1973)

FOREWORD TO THE FIRST EDITION¹

The rapid development of science, which marked the beginning of the twentieth century, stimulated the emergence of new experimental and methodological approaches to the study of biological objects. The discovery of radioactivity, the nature of X-ray radiation, the development of quantum theory – have allowed new approaches to the exploration of the vital processes of living organisms.

In light of these advances in science, especially at the intersection of different disciplines, the life and scientific contributions of Sergei Stepanovich Chakhotin – an outstanding biophysicist, tireless innovator, and the first to use focused ultraviolet radiation to affect a cell, developed a series of unique devices to investigate micro-objects and laid the foundation for a number of new experimental techniques in cytology, embryology, and physiology. His name is associated with advances in modern biophysics, especially in the areas of laser photobiology, radiobiology, and electrophysiology.

Albert Einstein wrote: “I know personally Professor Chakhotin as a very serious scholar who pursued his academic goals with energy and undismayed, despite the very large external interference”. Unfortunately, the activity of S. Chakhotin has not been adequately reflected in our scientific and social literature. That’s why the emergence of the research of Yuriy Posudin which is dedicated to the lighting the main stages of the life and creative activity of Sergei Chakhotin can only be welcomed.

T.M. TURPAEV,
Academician of the Russian Academy of Sciences

¹ Posudin Yu. I. 1995. *Biophysicist Sergei Chakhotin*. Agr.Univ.Publ., Kiev (In Russian).

FOREWORD TO THE SECOND EDITION

In 1979-1980 I was fortunate enough to obtain advanced scientific training at the Institute of Biophysics CNR, Pisa, Italy where I participated in research activities such as *in vivo* laser microspectrofluorometry of the photopigments in the alga *Euglena gracilis*.² This alga exhibits motor responses that are modulated by changes in ambient lighting. A specific organelle, called a paraflagellar body (PFB), plays a special role in responding to external light, acting as a photoreceptor. Since the PFB is fairly small ($\sim 1 \times 0.3 \times 0.4$ mm), the isolation and identification of its pigments is not a simple task. As a consequence, laser spectrofluorometer was used to identify the pigments in the PFB of *E. gracilis*. The device consists of a tunable dye laser, fluorescence microscope and recording system. This allowed determining the laser-induced excitation spectrum of fluorescence of the PFB establishing its flavin composition.

Naturally as a young intern I began with an intense bibliographic search of the literature on the subject. Among a number of authors associated with the technique of microirradiation of a cell, I repeatedly came across the name of Sergei Chakhotin³. Upon returning to Kiev, I tried to find additional information about Chakhotin's contributions to science, but was surprised to find in the Soviet literature virtually no information. Later I realized that the political activity of the scientist did not always coincide with the ideological concepts of the Soviet regime and his name had been banned. This naturally greatly stimulated my interest in Chakhotin resulting in a quest to learn everything I could about him.

As a lecturer, I appealed for help from my students with this endeavor. Since students are generally not keen on doing something for nothing, I proposed that those that could find information on S. Chakhotin would receive extra credit. It worked and after a while I had a copy of a newspaper article, with the names and entities associated with Chakhotin, which greatly facilitated the search.

I learned that A.S. Danilov whom lived in Alma-Ata (now Kazakhstan), was a unique person who collected information on all outstanding

² The author is grateful to Professor G. Lenci and Dr. G. Colombetti (Institute of Biophysics CNR, Pisa) for stimulating his interest in the photomovement of algae.

³ You can find such variants of scientist's surname in the literature as Tchakhotine, Tschakhotin, Chakotin, Chakhotin, Ciacotin, Tchakhotin, Chacotine.

scientists. This was well before the Internet so he did it by hand, making catalogs of information. I wrote to him and he kindly provided me the address of the Institute of Cytology, Academy of Sciences of the USSR (Leningrad), where S. Chakhotin worked after returning from abroad.

I immediately sent a letter to this Institute. After some time, the Chief of Staff of the Institute sent a copy of Chakhotin's personal file with his biographical information and a list of publications. The information made it possible to establish the main stages of his life, and the social and scientific contributions of this remarkable scientist – biophysicist, physiologist, and microbiologist – Sergei Stepanovich Chakhotin. The information available became progressively richer with the advent of the Internet.

As a result, the book “Biophysicist Sergei Chakhotin” (Kiev, Nat. Agr. Univ., 1995) and its electronic version (Electronic publishing “Analytical Microscopy”, Prof. A.Yu. Budantsev, ed., Pushchino, 2005) were published. In this edition, I would like to pay greater attention to the social contributions of Sergei Stepanovich Chakhotin who was deeply interested in the problems of social psychology, observations of the behavior of large numbers of people, clarifying the mechanisms of transforming and controlling large groups of people and their management by leaders by means of political propaganda. As a consequence, the title of the second edition of the book has been changed from “Biophysicist Sergei Chakhotin” to “Sergei Chakhotin – His Contributions to Social Psychology and Biophysics”.

The photographs in this book are provided courtesy of S. Chakhotin's family; drawings in Section III were taken from original works by S. Chakhotin.

Yuriy Posudin

Professor Yuriy Ivanovich Posudin was the first author to publish a book (in Russian) about Sergei Chakhotin, whom the scientific world considers to be the first scientist-biophysicist at the dawn of the twentieth century.

The book combines detailed biographical information with the scientific contributions of my Father. I express my great appreciation to the author of the book who has provided an very accurate critique of his work and I am convinced that it will stimulate considerable interest among those who will read the book.

Pierre Tchakhotine

*To twist a screw of brass,
so that, in the water's droplets,
the world would radiantly appear
minute – that is what occupied my day.
I'm fond of the serene alignment
of green laboratory lamps,
the motley of the complex tables,
the magic gleam of instruments.
And from descending all day long
into the microscope's dark well
you did not hinder me at all”.*

Vladimir Nabokov,
“*The University Poem*“, 1927

I. MAIN STAGES OF BIOGRAPHY AND SOCIAL ACTIVITY

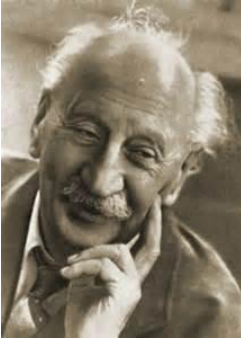
Sergej Stepanowitsch Chakhotin⁴ was born on 13(26)⁵ September 1883 in Constantinople (now Istanbul) in the family of the Russian dragoman (secretary-translator) Stepan Ivanovich Chakhotin.

His parents took him to Odessa when he was 10 years old where he studied in the Third Odessa Gimnasium. From 1899 to 1900 he resided in Italy due to an illness. After graduating from the Gymnasium with a gold medal, he entered the Faculty of Medicine of Moscow University in 1901. A year later, he was arrested for participating in student protests.

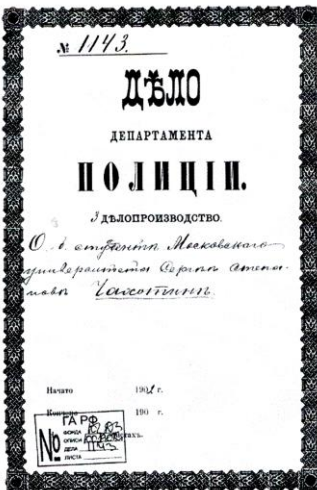
Chahotin describes this period in the preface to the book of L.V. Bobrov “*Shadows of Invisible Light*” (Moscow, Atomizdat, 1964): “*Expelled from Moscow University, I have chosed on the advice of A.F. Ioffe to continue my studies at the University of Munich, where the glory of Roentgen radiated even 6 years after his discovery of X-rays. A huge auditorium was always crowded for his lectures*”.

⁴You can find such variants of scientist surname in the literature as Chakhotin, Tschachotin, Tchakhotine, Tchakhotin, Chacotine, Chakotin, Ciacotin, Čachotin

⁵ Dates correspond to the Gregorian (in brackets - Julian) calendar



A.F. Ioffe (October 29, 1880 – October 14, 1960) was a Russian and Soviet physicist. Institutions: State Institute of Roentgenology and Radiology; Leningrad Physico-Technical Institute. The founder of the Leningrad Physico-Technical and Agrophysical Institutions. An outstanding expert in the field of electromagnetism, radiology, the physics of crystals, and thermo- and photoelectricity



The case filed by the police department on S. Chakhotin in connection with his participation in a student meeting on February 10, 1902, and the petition to Emperor Nicholas II for commutation of sentence [Sorokina, 2007].

№ 1173
 ДѢЛО
 ДЕПАРТАМЕНТА
 ПОЛИЦИИ.
 ДѢЛОПРОИЗВОДСТВО.
 О в. студента Московского
 университета Сергея Антоновича
 ЧАХОТИНА

Про Императора,
 Великого
 Государя Императора

Вашему
 Императору
 Государю
 Императору
 Николаю II
 изволю
 представить

Будучи арестован 10-го
 февраля 1902 года по обвинению
 в участии в студенческом
 собрании в Москве, являюсь
 по делу 9-го
 сентября 1902 года.
 Ввиду того что являюсь
 студентом Московского
 университета и желаю
 продолжать обучение,
 прошу Вашего Императорского
 Величества разрешить
 мне ходатайствовать о
 замене моего наказания
 на менее строгое.

James Imperatoro
 Chakhotin
 Спрос

And further: *“I am obliged namely to Roentgen in that research spirit which prevailed in his lab, excited in me as a biologist an interest in physics that have led me to the discovery of the principle and development of methods using an ultraviolet microbeam as a means for doing microscopic operations on cells”.*

Wilhelm Röntgen (March 27, 1845 – February 10, 1923) was an outstanding German physicist, who produced and detected X-rays that came to bear his name “Röntgen Rays”.

Roentgen was awarded the first Nobel Prize for this discovery in 1901.



W. Roentgen according to Chakhotin *“... was, one might say, the most honest, the most irreproachable man ...”.*

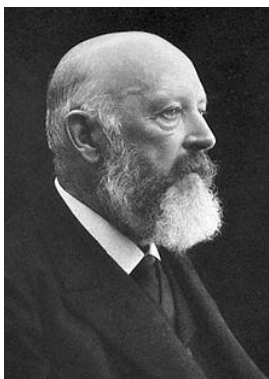
Chakhotin subsequently continued his studies in various medical and science faculties in Berlin, Munich, and Heidelberg, specializing in physics, chemistry, zoology, and physiology.



Ludwig Maximilian University in Munich - a higher education institution in Germany, with which Nobel laureates as Wilhelm Röntgen, Max Planck, Werner Heisenberg, and Otto Hahn were associated.

Among Chakhotin's teachers were the chemist A. Bayer who's expertise included the synthesis of indigo, the chemistry uric acid and acetylene, the stability of various cyclic carbon compounds and the properties of unsaturated compounds (Bayer was awarded the Nobel Prize in 1905); the biochemist A. Kossel, who discovered histidine in 1896 and conducted research on protamines and histones (awarded the Nobel prize in 1910); the Hertwig brothers, Oscar – a biologist, author of works on the morphology of invertebrates, cytology and embryology, who used new experimental methods in embryology, and Richard – a zoologist and embryologist, who formulated the laws of volumetric ratios of nucleus and protoplasm; and T. Engelmann who studied the physiology of the nervous and muscular systems, vision, and the heart. These scientists used innovative methods in their work that undoubtedly influenced the formation of Chakhotin's scientific outlook.

Chakhotin married Emma Haas in 1906 and they traveled to Corsica on their honeymoon.



Johann Friedrich Wilhelm Adolf von Baeyer (October 31, 1835 – August 20, 1917) was a German chemist who synthesized indigo and was the 1905 recipient of the Nobel Prize in Chemistry.

Albrecht Kossel (September 16, 1853 – July 5, 1927) was a German biochemist and physiologist. He was awarded the Nobel Prize for Physiology or Medicine in 1910 for his investigation of cell biology, chemical composition of cell nucleus and his work in determining and describing the nucleic acids.





Oskar Hertwig (April 24, 1849 – October 25, 1922) was a German zoologist and founder and director of the Anatomical Institute at Berlin University (1888-1921). He was a specialist in the fields of invertebrate morphology, cytology and embryology and was the first to apply experimental methods in embryology.

Richard Hertwig (September 23, 1850 – October 3, 1937) was a German zoologist. He was the first to describe zygote formation as the fusing of the spermatozoa inside the membrane of the egg cell during fertilization. His later research focused on protists, as well as developmental physiology of sea urchins and frogs.



Theodor Wilhelm Engelmann (November 14, 1843 – May 20, 1909) was a German botanist, physiologist and microbiologist. His research was characterized by highly ingenious and elegant staged experiments, and precise and clear descriptions of the results obtained. Engelmann invented and perfected many devices utilized in physiological research and described the experimental methods for these studies (e.g., experimental protocol for microspectrophotometry).

Chakhotin graduated from the Heidelberg University in 1907 with highest honors (“summa cum laude”). His thesis “Die Statocyste der Heteropoden”, involved the study of the structure and physiology of balance in Heteropoda. The author was awarded the academic degree of Doctor of Philosophy for this work.



The years of Chakhotin’s student study centered around the city of Heidelberg where he graduated with highest honors (“summa cum laude”) from Heidelberg University. Here he studied zoological problems with Prof. Otto Bütschli and oncology with Prof. Vincenz Czerny. In 1930-1933, Chakhotin was engaged in research at the Kaiser Wilhelm Institute for Medical Research

A special interest of Chakhotin, as well as other Russian scientists, was the Russian Zoological Station at the Bay of Villefranche (Fr. Villefranche-sur-Mer, It. Villafranca Marittima) on the French Riviera due to the exceptional diversity of zooplankton. The Russian Zoological Sta-

tion was created [Dolan, 2014]⁶ and used by Russian, French and American biologists.

The Russian scientist Sergey Fokin from St. Petersburg State University, describes the history of the zoological station in Villafranca, Italy [Fokin, 2008]: “*In the 1850–1870s, both Western European and Russian naturalists privately conducted zoological investigations at sites along the Mediterranean Sea (Messina, Naples, La Spezia, Villefranche-sur-Mer–Villafranca, Marseille, Banyuls-sur-Mer) as well as at some other marine locations.*”



Zoological Station, Villefranche-sur-Mer, France, that was repeatedly visited by Sergei Chakhotin [https://ru.wikipedia.org/wiki/Виллафранкская_зоологическая_станция].

There were among the Russian visitors not only students and magistrants but experienced zoologists such as A.O. Kowalevsky, W.A. Wagner, W.W.Salensky, M.A. Menzbir, K.S. Mereschkowsky, V.N. L'voff, A.N. Severtzov, W.M. Schimkevitsch, D.D. Pedashenko, M.N. Rymsky-

⁶ The history of the Russian Zoological Station, located near Nice, is intertwined with Italy and France. In 1860 it was transferred to France under the treaty between the two countries. Russia created a naval base and Oceanographic Laboratory by the end of the 19th century at what originally had been an old quarantine station.

Korsakov, E.A. Bihner, N.A. Ivantzoff, N.V. Nasonov, A.K. Mordvilko, V.V. Redikortzev, P.P. Sushkin, B.V. Sukatscheff, A.A. Ostroumoff, B.A. Svartschevsky, J.N. Wagner, N.K. Koltzoff, N.A. Livanow, S.A. Zernow, M.M. Novikoff, S.S. Tschachotin, and others [Davidoff and Spitschakoff, 1911]”.

Chakhotin visited the Russian Zoological Station several times (first as a student in 1904, 1905 and 1906.). From 1904 to 1938, Chakhotin also conducted studies at marine biological stations in Trieste, Naples, Helgoland, Monaco and others.

In 1907, the 24-year-old Sergei Chakhotin arrived with his family in Messina, Sicily at the invitation of the Italian Professor Alberico Benedicenti, who was studying multicellular marine organisms living in the Gulf of Messina.

In early in the morning (5:20–5:21 AM) of December 28th, 1908, a massive earthquake of 7.1 on the Richter scale took place in Messina. The main shock lasted 30–40 seconds and caused tremendous destruction within a 300-kilometer (186 mile) zone.

A subsequent 12-meter (39-foot) tsunami wave caused even more devastation. About 91% of the buildings were destroyed and some 70,000 residents were killed [The Messina 1908 earthquake. http://www.grifasi-sicilia.com/messina_terremoto_1908_gbr.html].



Messina was almost completely destroyed in 1908

Russian sailors from the ships of squadron under the command of Rear-Admiral V. Litvinov, that were on an educational cruise in that area of the Mediterranean, came to the help of the residents of Messina.



Russian sailors came to help the residents of Messina

During the earthquake, Chakhotin was buried alive under the rubble of his house, where he spent 12 hours. He described the situation as “being on the brink of life and death” in his memoirs, “Under the Ruins of Messina”:

“I crawl, pressing, clinging, pulling myself, more air around, the chaos of debris, planks with protruding nails, straw, roof, broken split, tiles, rusty iron sheets, everything just frozen in some wild whirlwind. Here, now close ... close ... I got out ... I’m on the roof.”

The original text (in Russian) was kept for a long time in the family archives, until it was presented for publication by the son of the scientist Peter Chakhotin (Pierre Tchakhotine). The book was first published in Italy a centenary after the Messinian disaster [Sergej Tchakhotine: Sotto le macerie di Messina. Racconto di un sopravvissuto al terremoto del 1908. Intilla editore Messina, 2008].

The presentation of the book was held in Messina in 2008, and subsequently in Russia in 2010 at the Solzhenitsyn's Russian Abroad House in Moscow.



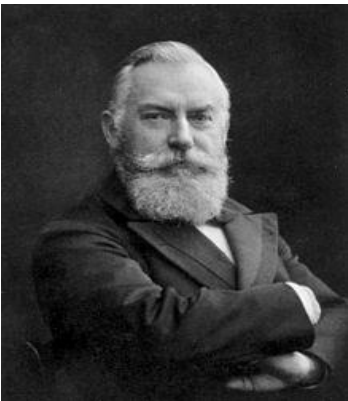
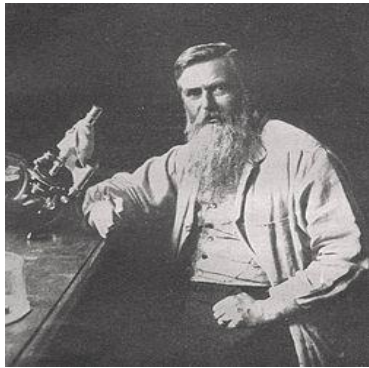
The presentation was attended by Marina Sorokina, a science historian; Professor Alexander Nikonov, the Institute of Physics of the Earth; Dr. John Biggart, a historian from the UK, and Pierre Tchakhotine, the son of Sergei Chakhotin.

After returning to Russia, Chakhotin left his family (two young sons and pregnant wife Emma) in Odessa and worked briefly at the University of Kazan, and then returned to Heidelberg, where he collaborated with colleagues on Zoological (Prof. O. Bütschli) and Oncology (Prof. V. Czerny) problems. Here he created in 1912 his famous Zeiss device for operating on cells using an ultraviolet microbeam.

In 1912, Chakotin, as a young scientist, was invited by I. Pavlov, the famous physiologist known for both his theoretical analysis and experimental activity. Pavlov had recently created a scientific school for his students and followers. Pavlov wrote: “... *If I excited, directed and focused our common work, I was at the same time under influence of observation and ideology of my co-workers ...*”. While working as an assistant at the

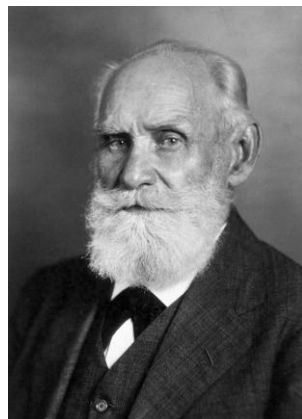
Institute of Physiology from 1912 to 1918, Chakhotin reorganized the physiology laboratory.

Otto Bütschli (May 3, 1848 – February 3, 1920) was a German zoologist, considered an expert in the field of cytology and protistology. He investigated the development of invertebrates and insects and first described many of the groups of protozoa.



Vincenz Czerny (November 19, 1842 – October 3, 1916) was a German surgeon, famous for his significant contributions to oncological and gynecological surgery. He described the dissection of tissue by means of electric current in 1910.

Ivan Petrovich Pavlov (September 26, 1849 – February 27, 1936) was a Russian physiologist and the founder of the science of higher nervous activity. He is known mainly for his development of the concept of the conditioned reflex. In 1870 Pavlov entered the Physics and Mathematics Faculty of St. Petersburg University, in order to attend a course on science. He was the recipient of the Nobel Prize in Physiology or Medicine in 1904.



The First World War was largely centered in Europe and over its duration (July 1914 until November 1918) resulted in over 37 million military and civilian deaths. The principal causes of World War I were political, territorial, and economic conflicts among the great European powers that were assembled into two opposing alliances: the Allies (based on the Triple Entente of the United Kingdom, France and the Russian Empire) and the Central Powers of Germany and Austria-Hungary. These alliances were expanded to include such countries as Italy, Japan and the United States that joined the Allies, and the Ottoman Empire and Bulgaria that supported the Central Powers.

The First World War had a tremendous impact on the economy and political life of Russia and caused the emergence of new state and public bodies, whose activities are aimed at maintaining the country's defense.

As the First World War began, a committee of military-technical assistance was created to study the mineral resources and ore needs of industry. It was headed by A.V. Fersman, an outstanding expert in geochemistry and mineralogy. Chakhotin actively participated in the work of the committee.

During this period, the scientist was adjacent to G.V. Plekhanov who organized the Russian Social-Democratic organization in 1914. With the beginning of World War I, disagreements between Plekhanov and Ulyanov (Lenin) concerning the positions of the committee on the war differed so much that Plekhanov formed its own social-democratic group in 1914. Plekhanov's group supported the war to the victorious end and were against a socialist Russia. The ideology of the group was reflected in the newspaper "Edinstvo" (Unity). The October Revolution was greeted by the group with hostility, and in 1918 the group disbanded.

Chakhotin organized the Committee of Military-Technical Assistance (KOVOTEP) in 1915-1916 to mobilize the scientific community for military purposes.

The Russian, Austro-Hungarian, Ottoman and German empires ceased to exist in their prewar form as a result of the war. The Russian monarchy collapsed and a struggle for power between the Provisional Government and the national network of Soviets, led by socialists, occurred.

The conflict lasted between the years 1917- 1923 and ended with the victory of the Bolsheviks. The Provisional Government intended to continue the war with Germany while the socialists (especially the Bolsheviks, "Majority") were opposed to the continuation of the conflict.

As a result, civil war broke out between the “Reds” (Bolsheviks) and “White” (anti-socialist faction).

The main weapons of the Bolsheviks before their seizure of power were deception and populism. They proclaimed freedom, the end of the war for soldiers and factory workers, land for the peasants, universal prosperity for everyone. With the start of land seizure from the wealthy, they initiated the use of intimidation and violence.

The overwhelming majority of the Russian intelligentsia had taken a neutral stance, refraining from any action. The fact of the violent coup d'etat and the government policies that fundamentally differed from the ideals of liberalism and democracy gave rise to anti-Bolshevik sentiments among the intelligentsia.

After the October 1917 revolution, the Russian writer Maxim Gorky wrote in his newspaper *Novaia Zhizn*: “*Lenin and Trotsky and their followers already have been poisoned by the rotten venom of power. The proof of this is their attitude toward freedom of speech and of people and toward all the ideals for which democracy was fighting*”. Some days later he wrote again: “*Lenin and Trotsky and all who follow them ... are dishonoring the revolution, and the working class...Imagining themselves Napoleons of socialism, the Leninists are completing the destruction of Russia*”!

The first steps of Soviet power infringed all the rights of the intelligentsia as non-proletarian social groups. This was especially true for the representatives of science and education such as high school teachers, professors and scientists. A fierce fight broke out with anyone who might be suspected of sympathizing with the ousted ranks, who had the misfortune to be born into wealthy families, who received a tolerable education and acquired the right to call themselves Russian intellectuals. The fate of intellectuals and scientists became tragic in these circumstances.

After the February Revolution, Chakhotin created the Committee of Social and Political Enlightenment under the Provisional Government in June 1917. Its tasks were the organization of political and cultural activities to support the Provisional Government (it ceased its activities after October 25, 1917).

Then he organized the Soviets of Deputies of the working intelligentsia [Sorokina, 2007]. The Soviets were elected by the population for a certain period as a collegial representative bodies of public authority. The whole system of the Soviets in 1917 was characterized by significantly chaos: in addition to the Soviets of Workers’ and Soldiers’ Deputies and

Soviets of Peasants' Deputies the following structures could also exist e.g., Soviets of Sailors and Officers' Deputies, Soviets of Cossack' Deputies, Soviets of Students' Deputies, Soviets of Working Intellectuals' Deputies, etc.

The Soviets of Working Intellectuals' Deputies opposed the Bolshevik seizure of power. The response of the Bolsheviks was rapid and Chakhotin was forced to flee to the Don in 1918 to avoid arrest.

The Russian Civil War in the Don region of southern Russia took place between the Don Cossacks allied with the White movement and the Bolsheviks from November 1917 until the spring of 1920. White movement used the Volunteer Army during this period. Here Chakhotin worked first in the Don Government and then in the political administration of the Volunteer Army, where he headed the Department of Propaganda "OSVAG" (**OS**Vedomitelnoye **AG**enstvo).

OSVAG was the information and propaganda agency of the Voluntary Army during the Russian Civil War that was created by General Denikin in 1918. The primary objectives of OSVAG were to inform the public about the White movement and its objectives; the dissemination of information about the crimes of the Bolsheviks; perpetuating the memory of the heroes of the White movement; providing information about the current state of affairs; and counter Bolshevik propaganda.

Examples of the Russian intelligentsia that assisted the OSVAG were the writers I.A. Bunin and E.N. Chirikov, philosopher and jurist E.N. Troubetzkoy, artists I.Ya. Bilibin and E.E. Lancer, the poet S.A. Sokolov and S.S. Chakhotin.

Overall, the White Movement was nationalistic [Kenez, 1980] and rejected ethnic particularism and separatism [Lazarski, 1992]. The White Movement generally believed in a united multinational Russia, and opposed separatists who wanted to create independent states instead of the Tsarist Russian Empire. Amongst White Army members, anti-Semitism was widespread.

Disappointed in the White movement and unable to return to scientific work in Russia, Chakhotin went to Paris in 1919. From that moment, his longest period of scientific exile began, which lasted almost forty years.

Chakhotin eventually returned to his research activities in Paris. Thanks to the support of French scientists, he received a subsidy from the Prince of Monaco, Albert I to conduct research at the Oceanographic Museum in Monaco. The facility (Fr. Musée océanographique de Monaco) consisted of the Museum and the Oceanographic Institute. The Oceano-

graphic Museum was founded in 1889, and the Oceanographic Institute was opened in 1906. Here Chakhotin studied ultraviolet micro-irradiation of sea urchins' eggs, the results of which were published in the Proceedings of the Paris Academy of Sciences and the Journal of the Biological Society.



Oceanographic Museum in Monaco.

During this period, he became one of the members of the social-political movement “Smena vekh” (translated “Change of Signposts”). This movement among the Russian émigré community supported cooperation with the Soviet government in the hope that the Soviet state would evolve back into a “bourgeois state”.

A collection of essays entitled “Smena vekh” (Prague, 1921) became a political manifesto of the movement. Among the authors of the collection were Yu.V. Klyuchnikov, N.V. Ustrialov (initiator of the movement), S.S. Lukyanov, A.V. Bobrishev-Pushkin, S. Chakhotin and Yu.N. Potekhin. The movement “Smena vekh” can be explained by the nostalgia of patriotic emigrants for the homeland.

In his article, “To Canossa!”, which was intended for the collection “Smena vekh”, Chakhotin tried to give his political assessment of the role of the Russian intelligentsia in the current events [Chakhotin, 1921]:

“... We are not afraid to say now: “We are going to Canossa⁷!” We were wrong, we were mistaken. Do not be afraid to recognize this openly and for ourselves and for others”.

“This recognition will not humiliate us, will not break our spirit. We have struggled honestly so far, since we considered that it was our duty. Events have shown us that we were wrong, that our way was in the wrong direction. And realizing this, seeing what the interests of the homeland require us, we are ready to admit our mistake and change our way. Will we become the Bolsheviks or the Communists, as some people think? Of course not. Communism as a practical doctrine in contemporary surroundings remains for us the same utopia as earlier, but it can and must be changed one way or another if it wants to become part of everyday life; and in many respects we, intellectuals, can assist this process”.

The main ideas of the movement “Smena vekh” were reflected also in the homonymous journal (Paris, 1921-1922), in the newspaper “Nakanune” (Berlin, 1922-1924) and in the journal “Novaya Rossia” (Moscow, 1922-1926).

Thus, the newspaper “Nakanune” continued the ideological line of the collection “Smena vekh”. But the editorial board of the newspaper paid special attention to the idea of regeneration of the Bolshevik dictatorship into the “labor state”. Special hopes are pinned on the “working intelligentsia” as an active force capable to carry out the revival of Russia [Biggart, 2012]. A dialogue between the the emigrant and the Soviet intellectuals took place on the pages of this newspaper, which was allowed to be distributed in Soviet Russia. The newspaper published outstanding writers such as M. Bulgakov, V. Kataev, E. Petrov, K. Fedin, V. Lidin, O. Mandelshtam. S.Chakhotin was included in the editorial board, A. Tolstoi edited the literary supplement.

⁷ “To go to Canossa” means in an allegorical sense to come with a confession head to the triumphant enemy, to admit mistakes, to repent of them and ask for forgiveness. Canossa is an ancient castle southwest of Reggio nell’Emilia in Italy. The historical events in 1076 that occurred were associated with the castle when Pope Gregory VII (1073-1085) cursed and excommunicated the German Emperor Henry IV (1050-1106), who declared that the Pope was deposed. The Emperor, once realizing he had lost, bowed to the Pope wearing clothing of a repentant sinner and was made to wait for three days on his knees until the head of the Catholic Church accepted him and absolved his sins.

It is not difficult to imagine what would have happened to Chakhotin who considered communism utopia if he, supporting the idea of “home-coming” (“vozvraschenchestvo” in Russian), returned to Soviet Russia. Five members of the “Smena vekh” movement were shot after their return from exile.

In 1920 Chakhotin worked at the Institute of General Pathology in Zagreb, Croatia. A year later he became a professor at the local university. The complexity of his situation with regard to immigration to Russia forced him to move to Italy for a year. There he participated in the Genoa Conference, highlighting its contribution in the newspaper “Nakanune”. At the conference Chakhotin met G.V. Chicherin, L.B. Krasin, and V.V. Vorovskiy who represented the Soviet state during the discussion of economic and financial issues. During this period, L.B. Krasin invited Chakhotin to participate in the organization of the Soviet trade mission in Berlin. In 1922 Chakhotin received his Soviet citizenship, but left the Soviet trade mission in 1925 due to disagreements with the leadership.

Chakhotin was not totally confined to his scientific work during these years. He wrote concerning the scientific organization of labor in “Organization, Principles and Methods of Industry, Commerce, General Administration and Policy” (Mocow, 1923), “European Literature on the Scientific Labor Organization” (Moscow, 1924), “The Rational Organization of Scientific Research” (Paris, 1938), and others. A Russia’s revival program based on neotaylorism principles was presented in these studies. Taylorism is the theory of management and scientific organization of labor, developed by Frederick Winslow Taylor, to increase economic efficiency and productivity. Details of Chakhotin’s ideas on the formation of “groups laboring intelligentsia” and the construction of socialism, based on the application of the Taylor system can be found in the work of J. Biggart (2012).

Chakhotin went to Genoa in 1927, where he worked in the Pharmacological Institute of the University, dealing with the oncology problems. In 1930, at the suggestion of Albert Einstein, he was awarded a prize by the Research Corporation. In the same year he returned to Heidelberg Institute in Germany to work at the Medical Research.



Sergei Chakhotin. Copenhagen. 1934.

The economic crisis in Europe after the First World War was a major cause of the emergence of fascism in the 1920s. While in Italy, Chakhotin observed the National Fascist Party march on Rome in 1922, which resulted in the King of Italy handing over power to Mussolini on October 26, 1922. Mussolini was appointed the Italian Prime Minister. The National Fascist Party became the ruling party. The word “fascism” derives from the Italian “fascio” (union) (for example, the name of the political organization of the radical Mussolini sounds like “Fascio di combattimento” (League of Struggle). This word, in turn, comes from the Latin “fascis” (bunch) which, in particular, represented a symbol of power in the era of the Roman Republic. The success of the march of Mussolini’s troops on Rome stimulated the activity of the German right-wing radicals.

As early as the early 1930’s the leadership of the Social Democratic Party of Germany (SPD) has been criticized by functionaries of Party for conservatism and bureaucratic hibernation, ossification of the party leaders, and boring and ineffective propaganda, which clearly lost out to Nazi propaganda’s appeals to the feelings and emotions of the people.

The Iron Front (Ger. Eisenre Front) was founded by Sergei Chakhotin, Carl Mirendorfom and other anti-fascists on December 16, 1931 to publicize the Nazi danger. During this period Chakhotin created an emblem for the Iron Front - three arrows that were directed against the enemies of Social-Democracy – National Socialism and its leader Adolf Hitler.



In March 1932, Chakhotin, being an expert in crowd psychology and the organization of mass demonstrations, became the head of the election campaign in several cities in the German state of Hessian and used his method of active political propaganda to try and stop the rise to power of Hitler and the Nazis.

Chakhotin used three arrows as a dynamic and aggressive symbol, similar to lightning; the triple repetition of arrow enhanced the action associated with collectivism. With regard to the worker's movement, this symbol can be associated with economic (trade unions), political and cultural (the party) and physical (selfdefense and sports) of the working class [Chakhotin 1933. Interview].



Propaganda materials with the emblem of Iron Front.

In Denmark Chakhotin organized in 1935 a translation of his book “Three Arrows Against the Swastika” from German (“Dreipfeil gegen Hakenkreuz”) to Danish (“Trepil mod Hagekors”). Here he was popular among Dutch youth. At the same time he felt the opposition of Danish leaders of the Socialist Party, who supported the leaders of the Social Democratic Party, in particular, Otto Wels who was in exile in Prague.

Chakhotin was skeptical of the Social Democratic Party bonze, who tried to cope with the wave of fascism with old “Marxist” dogmas, boring chatter, tearful complaints and pathetic declamations [Chakhotin 1933]. Faced with the opposition of Danish leaders, Chakhotin decided to move to Paris in early 1934 [Neil MacMaster, unpublished].



The cover of the German edition of Chakhotin’s book “Three Arrows Against the Swastika,” 1935.

There he was engaged with research activity in the Institute Evolution, the Prophylactic Institute, a research laboratory at the Hospital “Leopold Bellan”, and the Institute of Physical and Chemical Biology. The micro-irradiation of individual cells, the study of conditioned reflexes by Pavlov in unicellular organisms, and the etiology of cancer were the primary areas of his research focus.

The academicians L.A. Orbeli (founder of Evolutionary Physiology), A.A. Bogomolets (pathophysiology) and M.P. Konchalovsky (therapist) visited S. Chakhotin during this period and showed a keen interest in his new methods of research.

As a scientist Chakhotin worked very hard, speaking at numerous conferences on physiology, cytology, and oncology. He presented invited scientific reports to the Sorbonne (Paris) and the Faraday Society (London). His scientific successes were awarded prizes by the French Academy of Sciences (1936) and the Paris Academy of Medicine (1938).

During the period of 1930-1936, France was encumbered by the world economic crisis, which resulted not only in a decline in industrial production and a rise in unemployment, but also political instability. Right-wing parties and fascist organizations intensified their activity. The beginning of February (1934) was marked by riots in Paris. The leader of the fascist organization “Croix-de-Feu” de La Roque organized a march by the far right to the residence of the National Assembly Bourbon Palace,

and the 20,000th rally. The right-wing leaders openly demanded power. French Communist Party (Parti Communiste Français, PCF) and the French Section of the Workers' International (Section Française de l'Internationale Ouvrière, SFIO) have deduced on a 25,000th meeting.

Parisians in response to the actions of the right-wing. The confrontations and victims caused by these actions resulted in the resignation of the Daladier government.

In July 1934, both parties (PCF and SFIO) signed an agreement to unify their actions that created the prerequisites for the organization of the Popular Front (fr. Front populaire) as a coalition of political parties. The principal aim of the Popular Front was to protect the democratic forms of government in the state and to counter fascism which was a potential threat.

At this time, Chakhotin made contact with Jean Zyromski, the Secretary of the Commission for propaganda of SFIO and proposed to him a detailed plan for the modernization of socialist propaganda, rejecting the traditional French Socialist Strategy that focused entirely on achieving economic objectives. Chakhotin's plan was based on the scientific theory that he developed in Heidelberg. It envisaged a modern system of scientific labor organization in the field of agitation and propaganda, based on the ideas of Taylor, who proposed the modern methods of vocational training and production organization. Such a system would be able to display and analyze the "political weather map" ("Météorologie Politique") of the separate classes and regions, while decreasing total efforts, saving time and money.

Differences in position on foreign policy (for example, on the Spanish Civil War) led to a split within the Popular Front. The conservative position of the leadership of the Socialist Party of France disappointed Chakhotin.

At that time Chakhotin had been thinking about the relationship between the masses and political leaders, and trying to classify the instincts of crowds to explain how their support for dictators was conditioned by a specific historical period.

Investigation of the principles of mass psychology by sociologists and psychologists such as Gustave Lebon, William McDougall, Jean Gabriel Tarde, and Sigmund Freud and other observations were preceded by observations of Chakhotin. Lebon published his fundamental work "The Psychology of Peoples" (Fr. "Les Lois psychologiques de l'évolution des

peoples”) in 1894 and “The Crowd: A Study of the Popular Mind” (Fr. “La psychologie des foules”) in 1895, in which he gave a detailed analysis of the psychology of masses and considered the specific characteristics of the crowd. The people represent, according to the author, the human totality with its mental community and not a simple accumulation of people in a particular place. Lebon describes some of the characteristics of a crowd as: impulsivity; irritability; inability to make reasonable judgments; lack of common sense and a critical spirit; and excessive emotionality. An important provision of Lebon is the requirement for the crowd to have a leader who can affect the crowd by suggestion, using all possible means of propaganda.

The works of Lebon were subsequently used by politicians and scientists, among which were G. Plekhanov, J.E. Sorel, V. Lenin, G. Hanotaux, and S. Freud. Lebon’s work “The Psychology of Peoples” has become a kind of manual for leaders of totalitarian regimes such as Adolf Hitler and Benito Mussolini, who studied the methods of influencing a crowd.

Gustave Lebon (1841-1931) was a French psychologist, sociologist, anthropologist and historian. He had predicted the important role of crowds during this period of history and described the methods of influencing different types of crowds.



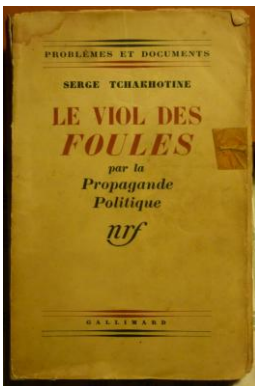
The psychologist William McDougall was the first who introduced the concept of “social psychology” in 1908.

Analyzing the psychology of a crowd, the French sociologist and social psychologist G. Tarde described the unconscious elemental crowd as clamorous and unable to control itself and is driven by dark destructive

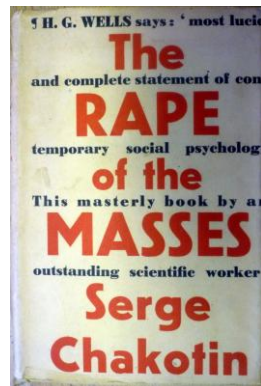
instincts. In contrast, the conscious public consists of intelligent social groups. Physical contact between people, their concentration in one place, and the unification for one action are requisites for the formation of a crowd. Tarde was engaged in the public psychology as a purely spiritual totality, as a group of individuals, physically separated and connected by a purely mental bond.

The famous psychoanalyst Sigmund Freud recognized that he relied heavily on the ideas of Lebon and McDougall. He noted that in a large mass of people or a crowd, some individuals immediately lose their moral principles, giving way to the most primitive and sordid mental state. Freud also emphasized the tendency of people to be divided into leaders versus followers.

Chakhotin appeared at the center of political, military and social developments in Europe, where he observed the emergence of totalitarian regimes. As a scientist, he was trying to understand the nature and mechanisms that influence the masses, turning them into a crowd with the help of propaganda. He put his observations, analysis and conclusions in the fundamental book “Le Viol des foules par la propagande politique”, that was published in Paris in 1939 and republished in English (“The Rape of the Masses: The Psychology of Totalitarian Political Propaganda”) in London and New York in 1940.

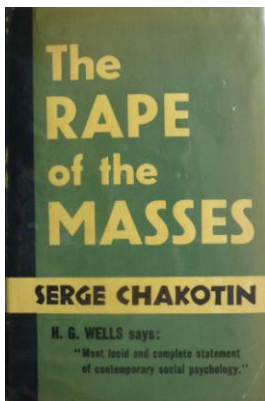


Serge Tchakhotine (syn. S. Chakhotin) *Le Viol des foules par la propagande politique*. Gallimard, Paris, 1939.

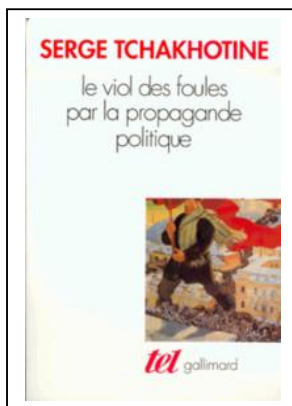


Serge Chakhotin. *The Rape of the Masses*. The Labour Book Service, London, 1940.

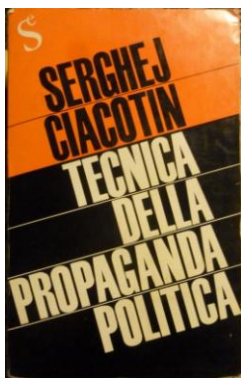
To Chakhotin the purpose of the book was to increase our understanding of the mechanism of psychic oppression that was utilized by the leaders of the masses and at the same time, providing an effective weapon in the hands of those who are ready to make any sacrifice to liberate humanity.



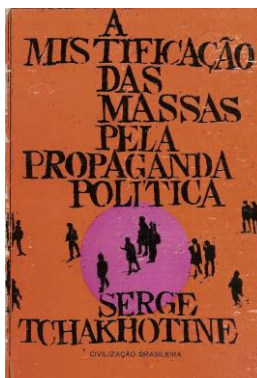
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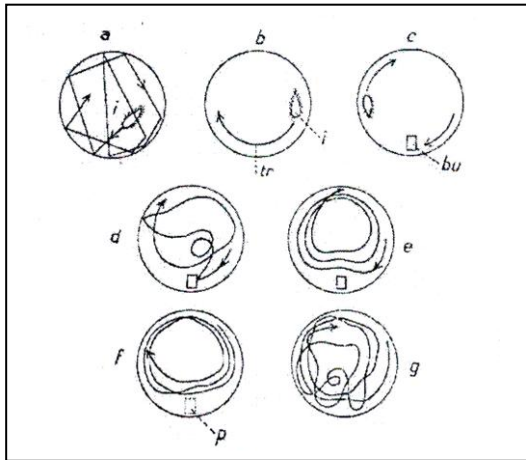
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The ideas presented in Chakhotin's books were based on the teachings of Pavlov who established the theory of conditioned reflexes as the response of an organism to external influences. Pavlov separated reflexes into unconditioned congenital reflexes and conditioned acquired reflexes. Conditioned reflexes occur under certain conditions and disappear in the absence of those conditions. For example if you allow a dog to sniff a piece of meat, it produces saliva which indicates the appearance of an unconditioned reflex. If the meat is accompanied by the ringing of an electric bell at the same time, the dog will associate ringing of the bell with meat and salivation will begin even if you do not offer the meat, indicating the appearance of a conditioned reflex.

Chakhotin began his observations of conditioned reflexes with protozoa. He studied the behavior of paramecium (Lat. *Paramecium*) which is a ciliate protozoan, whose body is covered with cilia.



Demonstration of a conditioned reflex in a Paramecium cell: *i* – infusoria; *tr* – the trajectory of the cells; *bu* – micro-barrier that is generated by ultraviolet radiation; *p* – the place corresponding to the previous exposure; *ab* – the cell floats at the periphery of the drop; *c* – the cell faced with space radiation; *d* – the cell feels a shock and changes the trajectory; *e* – the cell “recognizes” the place of exposure and goes around it (a conditioned reflex is created); *f* – micro-barrier is removed, but the cell continues to bend around the dangerous place; *g* – the cell gradually enters the danger zone, indicating a loss of memory (conditioned reflex disappears) [Tchakhotine, 1939; syn. Chakhotin]

Cilia beating allows the cell to move in the aquatic environment. In the absence of external stimuli, the ciliates move along a closed path within a drop of medium in the field of view of the microscope. Chakhotin used a beam of ultraviolet radiation focused through a microscope as the external stimulus. The cell exhibited a shock reaction (i.e., a photophobic reaction [Posudin et al., 2010]) due to its interaction with the ultraviolet radiation. This reaction results in a sharp change the cell's direction of movement in response to the stimulus. When you remove the stimulus, the cell continues to avoid the location that was previously irradiated due to the ciliate having developed a conditioned reflex. With the disappearance of the conditioned reflex, after an interval of time the infusoria renews its motion along a closed path.

Similarly certain external signals or stimuli are able to induce a response in large masses of people (a crowd) led by leaders. This is how the slogans and symbols of totalitarian regimes act on people. Well-chosen marches, hymns and songs, parades, rituals, easily understandable symbolism, gestures, and uniforms, all of which act as an external stimulus for a crowd. Such forms of propaganda generate reflexes and, as a consequence, the subordination of the crowd to its leaders. A special role in the management of the crowd is played by a leader who is seen as a man of action and is endowed with a strong will.

In his book, Chakhotin analyzed the reasons for the success of Hitler as a speaker. It should be noted that the rhythm of a speech before an audience – starts quietly, using a strictly calculated rhythm with a pace of 45-72 words per minute, climaxes with an explosion of anger, rage or hysterics that is accompanied with gesticulation – all this leads the audience into a state of ecstasy.

Chakhotin notes the special role of political propaganda, pointing out that it was important not **what** Hitler (or to be precise, his assistant, Propaganda Minister Dr. Goebbels) said and did, but **how** it was said – here is the clue [Chakhotin, Interview. 1933].

Based on Pavlov's research on conditioned reflexes, Chakhotin showed that all forms of life are struggling to survive with the help of four instincts that should be considered as complex unconditioned reflexes which interact with the environment. Among these instincts, numbered in

descending order of biological activity, are⁸: 1) struggle (combative); 2) nutrition (alimentaire); 3) sexuality (sexuelle); 4) maternity (parentale). The struggle instinct is the most important because every living creature is struggling for survival, against the threat of death.

Chakhotin concluded that the theory of conditioned reflexes and instincts, is the basis of objective psychology and is based on general biological laws. The theory can explain all complex forms of human behavior, including current phenomena of social life and political activity. Thus, the main periods of human civilization are based on certain instincts: Christianity – on the maternal instinct (“love thy neighbour”); capitalist society – on the instinct of nutrition (economic objectives); socialist society – on the instinct of struggle (opposition to the political systems).

Chakhotin warned of the possible deployment of the final struggle between two political systems, each of which is based on instincts of struggle. One of them is fascism which is a totalitarian, pseudo-socialist system; another is a socialist system which occupies the position of democracy.

Chakhotin also based his views of political propaganda on the concept of instincts. He distinguishes two forms of propaganda. The first (ratio-propaganda) uses persuasion, argumentation; the second (senso-propaganda) implements an appeal to feelings, enthusiasm, ecstasy. The first involves ordinary political instructions that are covered by the media at meetings and in the process of discussions. We can assume that since this type of propaganda reflects economic interests, it is based on the instinct of nutrition. The second type of propaganda uses symbols, flags, banners, uniforms, demonstrations, and noisy gatherings and thus it uses basically the instinct of struggle.

Strictly speaking the presence of a hierarchy among the instincts explains the greater efficiency of Nazi propaganda, that used the more powerful instincts of fear and aggression compared with the social-democratic propaganda that appealed to the civilized and humane themes of peace and harmony [Tchakhotine (syn. Chakhotin), 1939].

⁸ The translations of terms in French are given in parentheses in accordance with the edition of 1939.

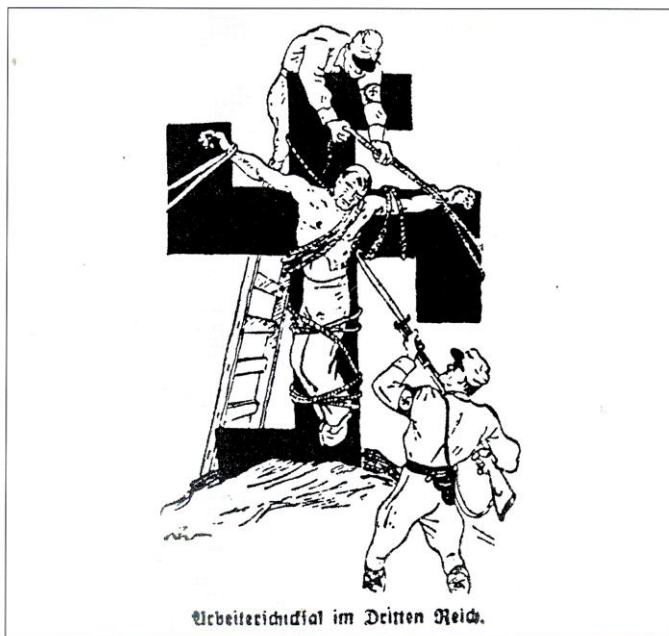


Illustration from Chakhotin's book *"The Rape of the Masses. The Psychology of Totalitarian Political Propaganda"*.

Chakhotin concluded that the ideas of freedom, peace, and clemency should become an integral part of our nature and reflexes that are fixed deeply in every human being. Achievement of this goal is possible in accordance with Pavlov's teaching through a reasonable formation of corresponding conditioned reflexes, propaganda, and especially education.

The Nazis that occupied Paris could not ignore the political activity of Chakhotin. He was arrested and imprisoned in a concentration camp at Compiègne as a Soviet citizen, where he had spent seven months in 1941. Only the intervention of German scientists who knew Chakhotin allowed him to acquire freedom.

After the liberation of Paris in 1944, Chakhotin lectured at the courses "Soviet Patriot" for immigrants. In addition, he organized a scientific and

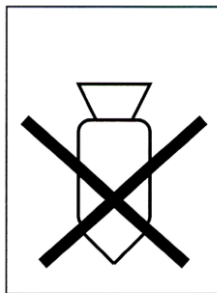
political society “Science-Action- Liberation”, presided over the French Confederation of Cultural Forces (COFORCES). The main task of these progressive movements was to spread correct, unbiased information about the Soviet Union and the fight against the threat of a third world war and resurgent fascism.

In 1939, Albert Einstein with the help of the Hungarian physicist Leo Szilard warned U.S. President Roosevelt that Nazi Germany was conducting active research in the field of nuclear physics, which could lead to the creation of an atomic bomb. In 1945, L. Szilard and A. Einstein turned again to Roosevelt to try and stop the production of the atomic bomb in the United States.

Chakhotin organized an emotive (causing strong emotions for or against something) propaganda campaign against nuclear explosions and missiles [Chakhotin. Memorandum]. With inherent enthusiasm and thoroughness he proposed outdoor pictorial posters (for example, the atomic bomb with a large inscription “NO!”) or small leaflets with short slogans.

Special attention is paid to graphic symbols, directed against the nuclear danger, to which Chakhotin required the following: the symbol must be simple, eye-catching, clear at first sight, easily recognizable and reproducible, reveals the principle of force and creates a sense of fear, struggle and, if necessary, even aggression.

The symbol to the right meets these requirements. Chakhotin used a schematized image of the atomic bomb, that is dissected by two crossed lines indicating NO!



During his work at the Institute of Physical and Chemical Biology, Chakhotin was actively involved in the organization of the First International Congress for Peace in 1949 (Paris). The idea formed the basis of the work of organizations such as SAL (“Science-Action-Liberation” – a scientific and political society organized by S. Chakhotin in 1944) and COFORCES (French Confederation of Cultural Forces). Chakhotin was the General Secretary of these organizations whose primary purpose was to fight against the threat of a third world war.

In 1955 Chakhotin moved to Italy, first to Genoa (Pharmacological Institute of the University), and then to Rome (Pharmacological Institute,

at the Higher Institute of Health and the Institute of General Physiology) where he worked until 1958.

A characteristic feature of Russian immigrants was that they were always against the current political regime in their home country, but always passionately loved their homeland and dreamed to go back there.

Chahotin expressed his desire to return to the USSR with his family for the first time in 1946 and again in 1952. Since there was no response from the Soviet authorities, he once again appealed to the Soviet Embassy in France in 1954 and received a positive response.

However, when the Soviet Foreign Ministry appealed to the Chief Scientific Secretary of the Academy of Sciences of the USSR (A.V. Topchiev) asking about using S. Chakhotin in the institutions of the Academy of Sciences, the scientist was refused, citing the lack of research in the field of experimental cytology. It was a bureaucratic formal reply.

The fact is that the leadership of the Department of Biological Sciences was under the influence of obscurantist investigations of Olga Lepeshinskaya, a protégé of Trofim Lysenko, who rejected genetics and defended the theory of spontaneous generation of life from inanimate matter. Of course in this situation the academic leadership refused Chakhotin, so it was not until 1958 that he able to return home.

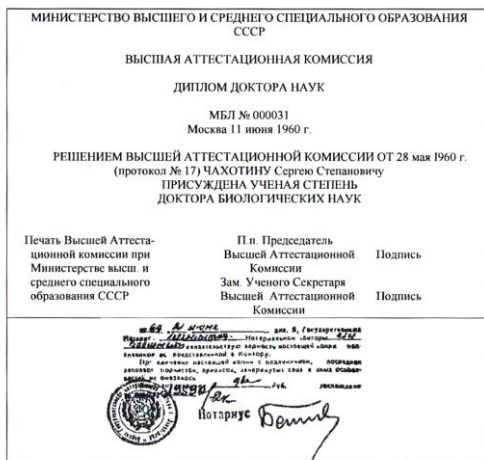
In front of me a copy of the order № 49 at the Institute of Cytology, Academy of Sciences of the USSR (Leningrad) on April 18, 1958: “Chakhotin Sergei Stepanovich is credited with a senior researcher position in the Laboratory of Cytological Bases of Reproduction and Development from 18 April of this year. Justification: Order of the Presidium of the USSR № 2-2865 on 24 December 1957” (Acting Director Professor Yu.I. Polyansky). The order of the Presidium of the USSR was signed by the chief scientific secretary, academician A.V. Topchiev.



**Institute of Cytology
Russian Academy of Science**

At the same time there was a question about the possibility of awarding Chakhotin with the degree of Doctor of Biological Sciences based on his scientific work (without defending a dissertation). Three reviewers (S.

Romanov, I. Sokolov and B. Paribok) gave a brilliant characterization of the scientist. Chahotin was awarded the degree of Doctor of Biological Sciences in accordance with the decision of Higher Attestation Commission dated May 28, 1960.



The decision of the Higher Attestation Commission of the USSR on awarding S. Chakhotin the degree of Doctor of Biological Sciences

Chakhotin continued to work in the field of ultraviolet irradiation of individual cells. He delivered reports at the Institute of Cytology, Institute of Microbiology, Institute of Cytology and Zoology (Leningrad), Morphology of Animals (Moscow), Moscow State University, Institute of Experimental Pathology and Therapy (Sukhumi), All-Union Congress of Anatomy, Histology, and Embryology in Kiev (1958), the Congress of Physiologists in Minsk (1959), and the Society of Physiologists in Leningrad (1959). Chakhotin demonstrated his methods and equipment at the International Symposium on Radiobiology (Moscow, 1960).

Chahotin was transferred to the Institute of Biophysics, Academy of Sciences to Moscow, according to the order of the Institute of Cytology of the USSR Academy on October 29, 1960, the where he worked until 1967.

On September 16, 1967, Chahotin was assigned to the Institute of Developmental Biology, Academy of Sciences of the USSR, where he worked as a researcher and then as scientific consultant from July 1, 1970 until his death on December 24, 1973.

RELATIVES⁹

Father: Stepan Ivanovich (1857-1920), a diplomat. Mother: Alexandrina Nikolaevna (eq. Umissa-Motzo von Gogenburg (1861-1942)).

Brothers, Ivan (1884, Constantinople¹⁰-1938, Odessa), Nicholai (1885, Constantinople-1972, Paris); Stepan (1888, Constantinople-1931, Odessa), artist and poet.

Wives: in the first marriage – Emma Vilgelmovna (eq. Haas, 1881-1942), sons: Sergei (1906-1976), Vladimir (1909-1943), Igor (1911-1993).

In the second marriage – Seraphima Nikitichna (eq. Omelaeva, 1885-1978), sons: Benjamin (1917-2014), Eugene (b. 1922).

In the third marriage Anna Markovna (eq. Svenchanskaya, 1907-1984), sons Andrew (b. 1939), Peter (b. 1943), an artist.

A brother of Chakhotin's, Stepan Stepanovich Chakhotin became the first victim in the family of repression; he was shot in 1931. His nephew, Peter Chakhotin, writes in his book "Stepan Chakhotin. Life in Pictures and Letters to his Mother": "*The life of a talented, intelligent, warm hearted, sympathetic person was forcibly cut short, as well as many other innocent citizens of our country*" [P. Chakhotin, 2015].

Irina Barancheeva reports in her work "Having emerged from beneath the ruins of Messina" that Vladimir died in the Vorkuta camp in 1943. Igor was arrested in 1941 and spent fifteen years in the Siberian prison camps. Sergei (a son of S. Chakhotin) fought during the sieged of Lenin-grad and he narrowly escaped the arrest by leaving as a volunteer in Siberia in 1948. Their mother, Emma Haas, a German by birth, was arrested and executed in 1942 in Novosibirsk.

"This was the fate of hundreds of thousands, maybe millions of Russian families destroyed by those who had usurped power in 1917".

⁹ This information was kindly provided by Pierre Tchakhotine.

¹⁰ The name Constantinople was officially changed to Istanbul on March 28, 1930.

In July 2005, according to the testament, the ashes of Sergei Stepanovich Chakhotin remains were dispelled by Eugene Chakhotin over a cliff into the sea near Monte Fumaiolo and the village of Cargèse that is located on the west coast of Corsica.



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II. MILESTONES OF LIFE PATH OF SERGEI CHAKHOTIN ¹¹



Chakhotin Brothers (left to right): Ivan, Stepan, Nikolai, and Sergei, in Constantinople (syn. Istanbul), 1891.



Sergei Chakhotin in Constantinople, 1891.



S. Chakhotin in the 3-d Odessa High School in 1893.



S. Chakhotin in Heidelberg in 1907.

¹¹ Photos from the archive of the family Chakhotin were kindly provided by Petr Sergeevich Chakhotin (Pierre Tchakhotine)



With his wife Emma Haas and his son Sergei in Messina, 1907.



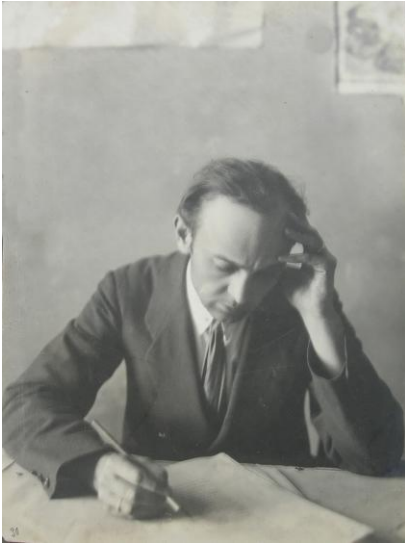
S. Chakhotin's wife Emma Haas with children - Sergei (on the left) and Volodya in Odessa, 1911.



S. Chakhotin in Berlin before the First World War in 1913.



S. Chakhotin at the sanatorium after the earth-quake. Schwarzwald, 1909.



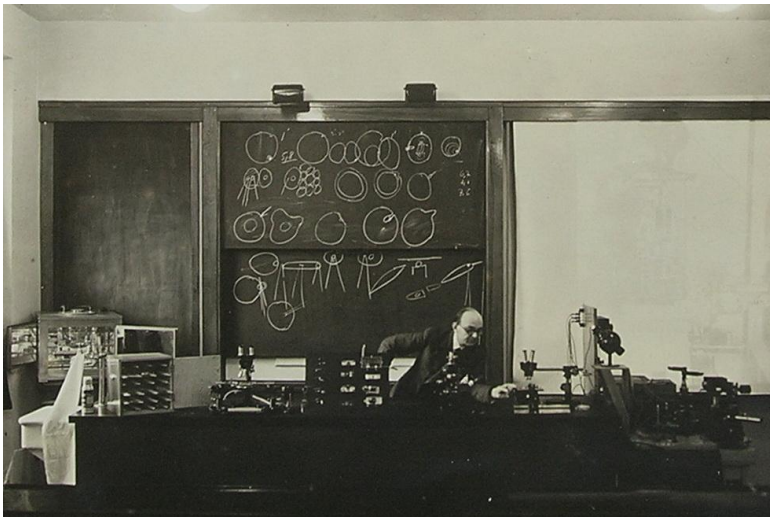
S. Chakhotin while working in OSVAG. 1918-1919.



Chakhotin reorganizing the Soviet trade mission in Berlin, 1922-1924.



S. Chakhotin (left) with Prof. A. Benedicenti (center) in Tuscany, Italy, 1926-1927.



S. Chakhotin demonstrating experiments in Heidelberg, 1929.



**S. Chakhotin in the laboratory of the Max Planck Institute
in Germany, 1931.**



**Sergey Chakhotin in
Heidelberg, 1931.**



**S. Chakhotin gathering sea urchins at the
Wimereux Biological Station in 1937.**



S. Chakhotin in the Nazi Royallieu-Compiègne concentration camp in 1941



S. Chakhotin with Prof. A. Benedicenti in Tuscany, Italy, 1955.



S. Chakhotin gathering sea cucumbers in Corsica, 1954.



S. Chakhotin with Prof. Alberico Benedicenti (on the left) in Genoa, 1954.



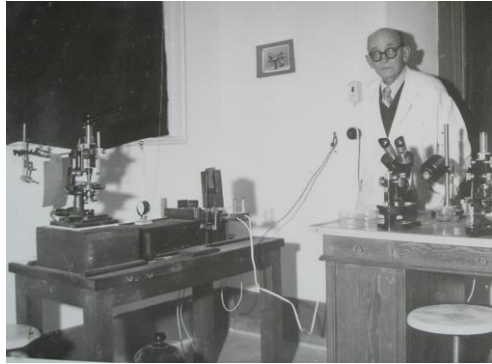
S. Chakhotin (right) with Prof. A. Benedicenti (center) in Tuscany, Italy, 1955.



S. Chakhotin behind the easel on the estate of Prof. Claudio Necci near Rome in 1957.



S. Chakhotin with Prof. Claudio Necci in 1957.



S. Chakhotin in the Laboratory of Prof. Pietro Di Mattei at the Pharmacological Institute in Rome, 1957.



S. Chakhotin working with card index in the Laboratory of Prof. Pietro Di Mattei at the Pharmacological Institute in Rome, 1957.



S. Chakhotin in the Laboratory of Prof. Pietro Di Mattei at the Pharmacological Institute in Rome, 1957.



S. Chakhotin visiting Prof. Claudio Necci with his son Peter in 1957.



S. Chakhotin after returning to Russia on the May Day demonstration in Leningrad, 1958.



Sergei Chakhotin with his son Peter in Moscow, 1961.



S. Chakhotin demonstrating the experimental setup for micro-irradiation in Moscow, 1960.



S. Chakhotin in Minsk, 1960.



Photo of S. Chakhotin in Moscow, 1961.



S. Chakhotin in Moscow, 1964.



S. Chakhotin in Moscow, 1966.



S. Chakhotin in Moscow, 1968.



**Sergey Chakhotin in the
seventies.**

III. RESEARCH ACTIVITY

Any scientific study begins with the formulation of the problem, selection and substantiation of methods for its solution. In this regard, it should be noted that the beginning of S. Chakhotin's scientific activity coincided with the development of idea that a cell represents an elementary biological unit within which basic vital functions are performed. The influence of such innovators as W. Rentgen, O. Hertvig, T. Engelmann and I. Pavlov, who used original experimental techniques in their research, helped to shape the scientific perspective of Chakhotin.

Introducing Chakhotin at the beginning of his assignment for the degree of Doctor of Biological Sciences, one of the reviewers (Dr. S. Romanov) wrote: "*The basic postulates that a cell is the elementary morphological unit of living matter and that the laws of physics and chemistry are the basis of vital activity of a living cell, became the methodological basis of scientific thinking of the majority of biologists. This strictly materialistic methodology was the basis of all his subsequent work. The problem that he has chosen for the study, can be formulated as 'Physical and Chemical Bases of Cell Behaviour'.*" The choice of this area of research indicates Chakhotin's erudition and deep understanding of the problems of modern biology.

His early research was on bioelectric currents in vertebrates. The author not only considers the nature of these currents, but also raises the question of the communication of bioelectric phenomena with the morphological structure of the tissue.

He also paid special attention to the problem of therapy for malignant tumors and established the connection between the cancer susceptibility of mice and blood leukocyte chemistry. Working in Heidelberg (1912) and Genoa (1928-1930), he published a paper on the role of leukocytosis cancer in laboratory animals caused by carcinogens. Violation of the glycolytic process in the developing eggs of the sea urchin allowed Chakhotin to initiate a phenomena similar to malignancy in tissues. In 1939, Chakhotin was give a scientific award by the Paris Academy of Medicine for his work entitled "The cell microscope and the problem of cancer".

Over his career, his primary area of scientific activity was the development of methods and tools for micromanipulation of individual cells, as

well as micro-irradiation of cells and their organelles using focused ultraviolet radiation.

While critiquing the necessity of development of experimental cytology, Chakhotin indicated a number of researchers that at that time had tried to examine individual cells (Balbiani E.G., 1888; Bataillon E., 1910; Chabry L., 1887; Maupas E., 1888; Roux W., 1895; Verworn M., 1889, etc.). However, these researchers used rather large objects such as eggs of frogs and other amphibians as their test models. In some cases, their approach was based on the study of regularities inherent in a homogeneous mass of cells with subsequent extrapolation of the results to individual cells.

Differential centrifugation, which is accompanied with the destruction of the cells and the distribution of content in accordance with a specific weight fractions, was beginning to be used at that time. This made it possible to study the properties of individual organelles – nuclei, mitochondria, microsomes, etc.

Beginning in 1912, Chakhotin developed a series of micromanipulators, microoperators and other devices to provide mechanical approaches and operations on individual cells under a microscope. He took into account the need to move, fix, immobilize, recover, and place them in different vessels.

The following critiques the main aspects of Chakhotin's contributions to microtechnology.

1. MANIPULATION TECHNIQUE

Allocation and Transfer of Cells

If necessary, the isolation of motile cells (algae, ciliates) can be accomplished using a long capillary with a funnel-shaped end. The end is positioned near the object which is captured using a pipette.

Another method involves the use of a glass mouthpiece the researcher holds in their mouth that is connected by means a rubber tube to a capillary-shaped micropipette. When working with a stationary object (such as a small egg) this precise microcapillary pipette method is used (Chakhotin named the apparatus a “mipetka”). The manufacture of such a pipette is shown in Fig. 1.

A glass tube about 15 cm long and 5 mm in diameter is stretched over the fire so as to form a capillary with a length of 20 cm and a diame-

ter from 0.5 to 0.7 mm (*a*). The capillary is cut and the cut end is melted to form a ball (*b*).

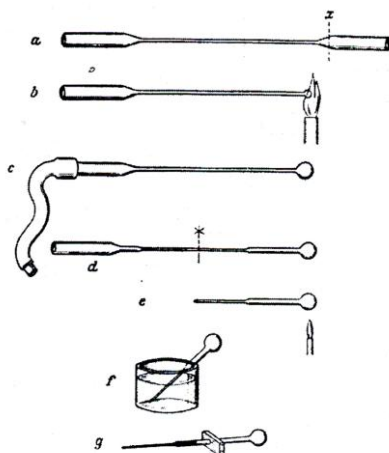


Fig. 1. **Manufacture of a capillary pipette with an air bubble** (explanation in the text).

The ball is then blown until a bubble is formed and a rubber hose is fitted on the tube (*c*). The capillary is stretched over the fire and then cut to an appropriate length (*d*). The bubble is then heated (*e*) and the air therein expands immediately after which the opening of the capillary is immersed in the prepared liquid and as the air contracts with cooling, it pulls the fluid into the capillary (*f*). A piece of cork is positioned on the pipette for insulation (*g*) which allows the apparatus to be held by the fingers.

An operation with a pipette involves holding the piece of cork between the thumb and middle finger (Fig. 2*a*) and by touching the glass bulb with the inner surfaces of the fingers, causing some of the liquid will come out of the capillary tube due to the heat transferred resulting in the expansion of the trapped air. The open end of the pipette is then immersed in a liquid containing the test objects and the capillary end positioned adjacent to the chosen object under the microscope. The fingers are removed from the bulb (Fig. 2*b*) and as the air in the bulb cools, the liquid is sucked in together with the object of interest. The pipette is transferred

into another vessel and by warming the bulb the test object is expelled. Instead of heating using one's fingers, Chakhotin suggests the possibility of using electric heating with a wire coil wound around the bulb.

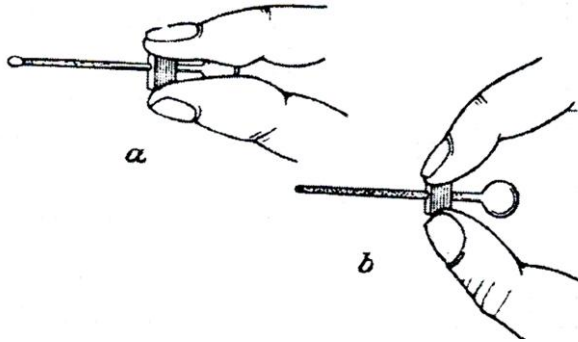


Fig. 2. **Transferring small, single celled organisms using the micropipette:** *a*) when expelling the object(s); *b*) when capturing an object for transfer.

Compression and Immobilization

Some researchers use a dense media for immobilization of microobjects whereby the velocity of the organism is significantly slowed or remains motionless for some time in contact with a solid object (so-called “sigmotaxis”).

Chakhotin proposed immobilization by using a small microcompression instrument. The microcompressor (Fig.3*a*) consists of a brass plate *D* 1 mm in thickness with a hole in the middle where a small piece of glass or quartz *S* is glued. The plate has a metal pin on each side (Fig. 3*b*). A second brass plate *D* has a larger diameter opening that is closed by a cover glass *dg*. The plate is equipped with soft gaskets *f* to prevent the organism from being crushed. Two small holes in the plate correspond to the pins of the top plate (Fig. 3*c*). A drop of water containing the organism is placed in the well using a micropipette and the upper and lower plates are connected.

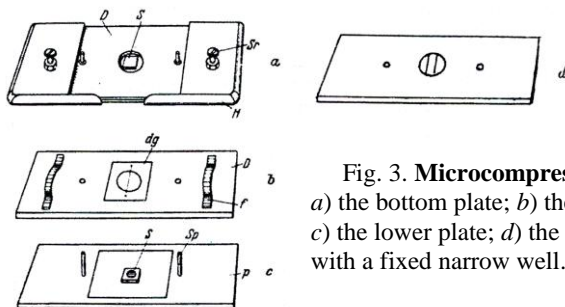


Fig. 3. **Microcompressor design:**
 a) the bottom plate; b) the upper plate;
 c) the lower plate; d) the upper plate
 with a fixed narrow well.

If a small narrow plate instead of the cover glass (Fig. 3d), is used, insertion and extraction of the cell is significantly simplified. The procedure involves the introduction of a drop of water into the device using a pipette, the end of which is positioned between edges of the holes and narrow plate.

Similarly, a microorganism *o* that is located under the plate *dg* can be introduced and the excess water is removed. This version allows the study of the combined effect of mechanical, light, heat and chemical factors on the microorganism(s). Placing on the plate a hair or thin thread makes it possible to cut the cells. Having a cell at the edge of the plate, it is possible to perform various micromanipulations such as injections, electrical irritation and so on.

The whole assembly is placed on the microscope stage (Fig. 4) and the plates are clamped together using small bolts *Sr*. The microorganism is then gently compressed and immobilized while remaining intact.

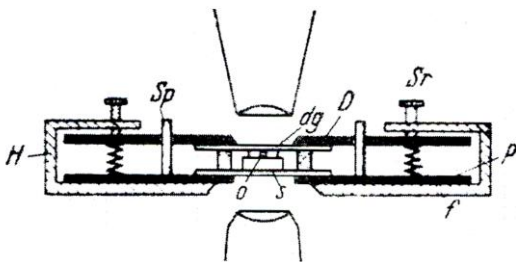


Fig. 4. **Use of a microcompressor on the microscope stage** (explanation in the text).

Operating Chamber

While operating on immobile microobjects (eggs, amoeba) it is not always necessary to press the objects being studied. It is possible that a chamber of a rather simple construction can be used as illustrated in Fig. 5.

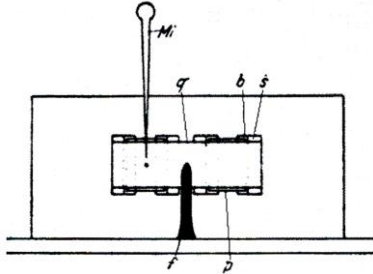


Fig. 5. **Operating channel chamber** (explanation in the text).

The chamber is a microscope slide on which there are four wide plates *b*, four narrow plates *s* and two plates *p* that create empty rectangular cavities or chambers. A long plate *q*, which is pressed by a spring *f* that is located above both chambers. The chambers are filled with a pipette, one with a solution of fluorescein, and the other with a liquid in which the object is adjudged. The object is introduced using a micropipette, the free end of which is supplied with a coverslip on the operating chamber. The cell is introduced into the chamber from the micropipette using a hand to warm the glass bubble (Fig. 6).

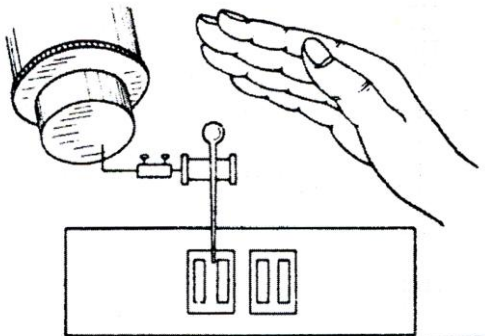


Fig. 6. **Introduction of a cell into the channel with the aid of a micropipette.**

Removal of the cell from the chamber is realised in reverse order after the cell has been manipulated. The design of the manipulator with a micropipette is shown in Fig. 7.

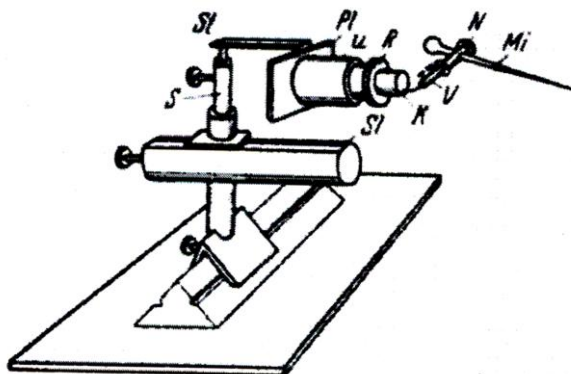


Fig. 7. **Micromanipulator with a micropipette** (explanation in the text).

A holder is moved on a trihedral base which has perpendicularly oriented sleds *Sl* with a column *S* and barbell *St*. A plate *Pl* with a sleeve *u* and pipe *R* are fixed on the barbell. The pipe can be extended and rotated about its axis. Stopper *K* of the manipulator, consists of a clamp *V*, a needle with glass head *N* and a pipette *Mi* which is inserted into the socket. This system allows the pipette to move in virtually any direction relative to the operating chamber.

One variation of the chamber involves a ring that is cut from paper with an outer diameter of 6 mm and inner diameter of 4 mm. Ring *r* is immersed in molten paraffin, then removed and allowed to cool after which it is placed on a slightly heated glass slide (Fig. 8).

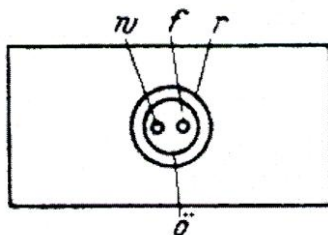


Fig. 8. **Operating chamber** (explanation in the text).

Thus the ring is attached to the glass and a drop of paraffin oil \ddot{o} is inserted inside the ring. The hole in the layer of paraffin oil is formed using a micropipette with a rubber tube and a drop of water w is placed on the bottom of the recess. The cell is transferred into the droplet and a drop of fluorescein f is introduced with the purpose of orientation using a second pipette. The advantage of the method is that the preparation is not necessary to close the cover slip, because the layer of paraffin oil protects the object from drying out while it is being observed.

Puncturing of Microobjects

The design of a microoperator that is intended for surgical operations on individual cells is shown in Fig. 9. The initial introduction of the tool is performed using a gear train and then the tool holder ih is moved nearer to the object by turning the head d and the clamping ring Kr allowing the surgery to be conducted.

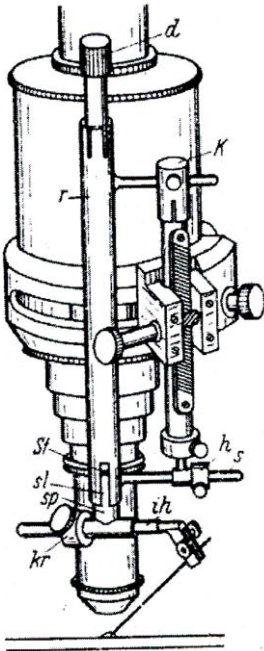


Fig. 9. The design of a microoperator that is intended for surgical operations on individual cells (explanation in the text).

Postoperative Chambers

Chakhotin proposed using postoperative chambers composed of 8-10 rings r of waxed paper glued to a glass slide (Fig. 10a). Paraffin oil p is placed on the bottom of each chamber using a capillary tube. A drop of liquid f with the cell under investigation is then placed on paraffin oil. If the droplet is too large and it protrudes from the surface of the paraffin oil, its size is reduced by multiple touch with a thin capillary K (Fig. 10b).

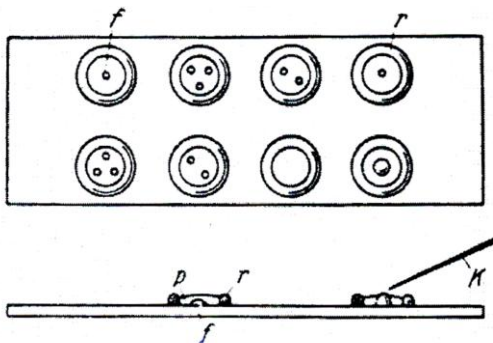


Fig. 10. Camera with waxed rings: *a*) top view; *b*) side view (explanation in the text).

Another type of postoperative chamber is shown in Fig. 11.

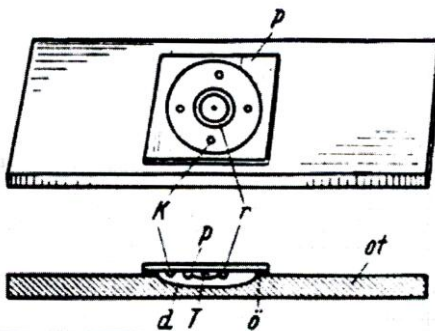


Fig. 11. Chamber with a suspended drop: *a*) top view; *b*) side view (explanation in the text).

Wax ring *r* is glued to a glass cover *p* and a drop of water *T* containing the cell under investigation is placed in the recess. The cover slip is then inverted and mounted with its recess facing down onto a glass slide *ot* with a spherical recess *d* that is glued by means a paraffin *g* to prevent evaporation of the drop. Additional drops *K* of solution can be deposited around the drop under investigation to help maintain the appropriate relative humidity.

In some cases, a series of thin capillaries containing the cells can be placed in a chamber (Fig. 12).

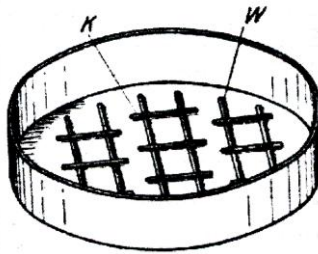


Fig. 12. **Capillaries in a postoperative chamber:** *K* – capillaries; *W* – paraffin barriers.

Another postoperative structure supplies the cell(s) with continuous aeration or fresh medium (Fig. 13). Two Petri dishes are inserted one into the another.

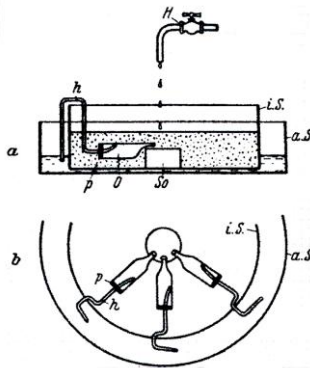


Fig. 13. **Postoperative chamber with a continual introduction of water or solution** (explanation in the text).

Water is in the outer cup aS and the base So with radially tapered glass tubes o is located in the middle of the inner cup iS . The ends of these tubes are closed with plugs p . The difference between the liquid levels in the inner and outer plates is created by the capillaries h and the inflow H of the liquid. The test object is introduced into the pointed end of the tube due to the pressure difference and pulled to the bottom of the tube where it can be observed. The medium is continuously renewed at the same time.

Microfixator

This device is a combination of a chamber with an upside down recess and a micropipette that are used to quickly fix of a cell. The design of the microfixator is shown in Fig. 14. Two brass plates R (4×8 cm and 1 mm thick) are arranged one above the other. The bottom plate has a 20 mm diameter hole; the upper plate has a hole 10 mm in diameter with a slot Sl width of 4 mm, which extends to the edge of the plate.

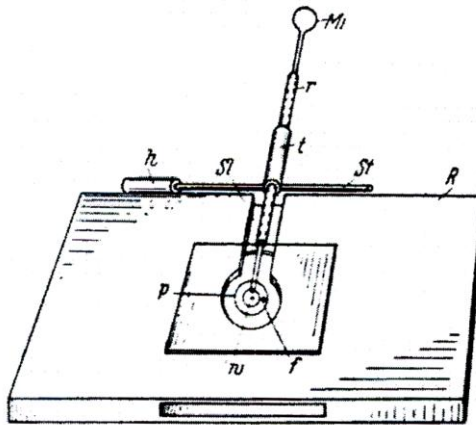


Fig.14. Microchamber with gas circulation (explanation in the text)

A sleeve h with a pin St supports a spring tube t that comprises a thin tube r that can be moved perpendicularly to the pin. A micropipette Mi is also located here and a cover glass is glued to the upper plate. A paraffin oil droplet p is applied to the upper plate of the cover glass and a water drop w is applied to the treated cell. The apparatus is turned over for this purpose. The pipette can include any reagent such as a dye, poison, medium,

electrolyte, etc. A Fluorescein drop f is introduced into the paraffin oil for calibration purposes.

Microchamber with Gas Circulation

This device is intended for the study of cell reactions to external physical factors under different gas compositions for studying the effect of the gaseous medium. The design of the device (Fig. 15) consists of a 1 mm thick brass plate p with a 15 mm in diameter hole and equipped with two slats l that are 5 mm in height. The dimensions of unit b are $30 \times 25 \times 5$ mm with a 10 mm diameter hole located at the edge of the plate. Tube r with hoses g are connected to this opening with the tubes passing through grooves e in the slats l .

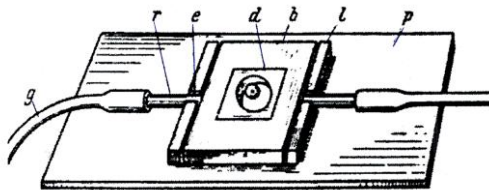


Fig. 15. Microchamber with gas circulation (explanation in the text).

A coverslip d is attached to the upper surface of block by the paraffin. The block is turned over and a drop of paraffin oil that contains a drop of water with the object under investigation is attached to the other surface of the glass cover slip. Then the block is turned over again and the hole closed using a quartz plate. Gas (illuminating or, if necessary to change the pH, ammonia or hydrogen chloride) is supplied through the hoses into the device.

Microperfusor

This device allows controlling the flow of fluids around the object under study in order to investigate the simultaneous effects of physical and chemical factors. The device (Fig. 16) consists of four parts ($a-d$). The lowest plate (Fig. 16a) is made of brass with dimensions of $45 \times 90 \times 2$ mm.

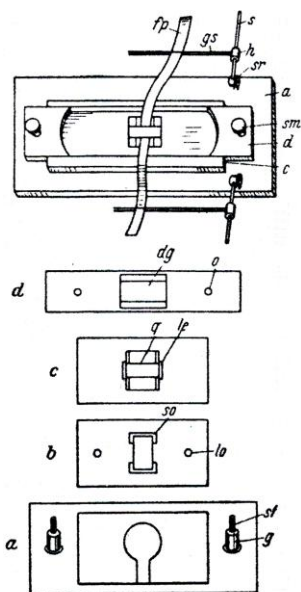


Fig. 16. **Compression microperfusor** (explanation in the text).

A quadrangular recess with dimensions of $27 \times 50 \times 1$ mm with a hole of 15 mm in diameter is attached inside. Two threaded bolts St with rubber tubes g are located on the right and left. The plate is equipped with hinges Sr , pins S , a sleeve h and rods gs . Strips of filter paper fp are used to transport the liquid; the strips are located between rods gs .

The second part of the device consists of a $27 \times 50 \times 1$ mm plate (Fig. 16b) that enters into the dimple in the first plate. A rectangular neckline 5×7 mm is located in the centre of the second plate. Two glass socles that act as supports are glued by the perimeter. The plate has two holes lo , that aline with the pins of the first plate. The third plate (Fig. 16c) has dimensions of $27 \times 50 \times 1$ mm and is equipped with a rectangular longitudinal neckline 7×13 mm at the centre. The strips le with a quartz plate q (5×10 mm) are located along the long edges of the slit. The fourth structural member (Fig. 16d) is a $20 \times 80 \times 1$ mm plate equipped with bars 1 mm high along the rectangular cut-out (14×13 mm) which is covered with a cover glass dg .

Liquid with the object under study is placed on the quartz plate of the third element of the design (Fig. 16c). The four elements are stacked and fixed in position with nuts sm . Precautions must be taken to not crush the object. The flow of fluid around the object is achieved by feeding drops of

water or a perfusion fluid into one end of the filter paper strips and the use of dried strips on the other side.

Microtransplantator

This device is designed for transplantation of cells, fragments of eggs etc. It consists of an aluminium object holder. A quartz plate *q* is fixed in the middle of the device with staples *K* (Fig. 17a). Three glass plates are glued to the initial plate. One plate (*a*) forms a channel with the movable plate *d*; the two other plates serve for guiding the movement of plate *d*. Another glass plate is used to close the channel. The plate moves using a pin *s* and lever *l* on the hinge *Sr*.

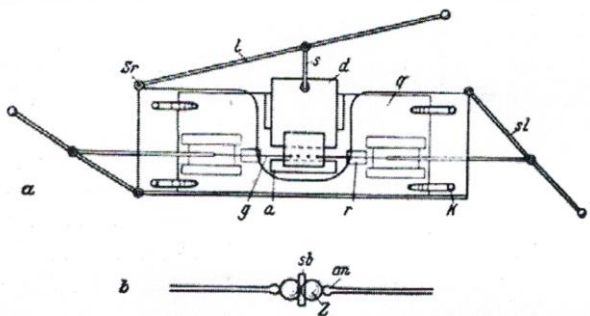


Fig. 17. **A device for cell transplantation:** *a* – general side view; *b* – two eggs pressed in the channel before micro-irradiation (explanation in the text)

The system is equipped with glass tubes *r* which have thin glass fibers *g* with spherical thickenings at the end (Fig. 17b). The other ends of the glass fibers are mechanically connected to levers *sl* making it possible to press together two eggs *Z* by moving the glass filaments. The contact *sb* is irradiated with ultraviolet light, causing the cells to be glued together.

Another version of the microtransplantator is shown in Fig. 18. A brass plate *p* (25×75×3 mm) is placed on a brass plate *P* (60×75×1 mm) with a hole *lc* so that both plates were tightly pressed to each other.

(a) The upper plate comprises an aperture with a diameter of 10 mm. A quartz plate *q* with a thin channel is placed on the hole. Short glass tubes *r* on the sides of the channel serve as guides for the pins *g*. In addition, the block *b* with a screw *sr*, pin *f*, hook *h* and rubber ring *gr* are in-

stalled on the upper plate. Guides *s*, plate *bl* with cover glass *d* acting as the cover of the channel, are mounted on the base plate.

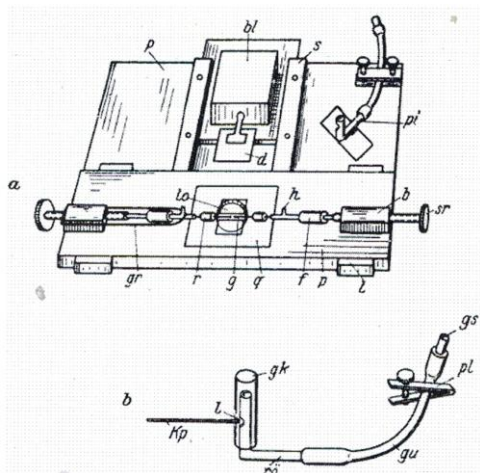


Fig. 18. **Another version of a microtransplantator** (explanation in the text).

(b) A compression pipette *pi*, consisting of a brass tube *ro* with the a hole *l*, rubber tubing *gk* and a glass capillary *Kp*, is set on the upper right corner. The brass tube is connected to a rubber hose *gu*, clamps *pl* and plug *gs*. The end of the capillary enters the channel, the width of which is regulated so as to connect the cells under investigation prior to irradiation. Cells are fed into the channel through a capillary and are exposed to radiation for transplantation.

Microstretching of Cells

Cells that have a certain plasticity can be deformed into a cylindrical shape making it more convenient to extract nuclei or for microirradiation. The procedure involves the suction of the eggs into a capillary tube using a syringe. The tube's inner diameter is slightly smaller than the diameter of egg. Suction is created by rotating the head of the syringe. Ejecting eggs from the capillary is achieved by rotating the head of the syringe in the opposite direction (Fig. 19a,b). The cell can be cut after the operation using a razor blade *r* (Fig. 19c). The end of the blade is inserted into the plate (Fig. 19d).

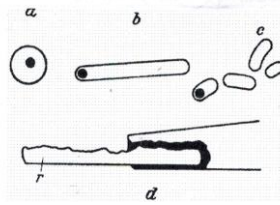


Fig. 19. **Microextraction apparatus** (explanation in the text).

Capillary Chambers

These devices move the cells into and out of a capillary tube. The design of the capillary chamber is shown in Fig. 20a. Plates *l* and *r* are located on the slide. Capillary *k* is between them and two liquid droplets *f*. A cell is injected into the left droplet. The right end of the capillary is lowered relative to the left capillary by the rotation of plate *r* causing the cell to be pulled into capillary. When the cell reaches the middle of the capillary, the capillary is separated from the two drops by lifting it with lever *h* and fork *g* (Fig. 20b).

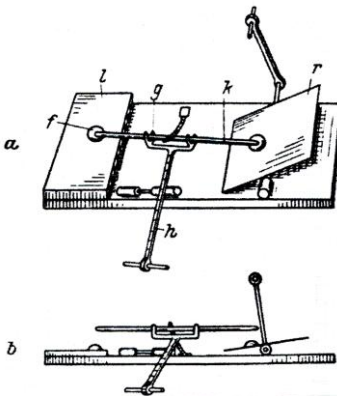


Fig. 20. **Capillary Chamber:**
a – top view; *b* – side view
 (explanation in the text)

After that the capillaries are arranged in a postoperative chamber (same type as that shown in Fig. 13) for studying and manipulating the cells.

Evaporation Chamber

This device allows immobilizing motile cells to investigate the effects of different gases on them. Its construction consists of a metal frame *r* (110×60×16 mm) that is closed at the top and bottom by glass plates with the top plate being removable (Fig. 21). The chamber is equipped with two seat slats and a holder *ot* for the object with a hole at the centre, guides *sl* and a quartz cover slip *q*.

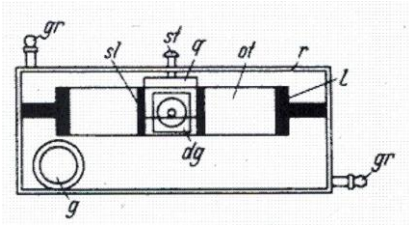


Fig. 21. The vaporization chamber (explained in the text)

The space that is formed by a hole in the holder allows the object (e.g., a cell) to be sealed using paraffin oil. Coverslip *dg* is located at the top, also greased with paraffin oil, and a drop with the cell transferred into it. A brass tube with a pin *st* is soldered in front of the chamber. Tubes *gr* are used for the supply and removal of gases. A receptacle *g* at the bottom of the chamber with concentrated sulphuric acid is used to facilitate evaporation and is controlled through a microscope visually. The chamber is closed when the cells are immobilized by a pin and evaporation ceases.

A Device for Measuring Respiration

It is often interesting to investigate metabolic process in a single cell and to establish the role of cellular organelles in these processes. For example, it is possible to carry out microirradiation of individual organelles (such as nuclei) and to measure simultaneously the cell respiration. For this purpose Chakhotin proposed a device (Fig. 22) that consists of a thin quartz capillary 10 mm in length and an inner diameter of 0.2 mm. The individual components are a drop of paraffin oil p_1 that acts as a plug, a drop of liquid *W* with the cell *Z* under investigation, gas bubble *g*, a drop of potassium hydroxide *R* and another drop of paraffin oil p_2 that are placed sequentially in the capillary.

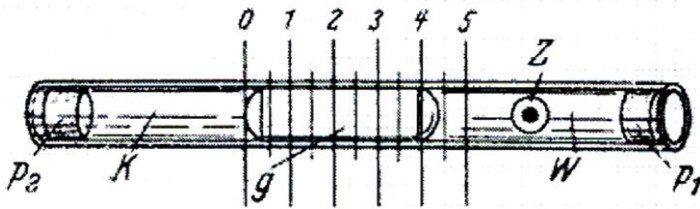


Fig. 22. A device for measuring respiration (explanation in the text)

The capillary is mounted on the slide next to another capillary with a solution of fluorescein that is necessary for focusing the ultraviolet radiation. The measurement process involves the determination of the volume of the gas bubble using an ocularometer. As oxygen is utilized from the gas mixture during respiration, the resulting carbon dioxide released is absorbed by potassium hydroxide and the volume of the gas bubble decreases giving a measure of the rate of respiration.

A Device for Filling the Capillary

The process of filling the capillary involves notching, breaking the capillary and filling. The following constructions are used for the first two operations. The first (Fig. 23) consists of a brass plate with a molding *l* and a capillary that is fixed by plates *pl* by means of screws *Sr* threaded into the plate.

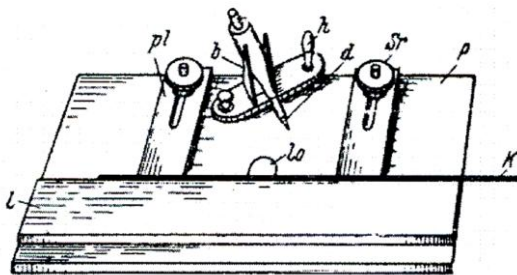


Fig. 23. A device for microincision (explanation in the text).

A hole lo 5 mm in diameter is placed at the centre of the plate and a diamond cutter d at the rim b is located above the hole. Turning the cutter is performed via a holder h . The capillary is scored with the cutter in the right place and then broken with a second tool (Fig. 24) that consists of a brass plate W with a rectangular depression f in the centre, closed by cover slip. The capillary K is fixed on the plate S at the stop bar a . The score on the capillary is positioned over a hole and the capillary K is broken using brass block b with a pipe, pin st and spring sf by pressing the pin.

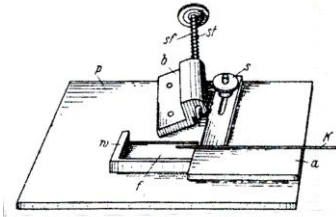


Fig. 24. **Microbreaker of the scored capillary** (explanation in the text).

The process of filling the capillary is facilitated by using the device shown in Fig. 25. A carriage pl is positioned on the brass base p (40×90 mm) with a hole in the centre lo , such that the plate moves along the base.

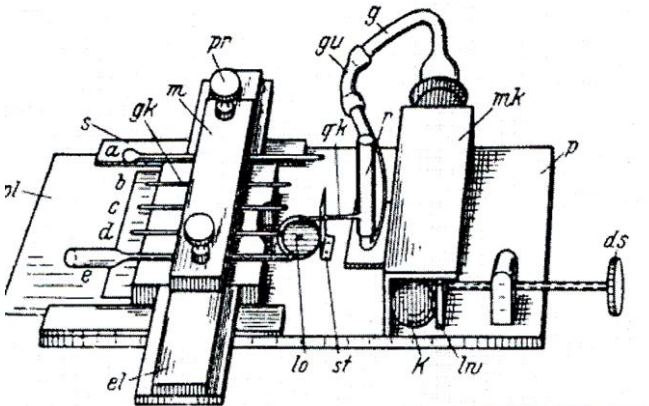


Fig. 25. **Device for filling a capillary** (explanation in the text).

A second carriage with a narrow plate *el* moves across the base. A clamp *m* is attached to the centre of this plate via screws *pr* which press down on the four glass capillaries *gk* and pipette *d* containing the cell. One of the capillaries (*b*) is filled with paraffin oil and the second (*c*) with the liquid containing the treated cells. The third capillary (*d*) contains 5% potassium hydroxide solution and the fourth (*e*) is filled with gas. The device consists of suction and compressor strips *st*, a brass tube *r*, hose *gu*, glass tube *g* and cap *K*. A brass box *mk* is used for compressing. One of the walls *lw* of the box can be pulled out using a screw *ds*. A quartz capillary *qk* is consistently filled with the content of these capillaries by moving the carriage with the capillaries, allowing respiratory experiments to be conducted.

A Device for Thermoelectric Experiments

The tool that is inserted into the holder micromanipulator is comprised of a trunnion *Z* with a flat asbestos ring *A* (Fig. 26). A wire spiral *S* is attached to its lower side. The free ends of this spiral enter the clamps on an ebonite plate *E* and voltage (4 V) is applied to the clamps. The temperature is monitored by means of the paraffin drops stained to varying degrees, that melt at different temperatures.

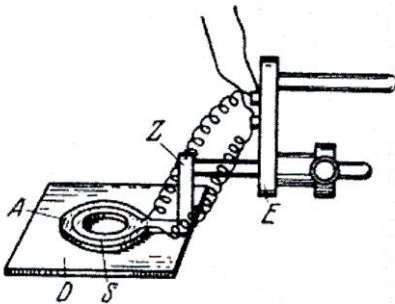


Fig. 26. A device for thermoelectric experiments (explanation in the text).

Dual microneedles *M* are inserted into the holder during the thermoacoustic experiments (Fig. 27). Both microneedles are insulated from each other and connected to the voltage source.

Chakhotin also designed a combination of a micropipette and an electric heater. A glass pipette with a balloon is heated when an electric current is passed through the coil with the pipette acting as a compressor.

Removal of the coil causes cooling and contraction of the air in the pipette which pulls the object into the pipette.

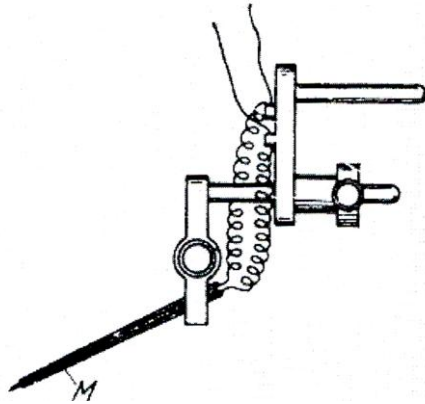


Fig. 27. **Device for thermo-acoustic experiments** (explanation in the text).

Method for Producing Microdroplets

Chakhotin proposed a method for using microdroplets to storing cells, observing them for several weeks, for cell extraction, and studying the effect of various physical and chemical factors, etc. A glass slide in which a depression is created is filled with a clean and transparent liquid paraffin or vaseline oil (Fig. 28). The heated layer of oil is punctured with a 10 micron diameter micropipette. A drop of water is deposited in the punctured layer. This operation is repeated several times to get a few drops spaced in a series into the oil layer. A test cell is incerted into each droplet. The drops are then coated with a thin layer of oil to prevent evaporation. Chakhotin named such a set of plates with wells containing rows of drops a “microclinic”.

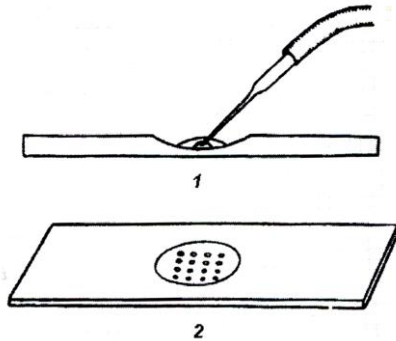


Fig. 28. **Method for producing microdroplets:** 1 – filling the depression in the slide with paraffin oil; 2 – inserting a number of droplets each containing a cell into the oil in the depression.

2. ULTRAVIOLET IRRADIATION OF CELLS

Experimental Technique

One of the most innovative approaches used by Chakhotin in his research was studying the impact of ultraviolet radiation focused on an individual cell or its organelles. The task seems to be far from easy, even at our current level of technology, which is why it is interesting to examine how he solved the methodological and experimental problems facing him.

Mechanical intervention into a cell with a needle or micropipette has a disturbing and damaging impact that can not be quantitatively or qualitatively assessed with an adequate degree of precision. Mechanical manipulation of cellular organelles is not possible without some mechanical damage to the cell membrane. All these considerations caused Chokhotin to try and replace mechanical manipulation with focused ultraviolet radiation. In 1912 the bactericidal action of short-wave ultraviolet radiation was already known and had been used to sterilize drinking water.

Chakhotin had developed a method, termed the “Ultraviolet Prick”. A number of structural and methodological problems had to be solved for the method to be successful. A microscope had to be converted from a strictly observational instrument to an operating unit that could focus shortwave ultraviolet radiation on a spot commensurate with the size of the cellular components. Since ordinary optical glass elements do not pass ultraviolet radiation, it was necessary to use quartz optics.

The the ability to manage, manipulate the focused beam and determine its location were critical.

The issue with the source of the ultraviolet radiation was originally solved at the beginning of the 20th century. A spark between magnesium electrodes was used as a source of ultraviolet radiation. There were a number of closely spaced spectral lines at 280 nm in the emission spectrum of the spark. A schematic representation of the optical part of the experiment is shown in Fig. 29.

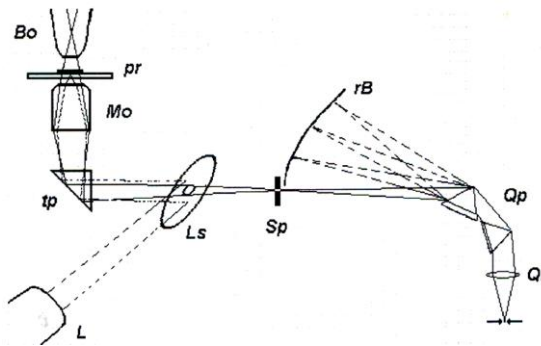


Fig. 29. Schematic representation of the optical-spectral portion of the ultraviolet micro-irradiator of Chakhotin (explanation in the text).

The radiation was focused by a quartz lens *Ql* and directed at two quartz prisms *Qp* that convert the radiation into individual spectral lines *rB*. The lines in the 280 nm region were directed to the microscope. Isolation of these lines, as well as the adjustment of ultraviolet ray intensity were performed using a precision slit *Sp*. After the slit, radiation passed through a hole in the mirror *Ls*, which was used to reflect the light of lamp *L* and observe the light field. Radiation was directed by a reflective quartz prism *tp* through a monochromatic quartz lens *Mo* to the object *pr* that was located on the microscope

stage *Bo*. The system allowed focusing the radiation in an area of 5 to 2 μm in the plane of the target.

A solution of fluorescein in combination with a movable mechanical arrow *Z* were used to determine the location of microbeam relative to the cell (Fig. 30, *a-d*).

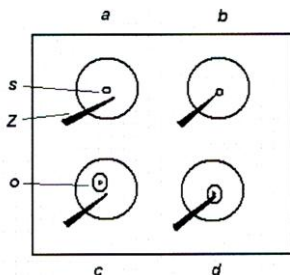


Fig. 30. **Determining the location of microbeam using a fluorescein drop:** *a* – application of fluorescein; *b* – placing a mechanical pointer on the drop; *c* – replacing fluorescein by treated cell; *d* – placing cell to the tip of the pointer

Chakhotin also faced a number of problems in addition to the photochemical nature of the instrument. He placed cells in mixtures of solutions rich in calcium ions in order to prevent damage to the cell surface during microirradiation of the nucleus. These mixtures had astringent properties. In addition, centrifugation of the cells was used by Chakhotin to exclude the effects of ultraviolet radiation on the cytoplasm. As a result, the nucleus was shifted to the periphery of the cell and a layer of cytoplasm between the nucleus and the cell surface was greatly reduced as the cell was flattened (Fig. 31, *a-c*).

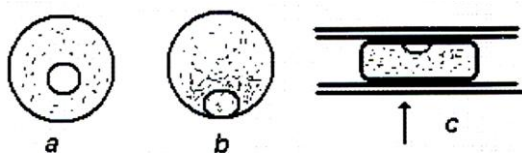


Fig. 31. **Centrifugation of a cell under study to shift the nucleus to the periphery:** *a* – cell before centrifugation; *b* – cell with a displaced nucleus; *c* – flattened cell during irradiation of a nucleus.

The micromanipulation techniques and operational and post-operative chambers were described in the previous sections.

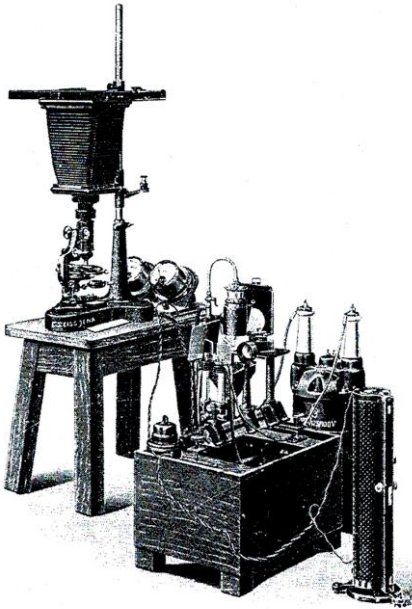


Fig. 32. Experimental installation of Chakhotin that was designed for the micro-irradiation of cells.

In general, the experimental installation for microirradiation of the cells consisted of a combination of a microscope, a source of ultraviolet radiation and the optical elements (Fig. 32).

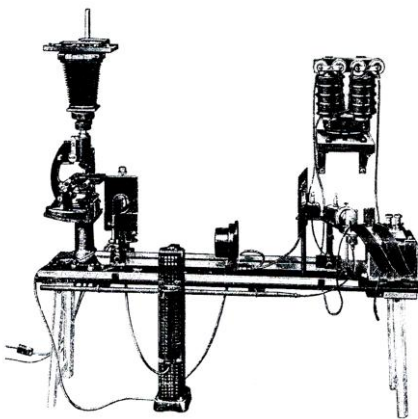


Fig. 33. The micro-irradiation apparatus on an optical bench (from right to left): inductor, power source, diaphragm, optical elements, microscope and camera.

An alternative installation of the elements arranged on an optical bench is shown in Fig. 33. The inductor consisting of a spark gap that is formed by the electrodes bF , capacitors K , inductors S , fuse F_s , transformer T , ammeter A , rheostat W , and switch Sch were used to produce a spark as the source of ultraviolet radiation (Fig. 34).

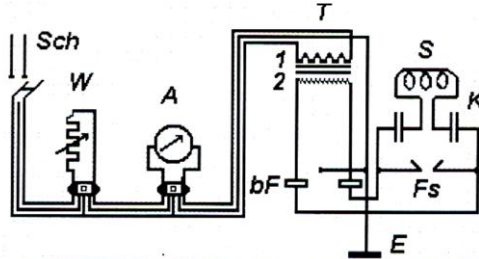


Fig. 34. The arc power supply circuit used as a source of ultraviolet radiation (explanation in the text).

Variations in the optical scheme were used if necessary to implement a relatively low ultraviolet light intensity (Fig. 35a). Here f is the arc; lk and Kk are the quartz lenses; P is the prismatic table; T is the tube; Kh is the condenser; Sp is the slit; Sf is the tripod of the microscope; tr is the prism; and Ls is the mirror. The distance between the circuit elements are indicated in millimeters.

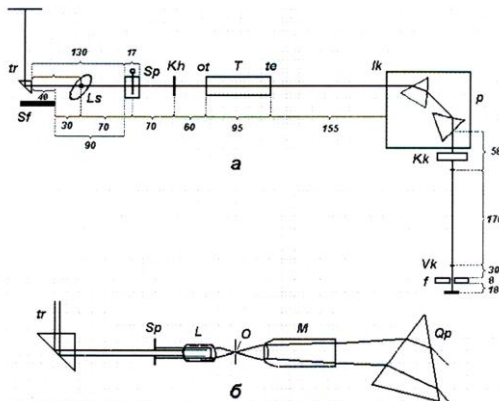


Fig. 35. Apparatus for micro-irradiation using high-intensity ultraviolet radiation (explanation in the text)

A variation in the scheme is depicted in Fig. 35*b* and was used for high-intensity ultraviolet radiation experiments. This option increases the amount of light entering into the lens. The combination of prism Qp , lens M , diaphragms O and Sp , quartz eyepiece l and prism tr were used for this purpose. An ultraviolet beam, which had a diameter of 6 mm, was narrowed to 3mm by passing it through the optical system increasing the light intensity.

Methodology of Micro-Irradiation

Guiding the focused radiation to the object was the first step in the process of micro-irradiation. A preparation with a drop of fluorescein was mounted on the microscope stage for this purpose. The sharpness of the preparation image was achieved by focusing under a bright field after which the light was switched off. The distance between the electrodes was set at 1 mm and the electric current was switched on. The optical system was tuned using a fluorescent screen (uranyl glass) so that the group of lines at 280 nm entered into the slit. A clear image of a glowing green dot on the preparation was achieved by moving the quartz lens. Then the uranyl glass was removed and the slit image was obtained. The pointer was supplied to the image and its end was combined with the image of the puncture. Then the arc was overlapped and the field of view became bright again. The cell under investigation was placed on the stage and was positioned at the end of the pointer. The ultraviolet radiation was then applied to the object.

Chakhotin used a cadmium photocell Z that was coupled with the power source E , extension fitting ro and the prism gp for dosage and quantitative estimation of the radiation intensity (Fig. 36). Adjustment of the radiation incident on the photocell was carried out with the help of the uranyl glass.

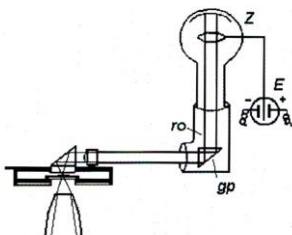


Fig. 36. Method for controlling the positioning and intensity of the ultraviolet radiation: Z – cadmium cell; E – power supply; gp – prism; and ro – tube.

Results of Micro-Irradiation of the Cells

Chakhotin reported on the results of tests using focused ultraviolet radiation for the treatment of malignant tumors during his early works, published in 1912. Experiments using X-rays and radioactive isotopes of radium for radiation therapy in conjunction with administering chemicals such as choline were conducted at that time. It was found that ionizing radiation and choline solutions induced selective individual lesion in tissues, especially those rich in lecithin. Experiments that were carried out by Chakhotin indicated the splitting of lecithin into components during irradiation with optical and ionizing radiation. Several hypotheses such as the destruction of enzymes and changes in the permeability of the protoplasmic membrane were put forward to explain the harmful effect radiation on cells.

Chakhotin's experiments had the objective of trying to determine the possible mechanism of action of radiation on cells. If lecithin is split by irradiation, then choline that is formed at the same time as lipoid soluble component should be kept longer than other decomposition products of lecithin, that are not lipoid soluble. Choline can be determined by alkaline reaction. Chakhotin chose as an indicator of this reaction a lipoid soluble dye (neutral red) that gives a red-purple colour in an acid reaction and a straw yellow colour in an alkaline reaction. The dye did not change its colour when exposed to ultraviolet radiation.

Sea urchin eggs, blood cells of frogs and birds, and cancer cells of mice were chosen as the objects of study. All these types of cells are rich in lecithin. It was found that cells previously stained a bright red colour with a dye, became yellow and decayed after irradiation. Adding lipid soluble acids to neutralize the reaction presumably formed bases that caused a red colour. This result supports the hypothesis that the dye is not leached from the cells during decay nor causes a change in permeability of the protoplasmic membrane. Adding choline in the cells caused a colour change from red to yellow.

Chakhotin concluded that irradiation of cells caused the appearance of OH ions and discoloration of the dye. It was necessary to clarify the origin of these ions – either they were the result of the degradation of some substance or they entered the cell from the surrounding environment as a result of a change of cell permeability. To solve this

problem, Chakhotin placed the blood cells, whose nuclei have been stained in a red colour, into an isotonic solution (0.6% solution of NaCl), which did not have OH ions and observed the colour change of the cells. Destruction of the shell of sea urchin eggs dyed red did not lead to a change in colour.

All of these factors indicated the formation of choline from the degradation of lecithin during the irradiation. The presence of OH ions together with choline caused a destructive effect on the cell. It was quite obvious that the effect of destruction of the cell membrane under the influence of irradiation changed the permeability to ions entering the cell from the surrounding environment. The decay of lecithin and cytolysis due to the impact of fission products were the crucial factors in determining the mechanism of selective irradiation on cell malignancies. Chakhotin came to the conclusion that the simultaneous effects of radiation and chemicals (chemotherapy) would be important in the destruction of tumors unavailable for surgery.

A series of experiments was devoted to the study of micro-irradiation of the nucleus on sea urchin eggs. Preliminary experiments had shown that it was necessary to only expose the cells for a short time in order to avoid destruction of the cytoplasm layer. He found that nuclear substances absorb radiation more intensively than the cytoplasm. In addition, information on the impact of various ions on the permeability of the plasma membrane (J. Loeb, R. Lillie et al.) was taken into account. Whereas K and Na ions increased the permeability of the plasma membrane, Ca ions had the opposite effect. The impact of focused (i.e., $\leq 5 \mu$) ultraviolet radiation on sea urchin eggs rapidly destroyed the process of fission which stopped virtually immediately. It was necessary to use micro-irradiation in conjunction with a medium that contained an excess of calcium to reduce the possibility of plasma membrane destruction.

Chakhotin found during micro-irradiation of a sea urchin egg that the permeability of the plasma membrane increased due to exposure to radiation. This allows the surrounding ions to penetrate into the cell and to cause a characteristic reaction. The experiments with cells stained red in colour and placed in a solution containing OH ions, supported this hypothesis. The eggs acquired a yellow colour after micro-irradiation and the permeability of the surface layer of the plasma dur-

ing egg activation and division increased. Local permeability in fertilized eggs fissile had different values. Micro-irradiation by its nature resembled, according to Chakhotin, the natural stimuli of fertilization that caused certain stages of egg activation such as membrane formation, division of the egg and segmentation of the cytoplasm. The process of stimulating the cells by micro-irradiation passed so slowly that it was possible to analyze the autonomous division of the cytoplasm.

Chakhotin was also interested in the functions of the stigma in the algae *Euglena gracilis*. It was known that some algae exhibited specific reactions to light, changing their characteristics, such as velocity, direction, trajectory of movement, etc. Some algae, in particular *Euglena gracilis*, exhibit a modulation mechanism for the orientation of the cells relative to light. In *Euglena gracilis*, photoreception and photoorientation are based on the periodic shading and illumination of the photoreceptor in the stigma during rotation of the cell. The stigma is a specific organelle that is located at the edge of the vacuole and acts as a light modulator in photoreception (Fig. 37). The rotational movement of the cell modulates the light striking the photoreceptor. The amplitude of modulation depends on the direction of movement of the cell relative to the direction of propagation of the light.

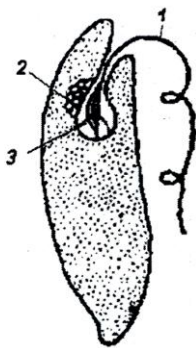


Fig. 37. Schematic representation of the unicellular alga *Euglena gracilis* (1 – flagellum; 2 – stigma; 3 – paraflagellar body)

The first attempts to clarify the functions of the photoorientation apparatus of *Euglena gracilis* were carried out by Chakhotin using ultraviolet micro-irradiation. His studies showed that the cell's flagella was discarded when exposed to radiation at a wavelength of 280 nm, the cell shortened rapidly, taking a spherical shape and swelling. It was noted that there was a differential in the sensitivity to the radiation between the front and rear ends of the cell. The sudden decrease in cell length was more significant when the front of the cell was exposed to the radiation. Perhaps the most interesting part of the research on the phototaxis of *Euglena gracilis* concerned the filigree. To study this he used two parallel narrow quartz capillaries that passed ultraviolet radiation that were placed on the microscope stage. One contained a solution of fluorescein, while the other contained the cells of *Euglena gracilis*. Initially the ultraviolet radiation was focused on the capillary with fluorescein and its location was fixed using a mechanical indicator. Then the first capillary was substituted with a second one containing a cell; a limited portion of the capillary was illuminated with white light from the microscope condenser. The cells moved to the "light-shadow" border, returned to the next boundary, and thus performed a reverse movement, which was determined by the spatial gradients of light.

Micro-irradiation of the stigma of *Euglena gracilis* made it possible to disrupt the photoorientation mechanism in the cell which was accompanied by crossing the border of the illuminated area. Moreover, excitation of the stigma with monochromatic light of different wavelengths showed that the red, yellow and green regions of the spectrum did not cause photomovement of the cells, in contrast to the blue and violet regions which were active.

Chakhotin established with his research that the stigma is indeed a primitive organ of cell vision that governs its photomovement. Ultraviolet (280 nm) irradiation striking the stigma "dazzles" the cell; the stigma also responds to the blue-violet region of the spectrum. Localized micro-irradiation causes cell contraction with the front part of the cell being more sensitive to the radiation. It should be noted that our understanding the functional and spectral properties of the stigma of *Euglena gracilis* established by Chakhotin have changed little since his work. Chakhotin conducted joint research with P. Gavaudan, the French naturalist from the Sorbonne, on the effect of micro-

irradiation on the fungus *Ascoidea rubescens*. Preliminary experiments had shown that certain structural modifications of the fungus were induced by its exposure to vital dyes; for example, the vacuolar system was reduced to a few spherical vacuoles in the cell. This state lasted for only several minutes and was reversible.

Irradiation of the cells using Chakhotin's method led to the above-mentioned vacuolar reaction after 10-15 seconds of exposure to ultraviolet radiation; the reaction occurred through the irradiation of part of the cell during a 15-30 second exposure and the reaction took place best when the cell was placed in drinking water at pH 7.2. The irradiation time required for realization of the response changed with pH, e.g., 11 s at pH 7.2 to 105 s at pH 4.2.

It was shown that the perturbing effect of ultraviolet irradiation on *Ascoidea rubescens* was effective in the presence of OH ions. The combined action of acids and bases on the one hand, and the ultraviolet radiation on the other, demonstrated that the vacuolar reaction occurred when using an alkali and did not take place with the addition of acid. The authors concluded that it was necessary to distinguish between the effect of pH on the permeability of the cytoplasm surface layer and the vacuolar reaction that was associated with internal changes in the cytoplasm. Micro-irradiation increased the permeability of the cell to components from the surrounding environment, which led to the vacuolar reaction.

The research by Chakhotin that was conducted on *Paramecium* (a genus of ciliated protozoa) was also important. Effects of ultraviolet radiation at a wavelength of 310 nm on the cells did not lead to any disturbances or changes of their vital processes. However, the addition of a solution of eosin in combination with micro-irradiation led to a contractile response by the cells. The dye acted in this situation as a photosensitizer that increased the sensitivity of the cells to radiation.

Chakhotin continued with a series of experiments on *Paramecium*, irradiating one of the contractile vacuole, and then a second. The cells ceased to pulsate and allocate water. If the cytostome was subjected to micro-irradiation, water was released by diffusion through the cell surface and *Paramecium* cells again "revived". Chakhotin compared this phenomenon with edema during uremic crisis in higher animals and humans.

The primary results obtained by Chakhotin using ultraviolet micro-irradiation technology (“micro-prick”, “radiation micropuncture”, “micro-photosurgery”), can be summarized as follows.

1. It is necessary to distinguish between modifications in the permeability of the cell surface layer and the coagulation of proteins and other components of the cytoplasm due to the action of radiation on living substance. Changes of permeability of the cell membrane causes ions to flow into the cell from the surrounding environment.

2. The mechanism governing the selective action of radiation on cancer cells was established as due to the degradation of lecithin and by the cytolysis due to the impact of fission products. Experiments on the combined action of radiation and chemicals (photosensitizers) indicated the potential use of photosensitivity for the diagnosis and treatment of malignant tumors.

3. Focused ultraviolet radiation (275-280 nm) on sea urchin eggs stimulated parthenogenesis, which allowed studying the process of fertilization.

4. The functions of the stigma when exposed to ultraviolet (280 nm) and monochromatic radiation in the visible spectrum were studied. The participation of stigma in the photomovement of *Euglena gracilis*, the sensitivity of the stigma in the blue-violet region of the spectrum and the influence of local micro-irradiation on contractile responses of the cell were confirmed.

5. The vacuolar reactions of the cells of the fungus *Ascoidea rubescens* (Hemiascomycetes) were found to depend on the duration of exposure to ultraviolet radiation, pH, and the presence of OH ions.

6. Effects of micro-irradiation on *Paramecium* such as the local abaissement of cilia, deformation of the surface, changes in the discharge of water, reflex reactions, the photosensitizing effect of irradiation (310 nm) and the affect of eosin on the contractile response of the cells were detected.

7. The role of the nucleus in cell respiration was studied and photometric measurements in the area the nucleus and cytoplasm were performed.

These results as well as the micromanipulation techniques and a the series of physiological and embryological studies developed by Chakhotin remain of undeniable value today.

Chakhotin saw further progress in his scientific activities with the implementation of the following plan (1935):

1. Continuation of studying the role of the cell nucleus with the development of cellular processes; and the effect of micro-irradiation of the cell on its development, movement, secretion, etc.

2. The study of artificial initiation of egg development by peripheral micro-irradiation as a method that is an intermediate by its nature between chemical intervention and normal fertilization.

3. Understanding determinacy in development by micro-irradiation of individual blastomeres in fertilized eggs and subsequent analysis of the results.

4. Destruction, damage or irritation of cellular organelles in order to clarify their roles and participation in the life activity of the cell.

5. The artificial fusion of eggs, egg fragments and blastomeres; and conducting experiments on transplantation and vegetative hybridization.

6. The removal of the nuclei of a cell after micro-irradiation and its transfer to another cell.

7. Separation of cells using focused radiation.

8. Further investigation of the permeability of the cell surface and its changes during segmentation and local micro-irradiation.

9. Study of the effect of various ions and toxic substances on the permeability of the cell.

10. Determination of intracellular pH by way of colour changes after irradiation.

11. Selective effects of ultraviolet radiation focused on individual embryo cells in a weak toxic solution and subsequent control of the development of the organism.

12. Combination of micro-irradiation and ultramicroscopy to study the localized changes in cellular colloids after irradiation.

13. Conduct research with focused monochromatic radiation and determine of the effect of wavelength.

14. Photometry of the cell associated with energy both absorbed and passed through different parts of the cell.

15. Consideration of the use of ionizing radiation for local effects on the cell.

IV. IMPACT OF THE IDEAS OF S. CHAKHOTIN ON SCIENCE TODAY

1. MASS PSYCHOLOGY

Sergey Chakhotin was actively involved in the problems of social psychology especially with regard to conditioned reflexes and instincts, the study of the behaviour of large masses of people, and the elucidation of the mechanism of transformation of these masses into a crowd and its management by leaders with political propaganda.

Chakhotin explained the presence of a hierarchy among the instincts and the superior efficiency of Nazi propaganda which used the more powerful instincts of fear and aggression in contrast with the social-democratic propaganda that appealed to the civilized and humane themes of peace and harmony.

The method of “psychological violence”, that was used by the leaders of totalitarian regimes to justify anti-human goals such as national isolation, racism, anti-Semitism and revanchism, can be adopted by Socialism and true democracy acting in this case no longer based on fear but on enthusiasm, joy, and love. A violent propaganda of non-violence!

Chakhotin notes how much more attractive and humane were ideas of morality, socialism, and peace. However, one must act and this requires either decisiveness and will or the organization of action, i.e. propaganda.

Chakhotin also based his views of political propaganda on the concept of instincts. He distinguishes between two forms of propaganda. First, ratio-propaganda which uses persuasion, argumentation; the second, senso-propaganda which stimulates feelings, enthusiasm, and ecstasy.

The first type uses political instructions that are communicated in the media, at meetings, in the course of discussions. We can assume that as this type of propaganda reflects economic interests and it is based on nutrient instinct. The second type of propaganda uses symbols, flags, banners, uniforms, demonstrations, and noisy gatherings and uses basically the instinct of struggle.

Chakhotin book, “Rape of the Masses: The Psychology of Totalitarian Political Propaganda”, was very popular during the years of its publication. The ideas proposed by the author continue to be relevant today,

especially when we see the formation crowds due to the influence of external stimuli [Moncomble, 1983; Volkoff, 1986].

The effect of external stimuli can be seen with soccer hooligans and raging fans of punk and rock bands. The action of such crowds are accompanied by acts of vandalism, public disorder, riots and scuffles. In contrast, religious crowds are characterized by the adoration of some deity and are accompanied by irrepressible fanaticism.

Often politicians and dictators use the media and modern methods of political propaganda to control a crowd, using the instinct of submission. We have even seen democratic and totalitarian political regimes that are based on authoritarianism, kleptocracy, or plutocracy. These totalitarian regimes exert violence over the masses.

The predictions of Chakhotin about the possible deployment of a struggle between two political systems are still operative today in various locations around the world. Each of these systems, either democratic or totalitarian, is based on the instinct of struggle.

2. EXPERIMENTAL CYTOLOGY

Chakhotin developed an experimental method using ultraviolet micro-irradiation of cells that has received wide application in various fields of biology and biophysics. The following lists some of these applications.

Investigation of the Properties, Structure and Functions of Individual Cellular Organelle

Ultraviolet irradiation was used to study the cellular morphogenesis of ciliates, particularly in *Paramecium aurelia*, *Urostyla weissei*, *Stentor coeruleus* and others. St.-Petersburg scientists S.I. Fokin and D.V. Osipov (1975) used ultraviolet irradiation of ciliates to study nuclear dualism. Microorganisms as ciliates have two different types of nuclei: a large polyploid, somatic macronucleus (*MAC*) that controls cell regulation and a small, diploid micronucleus (*MIC*) that controls reproduction.

Selective irradiation of these organelles provided information on the functional features of the individual elements of the nuclear apparatus. Immobilized cells of *Paramecium caudatum* were exposed to a focused ultraviolet light beam square in shape and $70 \mu\text{m}^2$ in size (or in some cases $23 \mu\text{m}^2$) and a wavelength of $\sim 260 \text{ nm}$. The following clones were tested: M-3I with a normal organization of nuclear apparatus and M-3I-20-omega containing intranuclear symbionts (in this case Gram-negative bacteria).

The following samples were exposed: 1) pure *MIC* (diploid micronucleus) in the cells of clone M-3I (Fig.1, 1); infected *MIC* in the cells of subclone M-31-20-omega (Fig. 1, 2a); infected *MIC* with subsequent reactivation by visible light (Fig. 1, 2b); a section of *MAC* in the cells M-31-20-omega (Fig. 1, 3); a section of the cytoplasm of M-31-20-omega cells (Fig.1, 4); and finally, dividing cells of subclone M-31-20-omega (Fig.1, 5).

They established that the elements of the nuclear apparatus of ciliates were characterized by unequal sensitivity to UV radiation. Quantitative estimates of the sensitivity of the cytoplasm, macro- and micronuclei, and symbiotic bacteria were made. The reduction in cloning efficiency, loss of micronucleus bacteria, appearance of cells with several *MIC* occurred as a result of micro-irradiation. The cells displayed the ability to recover from damage that had been caused by UV irradiation after subsequent illumination with white light.

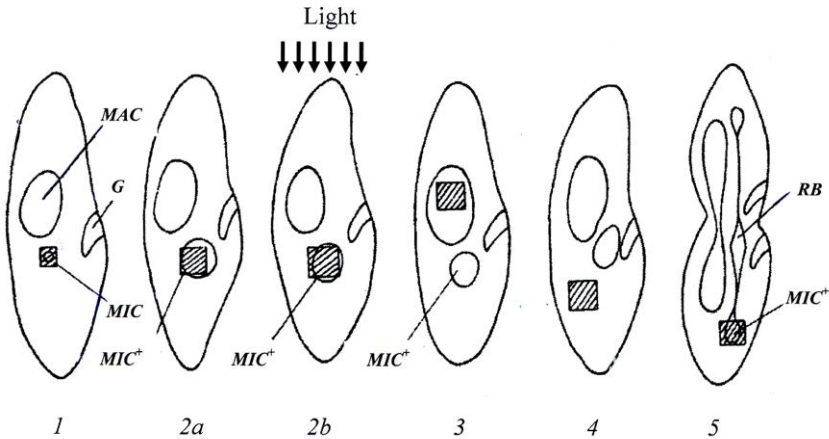


Fig. 1. A series of experiments conducted by S. Fokin and D. Osipov with ultraviolet irradiation of paramecia (1. clone M-3I, *MAC* is the polyploid macronucleus, *MIC* is the diploid micronucleus, *G* the gullet; 2a. an infected *MIC* in the cells of subclone M-31-20-omega; 2b. an infected *MIC* with subsequent reactivation by visible light; 3. a section of *MAC* in an M-31-20-omega cell; 4. a section of cytoplasm in a M-31-20-omega cell; 5. dividing cells of subclone M-31-20-omega, *RB* is the residual body of the division spindle of an infected micronucleus. Closed squares represent the irradiated areas (given not to scale).

Selective functional inactivation of the micronucleus in three clones of *Paramecium putrinum* was achieved with local ultraviolet micro-irradiation [Fokin, 1978]. Ultraviolet radiation resulted in the disappearance of the micronuclei, death of irradiated cells and fragmentation of the macronucleus. The author suggested that the fragments of the macronucleus were some kind of functional replacement for the lost or damaged generative nucleus.

The influence of the generative nucleus on the vitality in three clones of *Paramecium putrinum* was studied. The selective functional inactivation of the micronucleus (MI) was achieved with a localized ultraviolet microbeam. After irradiation of the micronuclei, it soon disappeared and the irradiated cells perished shortly afterward. In UV treated-subclones, macronucleus (MA) fragmentation was discovered. It is possible that the MA fragments are some kind of functional replacement for the lost or damaged generative nucleus. The data demonstrates the function of the MI in *P. putrinum* during its vegetative growth.

Micro-irradiation technology was subsequently greatly stimulated with the appearance of laser instruments. A laser is a device that generates coherent electromagnetic waves due to stimulated radiation by an active medium that is placed in an optical resonator. The principle of laser action can be explained by an acronym for *Light Amplification by Stimulated Emission of Radiation (LASER)*. The main properties of laser radiation are monochromaticity, coherence, directionality, and brightness.

Attempts to use laser radiation to affect individual cells was carried out shortly after the creation of this device. Work of Bessis and Ter-Pogossian (1965) using laser micro-irradiation of blood cells pioneered this new advancement.

Research targeting structural and functional features of cells were carried out by Burns and colleagues, who used focused laser radiation to study various cellular structures and functions. They established the exact location of cellular organelles as well as demonstrating their selective inactivation and removal [Berns et al., 1991; Berns, 2007; Berns and Greulich, 2007].

At present, the range of possible applications of laser micro-irradiation of cells is quite wide and as a consequence, it is difficult to highlight the results of all studies. The following are examples of basic research in this field [Bessis and Ter-Pogossian, 1965; Posudin, 1985, 1989; Berns et al., 1991; Curran et al., 2000; Curran and Murray, 2005;

Espina et al., 2006; Ahmed , 2006; Greulich et al., 2007; Berns, 2007; Berns and Greulich, 2007; Magidson et al., 2007; Gilbrich-Wille, 2013; Vandewoestyne et al., 2013].

Chakhotin's pioneering research opened the door to a broad range of research – two examples of which follow.

1) The effects of blue laser micro-irradiation on a single non-pigmented invertebrate neuron and the dynamics of its functional, structural, and metabolic changes were studied by Uzdensky [<http://photobiology.com/v1/uzdensky2/default.htm>].

Laser micro-irradiation of cancer nerve cells allowed investigating the ultrastructural and cytochemical changes in the mitochondria of irradiated neuron. The author observed the grouping of mitochondria in the perinuclear area, irregular profile changes of the nuclear membrane and localization of nuclear chromatin. Collectively this indicated differences in metabolic activity of the individual neurons in various areas of the soma during laser micro-irradiation.

Identification of Cellular Organelle

In his research, Chakhotin focused ultraviolet radiation on the stigma – a specific organelle of the unicellular alga *Euglena gracilis*, in order to clarify its functional characteristics during photoorientation of the cell relative to the direction of light propagation. This ability of *E. gracilis* to exhibit a characteristic reaction to light was known at the beginning of the 20th century. A number of researchers, including Chakhotin, were interested in the mechanism of photoorientation and the participation of cellular organelles in the process of photomovement in this species.

This alga exhibits locomotor responses to changes in environmental illumination such as a sudden increase or decrease in light intensity. Flagellum usually produce fluctuations relative to the longitudinal axis of the cell and changes its position, oscillating in a perpendicular direction. Cell turns by reorientation of the flagellum. According to some authors [Diehn, 1970, 1979], *E. gracilis* cells are able to accumulate in the areas of moderate illumination and to leave brightly illuminated areas.

Two organelles, the stigma and paraflagellar body (PFB), play a special role in the reaction to external light conditions. The PFB is a specific organelle that serves as the photoreceptor. The modulation mechanism of cell photoorientation during its rotation was mentioned earlier (p. #). The fact that the photoreactions of *E. gracilis* take place in a certain spectral region (from 350 to 500 nm) suggests the participation of certain pigments

in the photomovement response. It was of interest therefore to identify these pigments by extraction and subsequent spectral analysis. However, the PFB of *E. gracilis* are quite small ($1 \times 0.3 \times 0.4 \mu\text{m}$) in size making extraction quite difficult. The micro-irradiation technology developed by Chakhotin has allowed making this advancement [Colombetti et al., 1981].

Thus, a laser spectrofluorimeter was used to identify the pigments that are in the PFB of *E. gracilis*. The device used consisted of a tunable dye laser, fluorescence microscope and registration system (Fig. 2). The laser radiation within a tuning range of 380-480 nm was focused by the lens of the microscope and was directed on the PFB of *E. gracilis*, exciting the fluorescence of the pigments. The fluorescence emission passes in the opposite direction through a fluorescence microscope and liquid filter before entering a photodetector, the electrical signal of which is recorded using a multichannel analyzer.

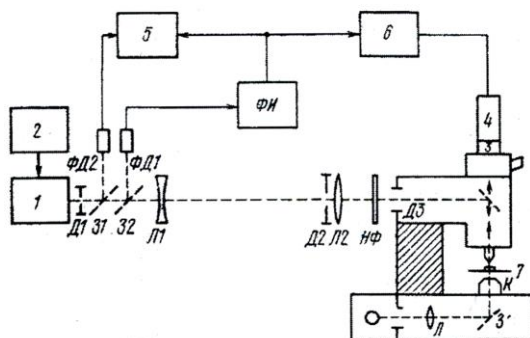


Fig. 2. **Scheme of the laser spectrofluorimeter:** 1 – dye laser; 2 – nitrogen laser of pumping; 3 – liquid filter; 4 – photodetector; 5-6 – multichannel analyzers; 7 – the object being studied; D1-D3 – diaphragms; L1-L2 – lens, M1-M2 – mirrors; NF – neutral filter; PD1-PD2 – photodiodes; FS – pulse shaper; and C – condenser.

This experimental setup allows comparing the pulse amplitude of fluorescence and excitation emission by using a second multi-channel analyzer and a pulse shaper. Each analyzer sorts arriving pulses by amplitude, distributing them through various channels. The system allows focusing the laser beam on a spot with a diameter of approximately $1 \mu\text{m}$. The fluorescence intensity of the PFB, cytoplasm, and slide was determined for each excitation wavelength. The dependence of normalized fluorescence radiation, determined by the contribution

of these three factors, on the wave-length represents the excitation spectrum of the *in vivo* pigments of *E. gracilis* (Fig.3).

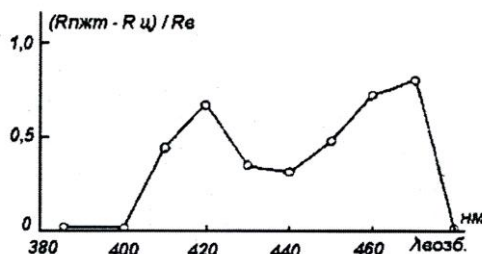


Fig. 3. Fluorescence excitation spectrum of PFB pigments of *Euglena gracilis*, obtained by laser microspectrofluorometry. R_{PFB} , R_c and R_s – are responses received during irradiation of the PFB, cytoplasm and slide respectively.

A comparison of this spectrum with that of an aqueous solution of riboflavin (Fig.4) indicated why flavins were determined to represent the photoreceptor pigments.

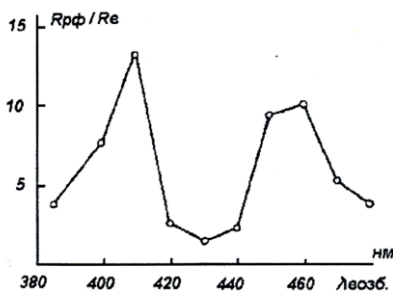


Fig. 4. Fluorescence excitation spectrum of an aqueous solution of riboflavin. R_{RF} and R_s are the responses received during irradiation of riboflavin and the slide respectively.

Fluorescence decay time is an important parameter, since the behaviour and the value of this parameter significantly depends on the means by which the energy is transferred from the excited molecule to its surroundings. The procedure for determining the fluorescence decay time requires focusing the exciting radiation on the cellular organelle receiving the fluorescence emission during excitation of the sample

using a signal of known temporal shape, transforming the fluorescence radiation into an electrical signal, and constructing the dependencies that allow determining the decay time for the fluorescence. The measurement of this parameter makes it possible to estimate the level of interaction of the fluorochrome with its neighboring molecules, intermolecular distances, structure of molecules, etc.

A group of Italian physicists (Sacchi C., Andreoni A., and Svelto O.) used laser microfluorimetry to study secondary fluorescence of complexes that were formed by a DNA molecule with a number of acridine dyes [Andreoni et al., 1979].

These complexes were created under appropriate conditions by intercalation of the dye between two adjacent base pairs of DNA. It was noted that the fluorescence intensity of these complexes depended substantially on the binding site – it increased when the dye was connected between the bases adenine-thymine and it decreased in the case of a combination of guanine-cytosine. It was found that illumination of chromosomes by ultraviolet radiation induced more intense fluorescence of the specific, relatively narrow ($\sim 1 \mu\text{m}$) bands than the others. This phenomenon can be used to identify the chromosomes. Likewise, analysis of the fluorescence decay of such complexes allows a variety of quantitative evaluations.

Laser Photochemotherapy

Photosensitization reactions are processes by which light energy absorbed by one molecular species (the photosensitizer) causes the chemical alteration of a second molecular species (target molecule). The phenomenon was described by Oscar Raab in 1900, when he studied the viability of ciliate *Paramecium* during light irradiation after acridine dye (acridine hydrochloride) treatment. The presence and absence of dye had no effect on the cells exposure in darkness, and light in the absence of the dye had no effect on the cells [Raab, 1900]. However, light in the presence of the dye killed the *Paramecium* cells.

The photosensitization that occurs in the presence of oxygen is called *photodynamic action* (some authors use the synonyms *photosensitization* and *photodynamic action*).

Laser photochemotherapy is based on the ability of the photosensitizer to be accumulated and retained selectively in malignant tissue to a greater

degree than in normal tissue; application of laser irradiation can induce the destruction of a tumor.

The photosensitizer molecule can absorb a quantum of light transforming it from the singlet to a triplet state; the triplet excited photosensitizer can produce either free radicals or singlet oxygen; the primary result of both of these pathways is the oxidation of the substrate (malignant tissue), its necrosis and destruction.

The photosensitizers commonly employed in the laser photochemotherapy of malignant tumors are hematoporphyrin derivatives. This type of sensitizers is known to be a mixture of porphyrins formed by acetic acid-sulfuric acid treatment of hematoporphyrin.

Currently a great number of natural and synthetic chemical compounds can act as photosensitizers. The main requirements of photosensitizers, besides their ability of selective control tumors, are the absence of toxicity, absorption in the visible part of spectrum, the high quantum yield of the triplet state, and photoreactivity.

Various types of lasers can be applied for the excitation of the photosensitizer. An excimer laser operates in the ultraviolet portion of the spectrum where the tissue has a significant absorption. An argon laser is suitable for the activation of hematoporphyrin derivatives. A continuous-wave dye laser can also be used because of its frequency tunability in the visible portion of the spectrum.

The research derived by numerous investigators [Spikes, 1985; *Lasers in Photomedicine and Photobiology*, 1980; *Lasers in Biology and Medicine*, 1980; *Porphyrins in Tumor Phototherapy*, 1984] document the efficient response of different types of tumors in animals to laser photochemotherapy.

PUVA therapy (**PUVA = Psoralens + UltraViolet A**) is one of the most promising directions for using ultraviolet radiation in medicine. Psoralens (furocoumarins) are the chemical compounds that were isolated for the first time from the seeds of psoralen (*Psoralea corylifolia* L.) [Jois et al., 1933]. Psoralens are found in plants such as parsley, parsnips, celery (family *Umbelliferae*), bergamot, orange, grapefruit (family *Rutaceae*), and figs (family *Moraceae*). PUVA therapy is used to treat psoriasis, eczema, atopic dermatitis, vitiligo, mycosis fungoides and a number of other diseases [*Light Therapy*, 2014]. Long-wave UV excimer lasers and frequency-tunable dye lasers are used as a source of radiation in these technologies.

Properties of Cell Membranes

Chakhotin paid considerable attention to determining the permeability of the cell membrane. An elegant technique based on the analysis of fluorescence photobleaching recovery (FPR) allowed studying the processes of lateral mobility, diffusion, and convection of cell membranes components [Axelrod et al., 1976]. The primary idea of the method is related to the photobleaching of a small spot on the fluorescent surface by a brief exposure to an intense focused laser beam. After a certain period of time, neighboring, unbleached molecules diffuse into the photobleached region, restoring the fluorescence. Excitation of fluorescence is performed using laser radiation attenuated about 10^3 times.

The character of the recovery curve contains information about the diffusion properties of lipids, hydrophobic peptides, and membrane proteins. The FPR method has high reproducibility and the irradiation does not affect the diffusion constants, cell morphology and membrane permeability.

Cell Sorting

The impact of the focused laser radiation on cells allows sorting the cells using flow fluorometry [Mullaney and West, 1973]. The principle of the method is based on the flow of a suspension of cells passing a laser beam. The method includes measuring the absorption and scattering or fluorescence of the cells with subsequent sorting according to certain criteria. The signal obtained after the interaction of a focused laser beam with the cell is controlled by an electronic system, which charges the cell. The cells thus processed, pass through the electric field, deflecting in one direction or another, in accordance with the charge.

Laser flow microphotometry and microfluorometry of cells can be used to calculate the content of DNA and proteins in cell populations, the presence of virus-infected cells and the determination of cell surface antigens. It is also possible to analyse the relative content of cells in various stages of development. The method also allows studying the mechanism of action of various chemicals on cellular activity, detecting malignant cells, white blood cells, etc.

Measuring Bioelectric Currents

Ions that move through a membrane have an electrical charge, so an electric current is generated in the membrane. Since the time when Chakhotin used a double microneedle to affect the cell, the technology of measuring

bioelectric currents has undergone significant changes. It should be noted that such experimental approaches as *intracellular recording* provide for dipping the electrode into the cell and measuring voltage and/or current across the membrane (A.L. Hodgkin and A.F. Huxley who developed this technology were awarded the Nobel Prize in Physiology or Medicine in 1963)[Hodgkin and Huxley,1952]; a *voltage clamp* is used to measure the ion currents through membranes of excitable cells while holding the membrane voltage under experimental control with a feedback amplifier; a *current clamp* records the membrane potential by injecting a current into a cell through the recording electrode making it possible to study how a cell responds when electric current enters a cell; the *patch-clamp technique* establishes a close contact between a polished glass microelectrodes (micropipette) with a diameter of 0.5-1 μm and the membrane; the small diameter of the micropipette make it possible to measure currents through individual ion channels (E. Neher and B. Sakmann received the Nobel Prize in Physiology or Medicine in 1991 for their discoveries concerning the function of single ion channels in cells and development of the “patch clamp technique” [Neher and Sakmann,1992].

Bioelectric Phenomena in Photomovement of Microorganisms

The term *photomovement* encompasses any movement or its alteration induced by light. Participation of bioelectrical processes in the photomovement of microorganisms is supported by the results of a number of investigations [Posudin et al., 2010 and refs. therein]. Measurement of these processes during the photomovement of microorganisms is possible with the help of the following methods [Sineshchekov, 1991].

The above-mentioned *intracellular microelectrode* measurement during the study of the photoreception in unicellular alga causes mechanical damage of the cell during microelectrode insertion.

The second method is based on the registration of a blue light-induced electrical response in flagellates using *extracellular electrodes* that do not penetrate the cell but are placed closely to the cell surface. This approach makes it possible to assess periodic local changes in the state of the cell membrane.

The more advanced method uses a *measuring suction micropipette* that sucks the cell into the tip of the micropipette and measures the potential difference between the inner and outer surface portions of cells separated by a pipette. This method allows measuring photoelectric responses of algae during several hours without damaging the cell.

Micro-Irradiation of a Cell by Ionizing Radiation

Chakhotin's ideas concerning the use of ionizing radiation for local effects on cell and cell organelles have been subsequently elucidated. Interest in this type of research has increased especially after incidents at nuclear power plants (Chernobyl, Fukushima). The annual report of the International Workshop on Microbeam Probes of Cellular Radiation Response (see, for example, Microbeam Workshop 2015), demonstrate the enormous scale of research in this area.

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CONCLUSION

In the preceding pages, my objective was to acquaint the reader with the milestones of life's path and the scientific and social activities of a remarkable biophysicist, microbiologist and cytologist, and the founder of modern forms of political propaganda, a scientist of international stature, Sergei Stepanovich Chakhotin.

Chakhotin was a pioneer in the development and use of micromanipulation, ultraviolet micro-irradiation technology and certain electrophysiology techniques. He has achieved results that continue to amaze and inspire scientists doing research on living cells to this day. It is important to remember that ultraviolet radiation can not pass through the microscope optics made of traditional glass; it was necessary to use quartz optics to focus ultraviolet radiation using a microscope. Likewise, it was not easy to find sources of ultraviolet radiation nor to determine its spectral composition in 1912.

His exceptional experimental approach as a scientist, his innovative solutions to solving methodological problems and his scrupulousness in trying to analyze the results engendered admiration among his peers. Many of the ideas and technical solutions developed by Chakhotin have evolved into a variety of modern instruments and methods

Chakhotin demonstrated, based on the teaching of Ivan Pavlov on conditional reflexes, that all life forms struggle to survive using four instincts that can be seen as a complex of unconditioned reflexes which facilitate the interaction of organisms with their environment.

Chakhotin came to the conclusion that freedom, peace, and charity should be integral parts of our nature and reflexes that are fixed deeply in every human. Achievement of this goal is possible in accordance with the teaching of Pavlov by the judicious formation of corresponding conditioned reflexes, propaganda and especially education.

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ABOUT THE AUTHOR:

Professor Yuriy Posudin, Doctor of Biological Sciences, National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine. He studied at the Kiev State University (Radiophysical Faculty) 1964-1969, the Institute of Radiotechnique and Electronics, Moscow (1972-1975), and the Agrophysical Institute, St.-Petersburg (1992).

Dr. Posudin's principal scientific interests are the investigation of photobiological reactions of algae and plants, and the application of the methods of optical and laser spectroscopy for non-destructive quality evaluation of agricultural products and environmental monitoring.

The academic duties of Dr. Posudin include lecturing on a cross-section of environmental topics, e.g., "Environmental Biophysics", "Methods of Measuring Environmental Parameters", and "Environmental Monitoring with Fundamentals of Metrology".



Dr. Posudin has taught and/or conducted research at a number of research institutes and universities around the world (e.g., Italy, Spain, U.S.A., Japan, and Germany).

The author is aware that probably not all the facets of the talents and contributions of Sergey Stepanovich Chakhotin were disclosed to the same extent. As a consequence, suggestions for improvement of the book and/or new information about this remarkable scientist will be received with deep gratitude.

Please send your comments on the contents of this book to the following address:

Prof. Dr. Yuriy Posudin,
National University of Life and Environmental Sciences of Ukraine,
Kiev, Ukraine
Geroiv Oborony St., 15, Kyiv, 03041, Ukraine
e-mail: posudin@nubip.edu.ua

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Monograph

YURIY POSUDIN

**SERGEI CHAKHOTIN
– HIS CONTRIBUTIONS
TO SOCIAL PSYCHOLOGY AND
BIOPHYSICS**

Edited by Pierre Tchakhotine and Stanley J. Kays

Kiev 2015

Yuriy Posudin

**SERGEI CHAKHOTIN
– HIS CONTRIBUTIONS TO
SOCIAL PSYCHOLOGY AND BIOPHYSICS**

This book is a biographical critique of the life and the scientific and public activities of Sergei Chakhotin, an outstanding biophysicist, tireless innovator, an expert in crowd psychology and political propaganda.

He was the first to use focused ultraviolet radiation to alter a living cell and developed a series of unique methods and tools for microscopic operations on test objects.

Chakhotin studied also the problems of social psychology focusing upon human instinct and conditioned reflexes, particularly with regard to the behaviour of masses, and to elucidate the mechanism of transforming a large segment of the population such that it could be governed by leaders by means of political propaganda.

The impact of the ideas of S. Chakhotin on science today is shown.

