

SURVEY OF CONTEMPORARY
KNOWLEDGE OF
BIOGEOCHEMISTRY

2. THE BIOGEOCHEMISTRY OF VANADIUM

DIDIER BERTRAND

BULLETIN
OF THE
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2. *THE BIOGEOCHEMISTRY OF VANADIUM*

DIDIER BERTRAND

Institut Pasteur

TRANSLATED BY VERA LEE

IN SCIENCE, THERE IS NOTHING SO SIMPLE AS THAT
WHICH WAS DISCOVERED YESTERDAY, BUT NOTHING SO
DIFFICULT AS THAT WHICH WILL BE FOUND TOMORROW

J. B. BIOT

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PREFACE

THE "SURVEY OF CONTEMPORARY KNOWLEDGE OF BIOGEOCHEMISTRY" owes its existence to the generosity of Robert Earll McConnell and George Monroe Moffett, and to the imagination and foresight of Albert Eide Parr, Director of the American Museum of Natural History. The purpose of the survey is to bring together, and publish as a series of monographs, the available information on all aspects of the interrelation of biology and geochemistry. The contact area between these two sciences, which have only recently begun to influence each other, is of direct concern in many of man's efforts to cope with his environment. This is obviously true in agriculture, in the utilization of aquatic resources, in human and animal nutrition, and in certain branches of medicine. It is equally true that aspects of biogeochemistry are involved in our understanding of the history of the earth and its inhabitants, in fact whenever questions arise as to the origin, potentialities, and fate of mankind. In order to consider adequately both the details of practical importance and the more general principles of theoretical significance, all contributors to the series are asked to cast their nets widely, and to report whatever of interest they may land. Much of the information that is available in theory is published in such scattered, miscellaneous, and inaccessible

works as to make it at present of very little utility. As such information is brought together, the interrelation of many previously disconnected facts becomes increasingly evident, and new generalizations emerge not previously available, because the facts known in theory were in practice never known by one and the same person.

The survey is intended as more than a synthetic summary. As much emphasis is to be placed on the lacunae in our present knowledge as on its established content. By defining the gaps carefully, a program for research is developing. Wherever well-defined problems can be investigated in the laboratory without prejudice to the development of the Survey, such studies are being made, but an enormous mass of uncertainty is bound to be disclosed without the prospect of any immediate investigation by those engaged in the project. It is, however, hoped that other workers will be attracted to the field by the inherent interest of the unsolved problems of biogeochemistry.

G. E. HUTCHINSON

Consultant in Biogeochemistry
The American Museum of Natural History
Professor of Zoology, Yale University

New Haven, Connecticut
July 7, 1948

INTRODUCTION

GABRIEL BERTRAND, in the first lecture of his course in biological chemistry at the Faculté des Sciences at Paris in 1905, said: "At first we believed life to be a sort of unfathomable mystery, something supernatural which we would never be able to penetrate. Then, little by little, with the progress of observation and experimentation, we came to think that life is perhaps the result of a specific mechanism, very delicate, very complex, but nevertheless accessible to our means of investigation." He also gave this definition: "Life is an integrated collection of phenomena by virtue of which creatures of complex structure feel, assimilate, and reproduce."

Since that time biochemistry, which was still in an embryonic state, has made enormous progress, especially in recent years, when the number of publications has taken on an "explosive" aspect, the speculative interest and useful consequences of which are incalculable. It is, however, curious that we still do not know the exact elementary composition of living organisms. Although no one would think of denying the biological importance of elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, iron, and even magnesium, which enters into the composition of chlorophyll, very few know that the elementary complexity of any sort of living organism is very much greater. This is probably due to the fact that the other elements found in plants and animals exist in only very small quantities. One might ask if these substances play a physiological role. They might, of course, only be impurities absorbed with the elements that are indispensable to life. Trinchinetti (1845) and later Deherain (1878) demonstrated that a healthy plant, watered with solutions of various salts, absorbed indifferently all the metals, even those that were capable after a time of bringing about the death of the plant. The experiments of Pfeffer (1886-1888) with certain aniline dyes confirmed and extended this idea. Since that time numerous experiments made with many of these metals or metal-loids indicate that a plant cannot live if they are removed completely from the medium in which it is grown; an excess, on the other

hand, becomes injurious. This is the law of optimum nutritive concentration of Gabriel Bertrand, which this scholar set forth as early as 1903 at the International Congress of Applied Chemistry in Berlin. He achieved absolute proof of this law for manganese in 1905 (1905b, 1912b), and in 1912 (1912a) he gave a lecture in New York entitled "On the role of trace substances in agriculture."

Analytical experimentation has shown why this fact had escaped the investigators; in most cases plants find in the media in which they grow a sufficient quantity of the elements that are indispensable to them. The significant but minute amounts of such substances may often be below the ordinary limits of chemical analysis. The same situation obtains for animals. We may agree with Lord Kelvin that our knowledge is very poor if we cannot apply a measure to it. Since then, there has been an increased interest in the study of such questions by investigators throughout the world. A few well-known examples may be mentioned: the deficiency in copper in plants in certain regions (Sjollem, 1933), in boron in sugar beets (G. Bertrand, 1939), such deficiencies entailing considerable agricultural losses, and the anemia in New Zealand sheep resulting from lack of cobalt (Aston, 1935-1936; Denham, 1937).

The constant presence of an element in an organism is not sufficient to demonstrate that this element is part of its living matter. Analytical observation must be supplemented by physiological experimentation. The latter, moreover, should not be limited to verification of the favorable action of the element studied; it should be capable of demonstrating that in the total absence of this element, life, or some physiological function, ceases. It is indeed possible that an element might have a useful physiological effect without existing normally in living matter.

To date, of the 92 known elements about 50 have been found at least once in animal or vegetable organisms. Among these vanadium, by virtue of its chemical nature, seems of particular interest.

Since I have had occasion to consider the distribution of vanadium in living matter, Prof. G. Evelyn Hutchinson, to whom I am especially indebted, has done me the honor of asking me to prepare a monograph on the biogeochemistry of vanadium.

Research in the literature was done in the absence of the bibliography published by Willis (1939-1942) and under rather difficult circumstances, namely, during the years when Paris was occupied by the Germans. This work has been completed since their departure, and I have thus been able to correct numerous errors in previous summaries, including some of my own. The work done by the allied nations since 1940 has been made available through the medium of Chemical Abstracts, and I wish to thank the American Documentation Services as well as Professor Hutchinson for the aid which they have given me in permitting me to read certain original works. As a rule, and

in accordance with a tradition honored in the Laboratory of Biological Chemistry of the Pasteur Institute in Paris, I endeavored to read the original publications. This, however, was not always possible. If some errors or omissions persist, I hope that they will be adequately compensated for by the other references.¹

May I be permitted, in concluding this introduction, to express my gratitude to my teacher, who is also my father, from whom W. I. Vernadsky in the course of frequent conversation drew many of the ideas for his book on geochemistry (1924a), especially those relating to the oligoelements.

¹ In order to render the results more homogeneous, all figures have been converted to parts per million (p.p.m.), e.g., 0.1% = 1000 p.p.m., 0.01% = 100 p.p.m., etc., of vanadium metal, save in a few cases where large proportions made it advisable to use grams per thousand. No reference has been made to frequently cited publications that contain neither original work nor new ideas or in which the bibliography is very incomplete.

VANADIUM IN THE GEOSPHERE

FOR VANADIUM to be found in plants and *a fortiori* in animals which, directly in the case of the herbivores and indirectly in the case of the carnivores, feed upon the former, this element must necessarily be universally distributed in the soil. The knowledge of its diffusion in arable soils is then fundamental,

but it is not wholly without interest to glance at its over-all geological distribution in rocks and waters which cooperate in the formation of cultivatable soils, as well as in sediments of certain biological origin such as the coals, or those of still disputed origin such as the petroleum.

VANADIUM IN ROCKS AND SEDIMENTS

We may probably credit the first observation of the presence of vanadium in an unmined substance to Beauvallet (1859), who found it in clay (or actually in ceramics) from Gentilly (the locality is erroneously referred to in the literature as Chantilly). This writer had in 1858 noticed yellowish efflorescences on certain pottery that had been insufficiently rebaked; analysis revealed the presence of alkaline vanadates. He notes that Terreil of the Paris Museum, using the same method, did not find any in various clays from the Paris region. Nevertheless one year later Terreil (1860) published a paper entitled "De la présence du vanadium dans les argiles de Forges-les-Eaux et de Dreux," which apparently passed completely unnoticed.

He found only traces in the Dreux clay, but as he had used material from the potteries he supposed that they might have been washed by waters which, according to the observations of Beauvallet, would have eliminated almost all the vanadium. On the other hand, in the Forges-les-Eaux clay he estimated the presence of a quantity between 100 and 1000 p.p.m. (rather closer to 100). He failed to find vanadium in other clays from the Paris region.

Forchhammer in 1864 again found vanadium in some clays. Although there were no other indications of the distribution of the element available in the world literature, it seems that, since 1875, vanadium has been regarded as widely dispersed outside ore deposits. Terreil wrote as follows in 1875: "A great number . . . of rocks and clays contain small quantities of vanadium." We must, however, wait for Hayes (1875) to find this metal in the rocks of Tinto District expressly mentioned (but with neither ex-

perimental details nor figures). Seger, in 1877, found it again in clay, and Bechi (1879) mentioned it two years later in the minutes of an academic meeting but without the slightest detail.

Sainte-Claire Deville in 1859 had shown that the bauxites were rather rich in vanadium, so that one might consider them as vanadium ores. Dieulafoy in 1881, basing his statement upon the preceding observations and having noted that argillaceous sediments which were rich in vanadium were also rich in titanium and belonged to Paleozoic formations, did not hesitate to establish an explanatory theory of the complete diffusion of titanium and vanadium in rocks of this era.

It is regrettable that the cult of precision, which is currently popular, has not had since that time some initiates to occupy themselves with this question. The result has been that the idea of the presence of vanadium in clays was considered common knowledge, so that it did not seem necessary to prepare further publications on the subject and to investigate more precisely the possible interrelationships between the amount of vanadium in clays and their geological origin. So that, except for a paper falsely attributed to Stolba in 1896 which in reality was only one of many from the pen of the reporter H. (1896) and in which without specifying his sources the author devoted himself to some considerations on the possible state of vanadium in bricks, it was not until 1903 that Mingaye announced that he had found 140 p.p.m. V in a white clay, confirming the figures obtained by Terreil 43 years previously. Fioletov in 1927 and again in 1929, studying some Russian clays and kaolins, found first from 11 to 210 p.p.m. V, later

often more than 280 p.p.m. Two writers inform us about Japanese clays. Sitoyasu Iimori (1930) found 600 p.p.m. V in a red clay. This is probably exceptional, as Sinya Oana (1938) found not more than 130 p.p.m. in three other red clays from the sea bottom. This order of magnitude was also given by Leutwein (1941) in a review of the geochemistry of vanadium in which he gave figures for clays varying from 110 to 140 p.p.m. Recently, Ahrens (1945), using spectrographic methods, indicated the presence of vanadium in 34 clays of different types from various South African localities. Until further investigation is carried on, we must consider that, save in exceptional cases, there are approximately 100 to 200 p.p.m. V in clays of various origins, and we shall see that this figure is not without interest.

We are much better informed regarding the consolidated rocks. Hillebrand (1898) in a remarkable paper gave a table of 64 groups of diverse rocks, in only five of which did he not find vanadium. His figures varied from 8 to 2100 p.p.m., the latter figure relating to an amphibole from California and being rather exceptional. The following year Vogt (1899) published his results, and adding his efforts to those of his predecessor he estimated the content of vanadium in the earth's crust to be 20 to 50 p.p.m. Having noted in passing that Mingaye (1903) found 170 p.p.m. V in basalt, we come upon the extraordinary work of Clarke (1908) based upon hundreds of analyses. This author estimated that the content of vanadium in igneous rocks is on an average 200 p.p.m. This figure was revised to include the sediments, giving 140 p.p.m. (Clarke and Washington, 1922). Later (Clarke and Washington, 1924) he gave 170 p.p.m. for eruptive rocks alone. His estimates for vanadium in the entire earth's crust rose from 100 p.p.m. in 1908 to between 129 and 133 p.p.m. in 1922.

Ramirez (1914) determined vanadium in numerous Argentinian clays.

Pondal (1930), by chemical means, determined vanadium in 15 rocks and found therein 17 to 282 p.p.m. V. The content is considerable in basic rocks and very slight in acid rocks.

After Pina de Rubies (1930) indicated by

spectrographic means the presence of vanadium in various Spanish minerals and rocks, including clay and marine sand, Hevesy, Alexander, and Würstlin (1930) gave more precise data by their analysis of 282 samples of eruptive rocks for which they found a mean of 200 p.p.m., in perfect agreement with the American estimates.

Jost (1932) provided some important data: if vanadium is present in calcareous and dolomitic rocks, there are normally less than 5 p.p.m. (24 samples), though one exceptional sample contained 36 p.p.m. On the other hand 45 samples of granitic rocks and sands contained from 10 to 170 p.p.m.; three contained from 220 to 500 p.p.m., and 11 samples less than 5 p.p.m., if any was present. The results obtained in the same year by Hill, Marshall, and Jacob (1932) who, in studying 46 samples of American rocks, found vanadium in only 20 of them (170 to 1350 p.p.m.), may be explained by the fact that the lower limit (170 p.p.m.) of their technique was too high. However, Sandell (1936), wishing to study siliceous rocks, made use of a more sensitive technique whereby he found from 5 to 150 p.p.m. V in 10 samples.

More recently, Friend and Allchin (1939), by spectrography which was confirmed chemically, found vanadium in a calcite from Carrington Park, England. Unfortunately, they gave no figures.

Bray (1942a) studied spectrographically with the aid of carbon electrodes the distribution of certain elements, including vanadium, in the igneous rocks of Jamestown, Colorado (and in another paper, 1942b, in the Tertiary rocks of the Front Range), and stated that there was more vanadium in muscovite and dark minerals. His figures varied from 6.5 to 18 p.p.m. for five samples. Shimer (1943) made similar studies of the granites and pegmatites of New England. Lundegårdh (1945), also by spectrographic means, found 15 to 500 p.p.m. V in nine rocks of eastern Upland, Sweden. Wager and Mitchell (1945), with a technique having a lower limit of sensitivity of 5 p.p.m., found 30, 30, 100, 100 p.p.m., respectively, in four samples of eruptive rocks of Skaergaard and none in two others.

If we know almost nothing of the form in

which vanadium is found in rocks, we may, despite the apparent contradiction among these various results, consider as conclusive the fact that in the earth's crust there are about 100 p.p.m. V, which is a concentration of the order of 0.01% in grams or of 10^{-4} as an absolute value. The clays would then be richer than the average rocks and sediments. With regard to recent sediments, a statement can be based upon unfortunately only two papers. Arkhangelskii and Kopchanova (1930) found from 30 to 170 p.p.m. V in certain Black Sea sediments and noted that this vanadium content varied in accord with the content of organic matter. Sinya Oana (1938) found in three muds of marine origin 50 p.p.m. V against 130 p.p.m. for red clays, as has been previously indicated. These muds and red clays while of the same geographical are evidently of very different geological origin.

The frequency of the presence of vanadium in the earth's crust is almost the same as that of Ni, Cu, Zn, or Pb, though it is more dispersed than are these elements (More, 1943). It is therefore more accessible in principle to plants than the other four metals, but, as we shall see with reference to its distribution in the biosphere, it is actually much less abundant than Ni, Cu, Zn, or Pb in living matter.

The fact that certain geological deposits are richer in vanadium than others has not been overlooked by investigators, and neglecting the purely chemical theories such as that of Dieulafait to which we have already referred, it is not without interest to recall that certain investigators have tried, perhaps prematurely, to integrate living organisms into the cycle of this element.

VANADIUM IN WATERS

The role of water, as much from the point of view of the biosphere as of the lithosphere, is considerable, and, if we avoid viewing it only as a carrier of suspended matter, we should expect to find in it vanadium which we have seen to be so well distributed in the rocks and sediments. Lindgren (1923), as we have said, did not even hesitate to suppose that this vanadium was to be found in the form of calcium vanadate. But in fact we know very little about this subject.

Hess (1914) probably was the first to put forward such a hypothesis. He considered the possibility of at least the partial intervention of living organisms in the origin of Colorado and Utah carnotites which are rather rich in vanadium. Phillips (1918), having found in a holothurian (*Stichopus mobii*) 1235 p.p.m. V in the dried animal, was more definite than his predecessor, in attributing an organic origin to vanadium in the sediments of Tortugas as well as in rocks and coal. Lindgren (1923), in a very detailed study of the cycle of vanadium, considered that a small proportion passed from the rocks into water, perhaps as calcium vanadate, and thence into the sea, where it was probably concentrated by the holothurians and the ascidians. After the death of these animals the metal returned to the sea and passed to the sediments. It is not impossible besides, he believed, for vanadium to be equally concentrated by other animals, perhaps by certain species of low phyletic position (possibly therein we may find the connection with petroleum, of which Lindgren was not cognizant). He surmised that vanadium should also be found in plants in replacing phosphorus, and he found evidence of this in the fact that the coals, especially the asphaltites, contain vanadium.

The following year Vernadsky (1924b) expressed again, at least partially, an identical point of view: "It is very probable that a large part of this vanadium (from different vanadic mineral layers) springs from living matter, for it is sometimes found in large quantities in organisms rich in vanadium (e.g., some holothurians) [*sic*]." At the end of this account, we shall see what opinion one must consider relative to such theories.

The first observation on the presence of vanadium in water was that of Hayes (1875), who wrote of having found it in the water of Brookline, Massachusetts. He gave no data either on the amount of water used or the quantity of vanadium found. The same is true of Witz and Osmond (1886) who examined the water of Deville, France. Bardet (1914), in a very thorough work on the French mineral waters, indicated in his preface that V. Mussi (no reference, date, or

details) had found vanadium in the waters of the Italian stations of Cortona and Rio, and that in the *Bäderbuch* published by the German Imperial Government vanadium was only indicated twice in 53 analyses of Italian and Austrian waters. Bardet claimed to have found vanadium by two-carbon-arc spectrography in the mineral waters of Chatel-Guyon (chlorinated, carbonated waters) in three out of eight springs in Cauterets in the water of the Parc Saint-Marie in Nancy, of Santenay in the Morvan, in the deposits of earthy carbonates at Vichy, as well as in the salts of Vichy water, though only in eight cases out of 45.

Although Bado (1917), studying by chemical means the drinking waters of Bell Ville (Cordoba, Argentina), found no vanadium in 50 liters from two springs of the River Tercero, he did find 1.32 p.p.m. in a third.

Bardet, Tchekirian, and Legrange (1938) found traces of vanadium in sea water, but they had to use 40 liters of liquid and a spectrograph to arrive at this result. In 1939 Charola, who did not cite his predecessor Bado, found by spectrographic means 1.46 p.p.m. V in the drinking waters of Bell Ville, a figure that is in accord with that of Bado.

The first accurate work came from Kazuo Kuroda (1939) who studied nine Japanese hot springs and found for five of them from 0.022 to 0.079 p.p.m. V and for three 0.208, 0.220, and 0.247 p.p.m. V. On the other hand, he found none at all in the hot spring of Marasugi. After Strock (1941) discovered it by spectrographic means in Saratoga mineral water, Kazuo Kuroda (1941) found 0.051 p.p.m. in the mineral water of Kinhei, Japan. However, with a technique specially adapted for soil, D. Bertrand (1942a) found

none in Atlantic sea water collected on the coasts of France. His method would have permitted detection of 0.001 p.p.m. Noddack and Noddack (1940) similarly could not detect the metal in sea water using a spectrographic technique of almost equal (0.003 p.p.m.) sensitivity.

The spectrographic results prove that in general there cannot be much vanadium in natural waters. Nevertheless we cannot state absolutely that sea water is as poor in vanadium as the results of Noddack and Noddack and D. Bertrand lead one to believe. In fact, though this last author recovered 0.001 p.p.m. V added in the form of ammonium metavanadate to the sea water studied, it is not certain that vanadium in another form might not remain undetected. With reference to zinc in sea water, G. Bertrand (1938) had shown that although the methods used up to that date only permitted one to find proportions of the order of 0.002 p.p.m., there was in reality more than 1000 times this amount present. This enormous difference was simply the result of an analytical error that had not been considered. Certain mineral springs in Japan and Argentina, moreover, may be rather rich in vanadium, but it is well to note that in the latter country there exist asphaltites and petroleum products that are exceptionally rich in vanadium and that, as a consequence, a geological exploration would possibly result in several surprises. The vanadium content of the Bell Ville water is sufficiently great to permit easy recognition of the element.

The problem of the percentage of vanadium in waters of diverse origins is evidently far from being solved and offers a vast field for study.

VANADIUM IN ARABLE SOILS

The study of the diffusion of vanadium in arable soils is in more direct relation to biogeochemistry than the preceding sections. On one hand, given a certain plant, we may ask what elements this plant takes from the soil, in what form and in what proportion these elements are presented to it, what the degree of assimilation is, and how this soil can be modified with a view to improving this to a maximum degree. On the other hand, the role played by the elements of the

living matter of plants in the formation of arable soils is of primary importance and has already been demonstrated. Unfortunately there is little information at our disposal in this field.

W. O. Robinson (1914) was the first to consider the vanadium content of soils. Analysis of 26 American soils gave a proportion of 28 to 220 p.p.m. V, which is on the whole quite analogous to quantities found in rocks and sediments. Thomas (1923)

in two additional American soil samples found at the surface 103 p.p.m. and at 1.06 meters 252 and 224 p.p.m. V.

Sitoyasu Iimori (1930b) did not find more than 14 to 60 p.p.m. V for some Japanese acid soils. We know nothing of the form in which this vanadium occurs, but Kame-taro Konishi and Toshihisa Tsuge (1933) showed by spectrographic analysis that one may find vanadium in the dialysable fraction of aqueous extracts of the soil and that as a consequence at least a part of this metal is not involved in a large silicate molecule. Byers (1934) found in the only American soil profile that he analyzed 132 p.p.m. V between 0 and 20 cm., as opposed to 129 p.p.m. between 20 and 45 cm.; if analytical errors are considered, the difference can hardly be significant. The problem was taken up again in the United States by Slater, Holmes, and Byers (1937) who, with a technique that permitted them to analyze for vanadium in soil in quantities of the order of several p.p.m., found the element in the 11 soils examined. Meanwhile in the same year Milbourn (1937) confirmed the presence of vanadium in soils, indicating important traces by spectrography (without giving the origin and number of soils). Hirai (1937) and Hirai and Takagi (1937) analyzed 61 different soils and found only two without vanadium. The others contained from 5 to 150 p.p.m. V, with a mean of 50, and Hirai concluded that according to his results the Japanese were less rich than the American soils. His work is especially interesting in that it gives the geological origin of the soils, and it may thus be seen that the soils of Tertiary origin are the poorest, those derived from Mesozoic rocks richer, but

less so than those derived from Paleozoic rocks, the respective means being 28, 65, and 90 p.p.m. We again find what has already been noted for the clays, namely, that those of Paleozoic origin are much richer in vanadium than the others.

In 1939, Rogers, Gall, Gaddum, and Barnette, contradicting the preceding results although they used graphite electrodes, detected vanadium in only 11 of the 76 Florida soil samples studied, using equal numbers of cultivated and virgin soils.

D. Bertrand (1942a), with a technique having a lower limit of 0.3 p.p.m., found, in 20 samples analyzed, from 3.1 to 68 p.p.m. V, these results relating to samples from a depth of 0 to 20 cm. in soils of various origins, namely, calcareous and granitic from France, as well as an alluvial soil from Italy, the muds of the Nile and Niger, and two soils from Denmark and Serbia. Two samples were particularly interesting as they were cultivated without manure, one since 1875 and the other since 1900, but nevertheless they contained 25 and 29.1 p.p.m. V.

The mean, which is approximately 22.5 p.p.m. V, shows that the French soils are clearly less rich than the Japanese, which are in turn poorer than the American soils, except for the soil of Florida.

It is apparent from the above data that there exists a very important geological and agronomical problem relating to the regional variability of the vanadium content of soils. The confirmation or disproof of this apparent variability would require an extensive investigation of the arable soils of the entire world by a single investigator in order to insure homogeneous results.

VANADIUM IN COALS

The vegetable origin of fossil fuels need not be discussed again, but we no longer believe in a simple transformation of tissues—branches, stalks, roots of *Cordaites*, arborescent or herbaceous ferns, etc. (or algae)—ending in humic matter, the principal element of coal. In the genesis of the different types of coals we find complex phenomena under the influence of various factors: oxygen, stagnation in water, increased temperature, pressure, and bacterial action. But as no one of

these factors can introduce new elementary matter into the future coal, it is absolutely useless to look for geochemical explanations to interpret the presence of the numerous elements that have been found in coals of diverse origins. These are simply derived from the plants (including algae) that have given birth to the coal, and if the proportions of the various metals or metalloids in coal ash do not correspond to those that may be found in the ash of modern plants, we must conclude

simply that the elementary composition of living matter at the end of the Primary epoch need not necessarily correspond to that of living matter in the Quaternary epoch. This is then the point of view that we shall adopt in the general conclusion of this study of the diffusion of vanadium throughout the world.

We are indebted to Torricco y Maca (1894) for the first observation of the presence of vanadium in coal (with reference to petroleum we shall see that the first observations often confused the asphaltites with the coals). This investigator found 2400 p.p.m. in the ash of lignites from Yauli, Peru. Baskerville (1899) in three samples of peat found, respectively, 3, 7.3, and 8.7 p.p.m. V, while Mingaye (1903) succeeded in finding up to 335 p.p.m. in coal ash. Although having relatively little information, since 1905 the Argentine Government envisaged the use of coal ash as a source of vanadium and estimated 680 p.p.m. as the vanadium content of the ash of Mendoza coal. No results were published relative to European coals until Jorissen (1905) announced the discovery of vanadium in the coals of the Liège Basin. Unfortunately the writer, who gave no data, carried on his research in the presence of a mixture of soot and dust, and it is difficult to determine the true source of the vanadium he found. This question was completely forgotten until Yoshimura (1930) determined vanadium in some Japanese samples, namely, a coal from Hujo, in the ash of which he found 310 p.p.m. V, and in a semi-anthracite in which he found only from 34 to 100 p.p.m.

Goldschmidt (1935) and Goldschmidt and Peters (1933) using X-ray spectrography, demonstrated the presence of vanadium in various English and German coals. Unfortunately they made no quantitative determination, and we must wait for the important work of Zilbermintz (1935) to be better informed, at least with regard to the Russian coals. More than 500 samples were examined; these contained from 56 to 80 p.p.m. V in the ash, except for one sample of Jurassic age which appeared to be exceptionally rich, containing from 1500 to 24,600 p.p.m. V. After noting the communication of Lefebvre and Boulant who, at the Seventeenth Congress of Industrial Chemistry in Paris (1937) indi-

cated that they had by arc spectrum discovered small quantities of vanadium in all the ash of coals from the French provinces of Nord and Pas-de-Calais, we find the work of Bader (1937b) who found 140 p.p.m. V, also using spectrography, in the ash of a graphite from Passau. Maucher (1938) had previously found considerably more in the same graphite, but in this paper noted his errors and after a new determination found from 100 to 500 p.p.m., which he stated was in accord with the results obtained by Bader! This demonstrates the inaccuracy inherent in some analytical methods.

Nazarenko (1937), in studying some coals from the Don, the Kuznetsk Basin, and the Ukraine, found in the ash of 21 samples vanadium in the same order of magnitude as in the earth's crust, and as the vanadium content increased with the amount of ash he concluded that this vanadium is of mineral origin. After the brief review of the origin of coals given above this cannot be true save in the sense that plants remove from the soil the mineral elements that we find in their ash. Vakhrushev (1940) studied various coals of Permian and Mesozoic age but did not find any quantities exceeding 56 to 170 p.p.m. V in the ash, the richest being the argillaceous coals of Samara. Vorob'ev (1940), again using Russian coals, found from 28 to 3600 p.p.m. V in the ash. His results are interesting since, as he noted, the Paleozoic coals of Asia Minor have a greater vanadium concentration than those of the Mesozoic.

Compared with the preceding data, the quantities of vanadium found by Abbolito (1942) in samples of coal from Carbonia, Vaccinella, and Ribola are quite ordinary, namely, 6000, 3600, and 1600 p.p.m. The presence of vanadium in coals has been recently confirmed by Legraye and Coheur (1944-1945) and by Gibson and Selvig (1944).

To be complete from a historical point of view, it is necessary to recall that in 1864 Rammelsberg had indicated the presence of vanadium in the lixivate from crude soda, confirmed the following year by Baumgarten (1865). Scheurer-Kestner believed in 1883 that the vanadium could be derived only from limestone or coal involved in the production of the soda, and this was confirmed by H. L. Robinson (1894) and Torricco y Maca (1894).

The important analytical results are classed then in two groups: first, the coals in the ash of which the vanadium content varies from 30 to 300 p.p.m., a content which, as we shall see, only slightly exceeds (and is in the same order of magnitude as) that found in the plants themselves; second, those in which it varies from 1500 to 6000 p.p.m. and more, the coal of Mendoza, Argentina, being intermediate between these two groups. The results are sufficiently homogeneous to permit the statement that the Paleozoic coals are much richer in vanadium than those of more recent formation. We have seen that the argillaceous sediments of Paleozoic origin are richer than those that are geologically younger; there is then a remarkable agree-

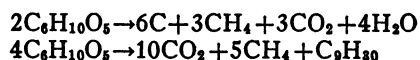
ment, but this is not enough to explain the great difference between the contents of the two groups, especially when Zilbermintz showed a sample of Jurassic and consequently Mesozoic origin to be in the category of coals that are richest in vanadium. He found indeed up to 24,600 p.p.m. V in this material, a figure that has never been equalled. A further explanation, which can be established only by a special study of the problem, is necessary. Either the plants that produced the coals rich in vanadium belong to families that are themselves exceptionally rich in the element, or the mode of origin of these coals is complex. An analogous problem will arise with reference to petroleum.

VANADIUM IN PETROLEUM, PETROLIFEROUS SCHISTS, BITUMINS, AND ASPHALTITES

At the present time the organic theories of the formation of petroleum have the most numerous supporters. As the study of these theories is somewhat beyond the scope of this paper, we shall only recall briefly some of the more recent ones, following Baud (1942). Anyone familiar with the English language may profitably consult the articles of Trask (1936), Brooks (1936), and Zobell (1946).

We no longer accept the hypothesis of a simple distillation of carbonaceous deposits brought about by igneous rocks coming in contact with bituminous schists or with coals rich in volatile materials. But vacuum distillation of coal and the study of carbides extracted with benzene have established in the eyes of Lewis, of Maberg, and of Richet a vegetable origin for naphtha and its association with coal. In certain oils from California, as in the "tar" of vacuum distillation, we have recognized the presence of hexahydromesitylene, C_9H_{18} , while melene, $C_{30}H_{60}$, figures among the constituents of Galician oils. In coal, as well as in petroleum, we find resins, sulfur, and phosphorus. Sands full of mineral oils and veins of coal are usually separated in horizontal beds (Pascoe, *vide* Baud, 1942). Finally, in Burma and in California, an "extraordinary abundance of fossil wood" preceded the petroliferous periods (Spielmann, quoted in Baud, 1942). The fermentation of "terrestrial resins" and the decomposition of "marine plants" may be

expressed respectively by the two reactions:



Methane would form, moreover, a link between coal and petroleum.

We may then formulate as follows the organic theory adopted today by the majority of geologists and chemists (L. Bertrand, Raguin, *vide* Baud, 1942).

Accumulated at the bottom of lagoons containing stagnant water or at the bottom of the sea, under the superficial zone of oxidation and agitation by waves and currents, an enormous mass of lower organisms (unicellular algae and the lowest animals)—plankton very specially adapted to this environment—might be deposited in a dense slime (sapropel) and become the seat of an intense anaerobic fermentation, acting as much on the cellulose and the lignin as on the fatty substances and the olefinic alcohols, the presence of multiple salts modifying somewhat the ordinary course of the putrefaction. In hardening, the "sapropel" might have become an argillaceous, siliceous, or calcareous rock, impregnated with the products of transformation and constituting the sapropelite or mother-rock, whence the oils might have reached the depot rocks by a series of slow migrations.

Despite the magnitude of the occurrences nothing is opposed to the view that the pri-

mary material from which petroleum is formed consists of successive accumulations of living organisms accumulating under suitable physicochemical conditions. In particular, the transformation of fatty matter under the action of anaerobic bacteria would seem important, this phase being followed, according to White, by a geochemical phase which is a function of pressure, of temperature, and of time. This view is still under discussion, as is also the theory of Taylor on the role of siliceous, calcareous, or argillaceous mineral sediments deposited with the organic matter.

The organic theory explains the presence of sulfur and of phosphate in crude petroleum as well as its optical activity. Moreover, the relative richness of the saline, which always accompanies naphtha and is of marine (Mazec) or of organic (Macovei) origin, is an indisputable index of the destruction of enormous masses of organic materials by their transformation into hydrocarbons.

Let us see how this relates to vanadium. We are indebted to Kyle (1891) for having directed world-wide attention to this matter by announcing that he found vanadium in an asphalt, rafaelite, which he erroneously described as coal from the province of Mendoza, Argentina. Not having enough samples, he was unable to determine the exact percentage of metal in the ash (0.63%). One year later (1892) he found 107.0 grams V per kilogram of ash (675 p.p.m. V for the asphalt itself), an enormous figure that was confirmed by Mourlot in 1893, who found 680 p.p.m. In spite of the interest that such a fact might have aroused, it was not until 1910 that Hewett analyzed a series of asphaltites from Peru and found on an average 530 p.p.m. V in the ash and 5.8 p.p.m. in the asphaltite itself, a considerably more modest figure and one that is not remarkable when compared with the concentrations that may be found in coals. Longobardi and Camus (1911), however, in a typical Argentine petroleum found 33,300 p.p.m. V in the ash (12.6 p.p.m. in the petroleum itself). Little further was noted until 1921 when Baragwanath, studying some asphaltites from Llaczacocha, central Peru, found 14,000 to 44,600 p.p.m. V, with a mean of 19,300 p.p.m. V (for some very high ash contents, as they varied from 10% to 20%).

He also stated, without giving the slightest reference, that the presence of vanadium in the ashes of these asphaltites had been known since 1892.

Hess (1922) found 2300 and 11,700 p.p.m. V in one of the two asphaltites from Utah, while Hackford (1922) confirmed the presence of vanadium in Mexican petroleum. Guthrie (1923) noted traces in petroleum from Hurghada, Egypt, and Dunstan (1924), using samples from Maidan-i-Naftum, found 14,100 p.p.m. V in the ash (1.41 p.p.m. for the petroleum). Porter (1924) was thus in a position to speculate on the association of vanadium with various organic deposits. Once attention was drawn to this subject, there was increased discussion, but unfortunately no serious comprehensive study was made.

Thomas (1924), however, at a time when the origin of petroleum was still being debated, attracted attention to the high vanadium content of the ash of certain mineral oils and to the fact that high vanadium contents had also been found in certain asphaltites, especially those of Peru.

Neubronner (1925) found 100 to 110 p.p.m. V (69% of the ash) in the bitumates of Schwaben, Germany.

Broz (1930) found the extraordinary amount of 380 to 450 grams V per kilogram of ash of crude petroleum from Maracaibo, Venezuela. The following year, using crude oil from the Texas Pacific Coal and Oil Company of Fort Worth, Shirey (1931) found for the ash of the six samples studied: Oklahoma 62, Persia 14.2, Texas Panhandle crude oil of the Prudential Oil Corporation 4, Kansas crude oil of the Vickers Petroleum Company of Wichita, Kansas, 1.2 grams per kilogram of ash, and only traces for the other two (mid-continent crude oil from the Standard Oil Company of Indiana and Wyoming crude oil from the Standard Oil Company of Indiana). This would indicate that the vanadium content is subject to considerable variation, not only for different regions but also for petroleum of the same origin. At the same time, Ter Meulen (1931), in the course of a study of the diffusion of molybdenum in nature, was surprised by the vanadium that he encountered in five petroleum of varied origin: heavy oil from Babaafshe Petroleum Maatschupij (138 p.p.m. for the ash), crude oil

from Persia (15.2), Mexican crude oil (164), Venezuelan oil (1.35), and oil from the Netherlands Indies (5.6); he thus encountered a range of concentrations from 1.35 to 138 p.p.m. V in the ash. Longobardi (1934), one of the first investigators to study this question, analyzed the various hypotheses put forward to explain the presence of vanadium, as well as the consequences of the deductions on the origin and the formation of petroleum. He also reported the work of Corti (1926) who found no vanadium in the petroleum of Juguy, Rivadavia, and Huineul, Argentina, although there was 191 p.p.m. V in the ash of a rafaelite from Auca Mahuida and 232 p.p.m. V in the ash of a coal from Marlagüe. Sürü (1935) in 14 Hungarian petroleum and in one from California found from 0 to 50.7 grams V per kilogram of ash. But he concluded that whatever hypothesis might be suggested with reference to the origin of vanadium in the petroleum, the latter is exogenous. His paper contains an interesting bibliography.

Vinogradov and Bergman (1935), analyzing 24 Russian bitumens and petroleum, found 1 p.p.m. V in the petroleum itself and 1000 p.p.m. in the ash. In two of their samples (Baku and Krasnovodsk) they found no vanadium, and only traces in two others. The positive figures varied from 100 to 28,630 for the ash but did not exceed 1000 p.p.m. in 10 samples. According to them, most significantly the quantity of vanadium is proportional to the quantity of organic matter in the petroleum and not to the ash, contrary to the theory of the geochemical origin of these substances and in favor of the view that petroleum originates from marine organisms such as the ascidians. They refer to Treibs (1935) who demonstrated the existence in a bitumen of a complex of vanadium and a porphyrin. This fact among others permitted Bader (1937) to conclude that the vanadium enrichment of organic sediments cannot be due principally to the accumulation of the remains of animals whose blood contained vanadium. He considered vegetable remains to be more abundant in the bitumens and the asphalt, so that it seemed to him that vanadium, after oxidation by atmospheric O_2 , might be combined with vegetable porphyrins in the form of complexes. The vanadium

content of organic sediments would thus be largely dependent upon the vanadium content of the solution in contact with the deposits and the porphyrins present that are able to combine with the vanadium. Ingenious as this hypothesis is, it does not appear to have gained great favor; in no case has an attempt been made to test it on the bitumens, so amazingly rich in vanadium, that had been discovered prior to Bader's paper. This matter of the origin of vanadium in petroleum and bitumens had moreover been the object of a study at the same time by Vinogradov (1936) and, in a more restricted manner, by Fester and Cruellas (1936) in a comparison between the asphalt of Peru and the observations made in the Argentina National Review by Fester, Cruellas, and Baron (1939). These authors, in their comparison between Argentine asphalt and the Peruvian material, found equivalent amounts in both, 310 to 2450 p.p.m. V. In the Argentine and Venezuelan petroleum, vanadium was found in the oil containing asphalt, the largest quantity being included in the last fractions of the oil and in the residues. These facts are given as a confirmation of the theory according to which vanadium would be the principal cause of formation of asphalt. Such results, though in direct contradiction to the analyses of Hewett, are in accord with those of Baragwanath and abolish a supposedly essential difference between the Argentine petroleum and asphalt and those of Peruvian origin. Bergman (1940), again taking up the comparative study of the vanadium contained in Russian mineral oils and bituminous rocks, found in the region of Saka 83.5 grams V per kilogram of ash in certain asphalt, as opposed to 8.35 grams per kilogram in the bitumens, demonstrating that in spite of the very high vanadium content of the latter the asphalt are incomparably richer in the element.

At the same time, Vakhrushev (1940) found from 2800 to 8000 p.p.m. V in the ash of bitumens from the Urals (57.5% to 78.7% in the ash), while in the coals the quantity present varied only from 56 to 170 p.p.m. for the ash, the greatest content being that of an argillaceous coal from Samara. One year later, Gulyaeva, Itkina, and Romm (1941) found vanadium in the ash of 17 petroleum

from Baku and in two from the peninsula of Aspheron, the highest content being 35.5 p.p.m. for the petroleum itself. Assarson (1941) demonstrated the presence of 200 to 2000 p.p.m. in the oil of Swedish schists. This vanadium is combined with sulfur in the schists and, contrary to the Russian observations, the schists rich in organic matter do not contain more vanadium than those that

are poor in organic matter.

At the end of this paper we shall see what conclusions may be drawn from these analyses. But it is well to notice at this point that the principal divergence that exists between the analytical results may perhaps be easily explained by noting that in all likelihood vanadium is found in the bitumens and not in the petroleums themselves.]

VANADIUM IN PLANTS

STATICS

RELATIVELY LITTLE WORK has been done on the vanadium content of living organisms. The considerable body of research on vanadium in the lithosphere, which we have just had occasion to analyze rapidly, and especially the occurrence of high vanadium concentrations in certain coals and asphalts should have attracted more attention. The lack of research is probably due to the fact that normally plants contain very little of the element and, because the analytical difficulties in its determination are great, the curiosity of investigators has been lessened.

For the sake of completeness we shall pass in review all the publications in which there is mention of vanadium in living organisms, though in many of these studies there is no proof that this vanadium really comes from the biosphere and not from analytical contaminants.

Bechi (1879) first mentioned the existence of vanadium in the ashes of plants. Unfortunately he contented himself with a mere statement, without any details and with no data. The statement indeed appeared only in the minutes of a meeting of the Italian Academy of Science, and we have proof that he had never published it in the fact that in 1900 he wrote to the Chemical Society of France to claim a priority with regard to the occurrence of boron in plants, an observation of which was included in the remark relative to vanadium. He then announced that he should have published the paper but never did so. We are entirely uninformed as to whether or not his observations were due to an analytical error.

We need not pay much attention to the observation of Ricciardi (1883) who, while he claimed to have found from 9.5 to 36.5 p.p.m. V in the lava of Etna, was content to state merely that the plants that grow on the lava of this volcano also contain appreciable quantities of the element. Von Lippmann (1888) is rather more precise, but contrary to what one often finds reported in the literature it was not in sugar beets that he found vanadium but in residues after industrial treatment of the distillation of molasses. This oc-

currence he claimed to have known of for eight years prior to its publication. The origin of the vanadium from the sugar beet is then by no means established, the metal being derived perhaps from the lime used industrially for the purification of the beet juice or, more probably, from the soil that remains in the incompletely washed roots.

The first scientific work is that of Demarçay (1900). He recognized vanadium by spectroscopic methods in the ash of various woods: *Pinus sylvestris*, *Picea*, hornbeam, oak, *Vitis*, and *Populus*. He expressed, however, some reservations with regard to his results, thinking that the vanadium might well have come from impurities of the reagents or from dust. In spite of his own criticism he did not attempt to perform controlled experiments. Since vanadium is very widely distributed in soils, dust must necessarily contain some of the element. The results of Demarçay, interesting as they may be, still cannot be considered as formally establishing the presence of vanadium in plants in general, or even in certain particular species.

Griffiths (1900), who is sometimes given as a reference for vanadium in plants (generally without indication in the bibliography), actually gives only two records of the presence of vanadium in traces following the findings of Demarçay whose work we have just analyzed. Ramírez (1914) determined vanadium in Argentine plants; his figures are unfortunately not known.

Robinson, Steinkoenig, and Miller (1917), using spectrographic methods, found only traces of vanadium in six of the 50 plant samples they examined. These were samples of kidney beans, clover, sugar beets, and pine needles. We may conclude from this that if vanadium exists normally in plants, there cannot be much of it. Cornec (1919), using a carbon-arc method, found vanadium in the ash of *Laminaria*. We must be even more cautious in accepting the results of Cornec than those of Demarçay, as Cornec, who used considerable quantities of ash, does not indicate whether the *Laminaria* were washed or whether precautions were taken to avoid con-

tamination. However, a much more important source of error, as we shall see, lies in the use of the carbon arc for the determination of vanadium. We have already seen that vanadium is to be found in all of the coals. Moreover, the carbons used in modern spectrography are often made from petroleum soot agglomerated by boric acid and consequently themselves contain appreciable quantities of vanadium. It is only recently that vanadium has been eliminated from graphite. The vanadium lines in the spectrum of the carbon are very fine, and certain investigators who recognize this fact consider that the vanadium is present in the ash analyzed when these lines are reinforced. This, as the following experiment proves, is completely false.

If, as pointed out by Webb and Fearon (1937), a crystal of NaCl is placed in the positive cup, before the carbons are used, and the arc is turned up until the sodium disappears and a series of exposures is made, the vanadium lines are seen to be considerably reinforced. Other salts, such as Na_2CO_3 , produce similar results. One might be tempted to conclude that the salts contained some vanadium, but the following experiment (D. Bertrand, 1942d) proved that there is none, or at least that the reinforcement of the vanadium lines already existing in the carbons does not arise from the salts added. If the following series of exposures is made: carbon alone, carbon plus 200 gamma V (as ammonium vanadate), and carbon plus a few milligrams of "pure" NaCl, the conditions of excitation and of exposure time being as nearly identical as possible for these different spectra, which are photographed on the same plate, upon examination the vanadium lines are much more intense for the NaCl than for the ammonium vanadate. A search for vanadium in the NaCl yields negative results both by the method using oxygenated water and by the technique given subsequently by the same author, being less than 1 gamma (or 10^{-6} grams). The variation in the reinforcement of the vanadium lines of the carbon being dependent on the salt mixture, we may readily understand that we may accept the presence of a variable quantity of vanadium in various ashes, while in fact there is none present. Certain writers, not having sufficiently luminous spectrographs or sufficiently fast

film, have not seen the vanadium lines in the proof negatives of the carbons alone and have thus recorded their presence only in the material studied, the reinforcement on addition of the sample being sufficient to cause them to appear.

Vernadsky and Vinogradov (1931), in the resumé that was prepared under their direction, stated that the various species of *Lemna* studied in their laboratory contained vanadium, but they give no data and the detailed report does not appear ever to have been published.

Turning to the work of Ter Meulen (1931), we find for the first time a definite indication of the discovery of vanadium in a plant. This vanadium was found occasionally by him in the course of a general work on molybdenum. This writer states, "*Amanita muscaria* may contain 3.3 mg. V per kilogram (3.3 p.p.m.); there was no trace of it in the other toadstools (six in all). In searching for vanadium in other plants, I found 0.8 mg. per kg. (0.8 p.p.m.) in garlic. Onions do not contain any." The quantity of toadstools used varied from 300 to 3500 grams (1500 grams in the case of *Amanita muscaria*). The author does not specifically indicate the state of desiccation of any of his samples, but it is clear from his report that he must have been working with fresh samples. The analytical technique that he used was rather laborious, but it does not seem likely that the vanadium finally obtained came from the reagents or the containers, for, starting with the same process and analogous control materials, Ter Meulen failed to find the element. The only criticism of this work that might be made is that the state of cleanliness of the samples is of little significance, as toadstools in particular are very hard to clean, while the cleaning of garlic is easy.

Boyd and De (1933) pointed out the presence of vanadium in some Indian plants, but having used a spectrographic method employing carbon arcs which, as they stated themselves, already contained vanadium, their work, as we have seen, furnishes no proof. The same is true of the work of Sullivan (1933) who indicated the presence of vanadium in wheat. The following year, Vinogradov (1934), in a memorandum communicated to the Russian Academy of Sci-

ences, did not hesitate to write in the English summary, without giving any evidence, "It turns out that vanadium is largely met with in small quantities of the order of about $10^{-5}\%$ in relation to living matter in various terrestrial and marine animals and plants." This, according to the Russian text, simply means that vanadium exists in the lithosphere in a concentration of the order of 100 p.p.m. and that there must be small quantities in plants and animals. It does not mean, as is implied in certain abstracts, that Vinogradov stated that the vanadium content of most plants is approximately 0.1 p.p.m.

Byers (1934), having found 130 p.p.m. V in a soil, analyzed the wheat growing on it and found 2.5 p.p.m. V. This soil, as we have seen, is exceptionally rich; nevertheless the information concerning wheat is interesting even if it does not refer to a normal concentration.

Němec (1936) discovered a fact of possible interest. While searching for several rare elements in the ash of *Polyporus fomentarius*, he found almost imperceptible traces of vanadium in the ash of the hornbeam and beech trees that supported the fungus. However, he found none in the fungus itself. Unfortunately the analytical method used was not indicated, nor whether this vanadium really came from the wood of the trees or from contaminants.

Konishi and Toshihisa (1936a, 1936b), using a spectrographic method, indicated very small quantities of vanadium in the roots of lucerne nodules; they did not find any in other samples. They did not state whether they employed a carbon arc or the copper electrode that they customarily used in the course of their work.

Rao Sundara (1940), although he employed a spectrograph with one carbon electrode which contained the material to be determined and one silver electrode, considered that the presence of vanadium was not conclusively proved.

Using an analytical technique especially developed for the purpose and sufficiently sensitive to permit determination of 0.002 milligrams V in 100 grams (0.02 p.p.m.) of dry plant matter, D. Bertrand (1942c, 1942d) found vanadium in every sample of the 62 species of plants that he analyzed. The fig-

ures varied from 0.152 to 4.2 p.p.m. V for the dry weight of the entire plants (aerial part only), leaves and fruits, from 0.100 to 12.14 p.p.m. for the dry roots, and from less than 0.010 to 1.2 p.p.m. for the seeds. The green leaves of cabbage were found to have a considerably greater vanadium content than the pale ones; but this was not true for escarole. It is not known whether vanadium acts in general as do certain oligoelements such as manganese and zinc, the content of which is greater in the green leaves than in the pale ones. For the kidney bean the root is richer than the aerial part, the latter being richer than the fruit, which is in turn richer than the seeds. This was confirmed for the sugar beet but, if it is a general fact, there would be a great difference between the sugar beet and the fodder beet, the latter being much poorer in vanadium than the former, confirming perhaps the observation made by von Lippmann in 1888.

It is probable that the roots are richer than the aerial parts in vanadium and that consequently if this metal is not simply absorbed but plays a physiological role, its importance may be greater for the parts of the plants that are underground. Indeed the roots of the wild carrot, the chickweed, the yellow lupine, the white lupine, the soybean, the broad bean, and the kidney bean have all been found to be richer in vanadium than the aerial parts. Similarly the seeds of the broad bean, the soybean, the white lupine, the kidney bean, the pine, the chickweed, the sugar beet, and peasant tobacco are clearly less rich than the fruits and the leaves of the aerial parts. The leaves of peasant tobacco are less rich than the entire aerial part and richer than the seeds.

A certain number of plants, grown under as analogous conditions as possible, have been analyzed twice with the object of finding out the order of variation of the vanadium content under different culture conditions. The result obtained corresponds to what one might expect: the plants growing in the soils that were richest in vanadium had greater amounts of this metal than those growing in poorer soils. The example of the white lupine, where the roots as well as the aerial parts were analyzed, is particularly instructive. Results are given in terms of vanadium in p.p.m.

	FRESH PLANT	DRY PLANT (OR SOIL)	ASH
Sample no. 1			
Soil	—	52	—
Roots	1.76	5.60	56
Aerial part	0.049	0.437	5.3
Sample no. 2			
Soil	—	21.7	—
Roots	0.840	2.76	25.8
Aerial part	0.026	0.208	2.97

These results are in accord with those obtained by Trinchinetti (1845), Deherain (1878), Pfeffer (1886–1888), and Ramírez (1914). It is regrettable that Ricciardi (1883) did not give data for the vanadium that he found in wheat, an example that would have been especially interesting to compare with the work of Byers (1934). However, we shall see in regard to the investigations made on the physiological role of vanadium in plants that the dependence of the concentration in the plant on that in the soil is a question of a purely physical phenomenon and is independent of any biological action.

Before the few rare studies that have been made of the possible concentration of vanadium by particular species of plants are discussed, it is worth while to consider the mean vanadium content of fresh plants and their ash. Vanadium varies from 0.010 to 0.870 p.p.m. for fresh plants, the latter figure being rather exceptional, as the mean is 0.160 p.p.m. For dry plants the mean content is very accurately 1 p.p.m. and for plant ash 7.1 p.p.m. If this last value is compared with the vanadium content of coal ash and if we consider the fact that the Paleozoic terrains were richer in vanadium than are the recent ones it is easy to explain the presence of vanadium without being obliged to admit that the plants which produced these coals had an elementary composition so very different from the plants of today. This conclusion is apparently contradicted by the group of coals in which were found 1500 to 6000 p.p.m. V, even more in the ash, and makes us wonder whether certain present-day plants are not vanadium accumulators. Only systematic study will provide an answer to this question. Fortunately Ter Meulen by chance provided the beginning of an answer. The vanadium found by that writer, 3.3 p.p.m. for a "fresh" toadstool, is well above the mean figure for plants

in general. We might conclude that generally the cryptogams are richer than the phanerogams, but this does not appear to be so; even if D. Bertrand (1942d) unfortunately analyzed only six cryptogams, the mean vanadium content of his fresh plants is exactly the same as for the phanerogams. As Ter Meulen found no vanadium in six other fungi, we might consider that his *Amanita muscaria* was exceptional, the high vanadium content being due to the nature of the soil on which it grew. D. Bertrand (1943b) therefore undertook the study of vanadium in fungi and especially in the genus *Amanita*. The results are given in table 1.

If the negative results of Ter Meulen on fungi are considered together with D. Bertrand's data, the following conclusions emerge:

1. *Amanita muscaria* is exceptional among the species of *Amanita* analyzed, and consequently very probably among the European species of the genus, in having a high vanadium content.

2. The vanadium content does not seem to be related to taxonomic position (older classifications of the genus were also considered).

3. The vanadium content of species of the genus other than *A. muscaria* is not of an order of magnitude different from that of the other terrestrial fungi or of plants in general.

4. The exceptional richness in vanadium of *A. muscaria* is not related to age or soil; indeed, the *Aspidella echinocephala* from Coyes was growing on the same soil right beside the *Amanita muscaria* from that locality.

5. The vanadium is not localized in any one part of the toadstool. *A. muscaria* is believed to live on the roots of certain conifers or of the birch tree, and since the matter has not been completely examined elsewhere, it seemed necessary to ascertain whether these species were especially rich in vanadium. Previous analyses proved that there was none present in the conifers. The analysis of the birch wood grown at Coyes beside the *A. muscaria* yielded 0.15 p.p.m. for the dry wood. An analysis of its roots was therefore unnecessary, since their vanadium content would vary only slightly from that of the aerial parts.

Amanita muscaria thus represents a defi-

TABLE 1

	Number Deter- mined	Dry Weight in Grams	Per Cent of Dry Weight	Per Cent of Ash	Vanadium in Milligrams per Kilogram, Dry
<i>Aspergillus niger</i> (Cramer van Tieghem), culture	—	32.8	12.2%	5.8%	0.15
<i>Russula queleti</i> (Fries), reg. of Paris	—	45.5	11.2	5.8	0.34
<i>Russula emetica</i> (Fries), reg. of Paris	—	14	5.4	12.4	0.27
<i>Lactarius torminosus</i> (Fries), reg. of Paris	—	8.6	8.9	7.4	1.2
<i>Chantarellus cibarius</i> (Fries), Vendée	—	31.3	7.2	5.6	0.43
Amanites, first group:					
<i>Amanitopsis vaginata</i> (Bulliard), Viroflay, Verrières, Fontainebleau	33	13.4	5.2	7.5	0.15
<i>Amanita caesarea</i> (Scopoli), Mlady, Mantes	1	10.6	8.1	9.6	0.58
<i>Amanitaria pantherina</i> (Candolle), f. typica, Viroflay, Coyes-Belleme	19	25.6	10.5	9.9	0.19
<i>Amanitaria muscaria</i> (Linnaeus), f. typica, Coyes	1 adult	14.4	9.6	4.8	60.8
Vendée	2 young	4.6	6.6	7.9	149
Verrières	1 adult	13.3	4.55	4.7	156
Cuticle of cap, Coyes	—	0.25	—	—	98.5
Stalk, Verrières	1	7.7	4.65	—	139
Cap, Verrières	1	5.7	4.4	—	181
<i>Amanitaria muscaria</i> (Linnaeus), f. <i>formosa</i> , Belleme	2 young	8.7	8.8	6.8	85
Amanites, second group:					
<i>Amidella ovoidea</i> (Bulliard), Exp. Museum	3	30.4	7.85	13.1	0.42
<i>Amanitina phalloides</i> (Fries), Fontainebleau, Marly	30	10	3.2	22.9	2.4
<i>Amanitina citrina</i> (Schaeffer), f. typica, Viro- flay	203	43.8	3.9	10.7	0.06
<i>Amplariella rubescens</i> (Fries), Viroflay, Verri- ères, Fontainebleau	15	25.5	5.8	11	0.21
<i>Amplariella spissa</i> (Fries), Verrières	9	18.6	9.4	5.7	1.0
<i>Aspidella solitaria</i> (Bulliard), Exp. Soc. Mycol.	2	11.5	8.4	14.5	1.8
<i>Aspidella echinocephala</i> (Vittadini), Coyes- Belleme	4	15.8	5.65	11.1	0.63

nite anomaly which is very remarkable as compared with all the other plants studied up to the present and poses a biochemical problem. The vanadium content of dry *A. muscaria*, which is 112 p.p.m. as a mean, 112 times higher than that of the other plants, with 1930 p.p.m. for the ash, permits us to accept the possibility that in ancient geological periods there were some plants with a vanadium content approximately 20 times higher (at the most) than the normal, which would explain how some coals contain in their ash 10 to 20 times as much vanadium as the majority of the others. This conclusion

may be subject to paleobotanical verification.

A more systematic study that would permit us to ascertain whether *A. muscaria* represents an exceptional case or if the same phenomenon also occurs in some phanogams would be desirable.

In connection with the studies considered in greater detail in the next section of this paper that have been made on different organisms living in the nodules of leguminous plants, it was necessary to know the true mean concentration of vanadium encountered by the nitrogen-fixing bacteria in such nodules. Work of this kind (see table 2) has been

TABLE 2

	Fresh Weight in Grams	Dry Weight in Grams	Per Cent of Dry Weight	Per Cent of Ash	Vanadium γ	Vanadium in Milligrams per Kilogram		
						Fresh	Dry	Ash
Kidney bean (Versailles)								
Soil	—	0.915	—	—	19.4	—	21.2	—
Roots	19.2	3.865	20.1%	9.55%	14	0.735	3.62	38
416,000 nodules to kg. dry (1100)	18.4	2.66	14.4	9.4	6.8	0.369	2.55	27.2
Aerial part	209	38.2	18.2	13.5	29	0.138	0.760	5.6
Black Tokyo soya (Versailles)								
Soil	—	1.090	—	—	23.7	—	21.7	—
Roots	22.1	3.85	17.4	11.6	12	0.544	3.12	27.6
140,000 nodules to kg. dry (383)	12.8	2.72	21.2	8.6	7.5	0.590	2.8	32.6
Aerial part	205	38.5	18.8	12.5	25	0.122	0.65	5.2
Yellow lupine (Vendee)								
Soil	—	1.046	—	—	54.3	—	52	—
Roots	10	3.67	36.7	7.2	6.4	0.640	1.74	24.2
18,400 nodules to kg. dry (52)	18.4	2.82	15.3	16.5	10	0.543	3.54	21.5
Aerial part	373	36.5	9.5	10.5	48.2	0.129	1.32	12.8
White lupine (Vendee)								
Soil	—	1.046	—	—	54.3	—	52	—
Roots	11.6	3.65	31.2	10	20.2	1.76	5.5	56
125,000 nodules to kg. dry (237)	22.1	2.84	12.8	19.6	11.4	0.515	4	20.4
Aerial part	336	37.8	11.2	8.25	16.5	0.049	0.437	5.3
White lupine (Versailles)								
Soil	—	1.090	—	—	23.7	—	21.7	—
Roots	12.4	3.76	30.0	10.7	10.4	0.840	2.76	25.8
25,000 nodules to kg. dry (68)	15.45	2.72	17.6	16.4	12.4	0.805	4.56	27.8
Aerial part	302	38.4	12.7	7.2	8	0.026	0.208	2.97
Broad bean (Grignon)								
Soil	—	1.086	—	—	32	—	29.1	—
Roots	10.5	3.095	28.8	16.3	37	3.52	12.4	75
53,500 nodules to kg. dry (2610)	27.25	4.883	17.8	23	54.6	2.00	11.2	48.5
Aerial part	302	45.7	15.1	9	18	0.060	0.394	4.4

started (D. Bertrand, 1942d).

In this work the various samples were collected at the same time, while the plants were flowering, so that certain comparisons could be made.

The vanadium concentration in the nodules has no relation to their size or to the richness of the roots in this metal. At the most, we might conclude that the large nodules are richer in vanadium per unit dry weight than are the roots, but this may be accidental. The only fact that seems certain is that in the six

samples analyzed the vanadium concentration in the dry matter of the nodules is from 3 to 4 p.p.m. (three times as much for the broad bean). Quantities of the order of magnitude employed experimentally by Bortels (1936, 1937, 1938) are clearly involved, but it must be noted that the figures given here relate to the nodules and not to those portions of the nodules that contain the bacteria.

In conclusion, even if much remains to be done, we may nevertheless conclude that the mean content of the element normally pres-

ent in plants is 0.16 p.p.m. for fresh samples, 1 p.p.m. for dry samples, and 7 p.p.m. for the ash. If the analyses are still insufficient to inform us as to the differences that may exist

between certain groups of plants, at least one species presents an exceptionally high content, namely, a mean level of 112 p.p.m. for the dry plant and 1930 p.p.m. for the ash.

DYNAMICS

THE PHYSIOLOGICAL ROLE OF VANADIUM IN PLANTS

Since it is now known that vanadium exists normally in plants, it is important to ascertain if this element is indispensable to the life of the organisms, that is to say, if it is, according to the expression of Gabriel Bertrand, a synergic oligoelement, or if it is encountered simply as an inert impurity absorbed from the soil, selectively or otherwise. Various authors have already inquired for various reasons whether vanadium played a physiological role in plant life. However, their point of view was rather arbitrary, as they knew nothing of the quantities of the metal existing in plants nor even whether the presence of the element were normal. Many of these researches were effected with a toxicological purpose, since vanadium exists in considerable quantities in certain fertilizers, in particular in Thomas slag. Although such a point of view is quite different from the one I take in this study, it has seemed necessary to cite all of the works relative to the physiological action of vanadium.

In 1886, following the statement of Ricciardi (1883) that he had found vanadium in wheat growing on the lava of Etna, Witz and Osmond (1886) became interested in determining whether this element affected the growth of the plant. They grew wheat on sterilized talc with and without "hypovanadic chloride, in small amounts" and found that this salt had a manifestly detrimental action.

Suzuki (1903) was more precise. He indicated that barley is poisoned if in the culture medium there are 100 p.p.m. V in the form of sulfate, whereas with 10 p.p.m. there is no action. These results derived from observations in liquid media were also confirmed on solid media.

Bokorny (1904), knowing of the use of vanadium salts in tuberculosis, endeavored to verify its toxicity for various microorganisms. He used a solution of vanadic acid

and stated that with 260 p.p.m. V the growth of yeast is halted, while many bacteria continue to multiply with 1300 and even 2600 p.p.m. V. In a five-hour test, the *Mucorineae* can grow in a solution containing 2500 p.p.m. V. The question there is of considerable quantities. The sole interest of these experiments with high concentrations is to demonstrate that vanadium is practically non-toxic for certain microorganisms.

Frouin and Ledebt (1912a) stated that vanadate favored the development of *Pseudomonas pyocyanea* but prevented the appearance of pigment (they used 5 grams of salt per liter). In a second paper (1912b), these authors indicated that with 0.4 grams of sodium vanadate per liter of nutrient solution (103 p.p.m. V) a considerable growth of tuberculosis bacilli may be obtained.

Frouin and Mercier (1914) extended this result to *Aspergillus niger* (*Sterigmatocytis nigra*, Cramer van Tieghem). Sodium vanadate was added in doses varying from 1×10^{-5} to 2.3×10^{-3} (2.5 to 645 p.p.m. V). This produced an increase of one-third in the weight of the product for the high concentrations, when grown in "matrasses." Working, as did Raulin (1870), in uncovered photographic basins, there was no visible effect. The most favorable action corresponded to 1×10^{-4} (25 p.p.m. V).

Javillier (1914) reconsidered certain aspects of a work destined to show that no metal can replace zinc in the metabolism of *Aspergillus* and tried among other elements vanadium. The concentration varied from 0.1 to 200 p.p.m., with equal quantities of ammonium vanadate and sodium vanadate, one of these being an oxydant and the other a reductant. The process used by the author to purify his culture medium (by recrystallization) is, as we shall see, generally very difficult in the case of vanadium and therefore necessitates the use of an analytical control, which was not employed here. Hence it is not surprising that this investigator found no fa-

favorable action for vanadium in quantities of less than 1 p.p.m. On the other hand, it must be noted that according to previous work on this plant or other lower organisms there is at high levels up to 200 p.p.m. a favorable action, but in those cases conditions are involved that these plants can rarely encounter in nature and therefore are different from those that concern us.

According to Longobardi (1934), Ramírez stated that vanadium is absorbed by plants. We may consider this as a partial confirmation of the experiments of Deherain. The phenomenon is moreover apparently independent of physiological action and is only of a physical nature. It is most regrettable that the work of this scholar is not available, as he also determined vanadium in some clays and plants from Argentina, where the petroleum (Longobardi, 1934) and certain waters (Bado, 1918) are known to be very rich in this metal.

Free and Trelease (1917) demonstrated that vanadium begins to be toxic at a concentration of 20 p.p.m. for young wheat, but indicated that at a certain concentration it has a slight stimulating effect.

Krioukov (1931) tried to determine whether sodium vanadate can serve as a catalytic fertilizer. With 50 milligrams of this salt per kilogram of soil (22 p.p.m. V), he obtained an 80% diminution in the crop of oats; the growth of the plant was halted completely with 150 milligrams (66 p.p.m. V). The salt was demonstrated to be equally toxic for mustard. Such a fact is in no way incompatible with a favorable action of this metal but may appear surprising in view of the fact that arable soils occasionally contain quantities of vanadium double that of the higher quantity employed in these experiments. Gabriel Bertrand (1931), as stated in the Introduction, showed that the oligoelements had a maximal favorable action at a given concentration and that at higher concentrations these same substances offered a more or less markedly toxic action which varied according to the plants, but in general was strikingly developed at concentrations only slightly in excess of the optimum, at least in the case of the higher plants. We already know that there is a decided difference between lower and higher plants with respect to vanadium toxicity, the element being relatively slightly

toxic to the former, and demonstrating very rapidly, even at low concentrations, a high degree of toxicity for the latter. Krioukov, in his experiments, did not trouble to find out the quantity of vanadium in the soils that he used and that we know always contained variable and sometimes considerable quantities. Plants, however, contain normally only very little vanadium; we are correct in thinking by analogy with the other elements already studied in plants, that plants need only very slight quantities of this metal. It is therefore more than likely that Krioukov exceeded the optimum concentration. As we do not know, unfortunately, the vanadium content of the soil that he used, we cannot deduce from his report the level below which the optimum concentration may be found, but the subsequent work will permit us to confirm this point of view.

Brenchley (1932) found vanadium to be toxic to barley cultivated in flasks. As this investigator used a concentration of 40 p.p.m. V, this result is not surprising, but it does fix a maximum which should not be exceeded. Moreover, studying the action of basic slags on the cultures, she stated that pulverization of the slags produced a definite reduction in growth and explained it as owing to the presence of vanadium.

Scharrer and Schropp (1935), using graded quantities of vanadium, from 10^{-10} to 100 milligrams in the form of sodium metavanadate, tested in sand (700 grams) the growth of wheat, barley, rye, oats, maize, and peas. Of these, the peas were most sensitive to the toxicity. For maize there was an increase in weight for the weak doses. For wheat all results were negative. Vanadium was, however, toxic for the others, especially the barley and oats. Experiments also were made using 1 liter of Richter's liquid medium. The action was unfavorable both on the aerial part and the roots. The lower limit of toxic action was from 1 to 10 p.p.m. according to the grain tested. The remark made with reference to Krioukov obtains here as well. These authors did not determine how much vanadium may have been present as a contaminant in their culture media.

The work of Shibuya and Saeki (1934) is more interesting. These writers found that vanadium has no direct effect on plants, but

stimulates their metabolism through its effect on the activity of *Azotobacter*. The same criticism applies in this case.

Burk and Horner (1935) demonstrated that, below 31° C., stimulation of the development of *Azotobacter* by vanadium was only produced in cultures where nitrogen was not added in a combined form. There was, then, a special catalysis of chemical fixation of nitrogen. These effects were obtained with concentrations varying from 0.0000005 p.p.m. to 0.00005 p.p.m., which produced the maximum effect. There was no toxicity for 5 p.p.m. On the other hand at the highest concentrations the action of vanadium was 70% greater than that exercised by molybdenum.

Bortels (1936, 1937a, 1937b, 1938) extended this work to *Azotobacter chroococcum*, *A. vinelandii*, and *Bacillus amylobacter*. The optimum concentration varied from 10^{-8} to 2.5×10^{-5} (0.01 to 0.250 p.p.m.). We are uninformed, as in the preceding cases, of the quantity of vanadium remaining in the culture medium after the purification. But here, as for the work of Burk and Horner, the media are pure enough with regard to the amount of this metal needed by the plants used in order to produce a favorable action.

On the other hand, in experiments with growth on natural soil, although Bortels had been able to observe an augmentation of the development of *Azotobacter* and an increase in the nitrogen content of the soils under the influence of vanadium salts, he failed with the leguminous plants, probably because the soils that he used were already sufficiently rich in this metal.

Arnon (1938) conducted some experiments on the influence of vanadium on certain higher plants, asparagus and lettuce, growing in a liquid medium. He stated that increased growth resulted from addition of 0.01 p.p.m. V, but as he added six other elements at the same time (Mo, Ti, W, Cr, Ni, Co), it cannot be concluded that this action was really due to vanadium.

Mazé and Mazé (1939) concluded that vanadium has a definitely favorable action on corn, but remarked that their experiments did not show whether or not this role was limited to an anti-infectious action. Such hypothesis does not seem very probable as

for the microorganisms studied up to this point, vanadium has only an extremely slight toxic action and in every case at concentrations that are definitely toxic for higher plants. As the culture medium is not always specially purified for vanadium, this experiment on the comparative development of two roots of a plant, one soaking in a solution without vanadium, the other in the same solution with vanadium added, succeeded only because the optimum concentration of vanadium for the root of corn is notably higher than that for the majority of the other plants studied, a fact which seems to be well established by the work of Scharrer and Schropp (1935).

Dmitriev (1939) considered that vanadium had no effect on the growth of clover in podzol soils.

Gericke and von Rennenkampff (1939–1940, 1940) made a series of very important contributions on the action of vanadium on certain higher plants. Vanadium added in the form of VCl_3 at a concentration of 100 p.p.m. produced an unfavorable effect on barley cultivated on sand, while in the form of ammonium metavanadate at the same concentration the action was favorable. These investigators found in a soil rich in calcium that, at the enormous concentration of 1250 p.p.m., vanadium as ammonium metavanadate was not toxic and gave a favorable effect. In aqueous culture, 1 p.p.m. vanadium in the form of calcium metavanadate gave a better result than 0.1 p.p.m., while 10 p.p.m. was toxic (the root grew with 2 p.p.m.). Comparing in liquid media the action of calcium vanadate and of Thomas phosphate on the growth of barley, the latter is more active and acts more favorably. The same is true for red clover.

In their paper published in 1940, these investigators continued their study of this question, again using plants grown in liquid media, and found that vanadium is a growth stimulant for plants up to a certain concentration, when toxicity appears. This is Gabriel Bertrand's law of optimum nutritive concentration. In addition the same concentration of vanadium as an anion is more favorable according to these authors than as a cation, and the vanadium contained in the basic slags (normally about 5000 p.p.m. V in

the form of calcium vanadate) is a superior factor in the fertilizing action. For the red clover, growth of the roots is due largely to vanadium, which has contrariwise an unfavorable effect on growth of the aerial part. We find again what has been noted for corn, where the optimum concentration for the roots was very high, but we also find again what had been found in the analysis, namely, that in general the roots are richer in vanadium than the aerial parts. Finally, according to Gericke and von Rennenkampff, the relation of nitrogen to phosphorus is not affected by vanadium which augments, nevertheless, the relative nitrogen content of the roots.

In 1940, Gericke observed that vanadium is of minor importance as a fertilizer and emphasized that an excess of it is harmful. Then in 1941, returning to the effect of vanadium on the growth of plants, he stated that vanadium, from 2 to 5 kilograms per hectare, either in the form of metavanadate or in the form of Thomas phosphate, has only little effect on the growth of the roots of red clover and a negligible effect on wheat. Analysis of the soil showed more nitrogen in the parts treated with the vanadate than in the untreated parts, which confirmed the observations of Bortels. The apparent contradiction in the publications of Gericke comes from the fact that this author, like his predecessors, neglected completely the vanadium contained in the experimental soil, the quantities of which vary notably, thus permitting a favorable action in a soil poor in this element and none or a toxic action for the same quantity of vanadium added to a rich or very rich soil.

In 1942, Horner, Burk, Allison, and Sherman, in an important paper, returned to the work done seven years previously by Burk and Horner. These authors used eight strains of *A. chroococcum* and one of *A. vinelandii*, and for almost all found an increase of five to 25 times for the nitrogen fixed in the presence of vanadium in the form of sodium orthovanadate or metavanadate. With about 0.00001 p.p.m. of the latter, there was already a detectable effect, and about 1 p.p.m. gave a maximum increase in six days. With young cultures, 0.005 to 0.01 p.p.m. gave a maximum increase in one day. In one with no vanadium (or molybdenum) very little nitrogen was fixed in 31 days, and usually 50% to

75% of the sugar in the culture medium was not consumed. The effect produced by vanadium was only 50% to 80% of that produced by molybdenum.

These results are particularly interesting and although we cannot generalize about them, let us recall the opinion expressed by Pasteur in 1862, with reference to his discoveries on the generation of the lower organisms, "These facts reveal to us a method with the aid of which plant physiology will be able to attack without difficulty the most delicate questions on the life of these little plants (Mucedineae), so as to prepare the way confidently for the study of the same problems in the higher plants.

"Even though we might be afraid of our ability to apply to the higher plants the results furnished by these very minute organisms, there should be no less great an interest in resolving the difficulties raised by the study of plant life, beginning with those in which the minimum complexity of the organism renders conclusions easier and more certain: the plant is reduced here in some way to the cellular state, and scientific progress shows more and more that the study of acts accomplished under the influence of plant or animal life, in their most complicated manifestations, leads in the last analysis to the discovery of phenomena peculiar to the cell."

This statement, taken word for word, even if it may no longer be considered as corresponding to the facts of modern science, nevertheless remains profoundly true in spirit. That is why we shall develop with regard to one of these microorganisms a point of view that the study of the physiological action of vanadium makes us adopt.

Raulin (1870) tried manganese, among other elements, to determine if it was beneficial to the growth of *Aspergillus niger*, but found no favorable action. G. Bertrand (1912b) demonstrated that this was because the culture used by Raulin already contained enough manganese to supply the needs of the plant. By adequately purifying the culture medium, he was able to show at the same time that a concentration of 1 by 10^{-10} (0.0001 p.p.m.) was enough to produce a very considerable increase in the growth of *Aspergillus niger* and succeeded in giving this result a more general application and a new direc-

tion by showing that manganese was not only useful but indispensable to the life of these plants.

The thought that an analogous situation might have occurred in the work of Javillier (1914) led D. Bertrand (1942e) to undertake an investigation of the action of vanadium in *Aspergillus niger*, in the same year that Horner, Burk, Allison, and Sherman returned to the study of the influence of the element on the fixation of *Azotobacter*.

A preliminary analysis showed that *Aspergillus niger* was very poor in vanadium, 0.152 p.p.m. for the dry plant. As this sample came from a culture grown in Raulin's medium, with unpurified commercial salts, and as its appearance and the weight of the product were normal, we may consider that the amount of vanadium found was sufficient to provide for the normal growth of the plant.

It was necessary, because of the impossibility of completely purifying the mineral salts, to employ a special culture medium which, tried with some impure salts, gave results as good as those obtained with Raulin's more complex medium. In spite of the preliminary purification of the salts, there still remained 0.0018 p.p.m. V in the culture medium which finally was reduced to less than 0.0007 p.p.m.

Under these conditions, 0.0043 p.p.m. V added to the medium was sufficient to obtain a 21% increase in the weight of the product. Unfortunately, the experiment was carried out at a difficult time in Paris and had to be done in a Pyrex container; it is not absurd to think that the Pyrex glass, which contains vanadium, might well have been able to furnish a very notable quantity of this metal to the mycelium. Indeed, with reference to manganese, G. Bertrand (1912c) had shown this to be true of the containers.

In the case of vanadium chemical analysis of the results corresponding to 4 liters of the medium (24.67 grams of dry matter) showed 3.2 gamma V (0.13 p.p.m.), a figure well above the amount of this metal that might have remained in the culture medium. *Aspergillus niger* grown in an unpurified culture medium contained 0.152 p.p.m. V, a figure that is of the same order of magnitude and as a consequence seems to represent accurately its normal content. Consequently, to obtain a

normal growth it is necessary that the culture medium contain at least the quantity of vanadium corresponding to the normal maximum yield, which would be 0.002 p.p.m. (a figure corresponding to a yield of 16.27 grams of dry matter, indicated by Raulin to be the maximum that can be obtained with 46.7 grams of saccharose and that has not been exceeded since).

The question here is one of concentrations considerably lower than those that we have found for vanadium in nature, but it must be noted that in fact the culture medium contained 53.36 grams per liter of dissolved substances and that the optimum level of vanadium would, when related to these constituents, be 0.04 p.p.m.

The quantities of vanadium involved are still so small that we might be tempted to be skeptical if it were only a question of an isolated case. Indeed, for *A. chroococcum* the optimum concentration was, as we have seen, 500 times greater. But there is nothing surprising therein, and the role played in life by trace amounts of metal or metalloids is increasingly the subject of investigation. Manganese, to which we have already referred in connection with the strains studied by G. Bertrand, had an optimum nutritive concentration of the order of 0.01 p.p.m., which is only five times as much as in the case of vanadium. This is equally true of the metalloids. One of the most remarkable examples is that of boron and the disease of the heart of the beet, where the quantity of boron necessary to prevent this ailment is extraordinarily small, only 3 to 5 kilograms per hectare being required (G. Bertrand, 1939), which would be for about 7500 tons of soil assuming a useful depth of 0.3 meter.

These experiments not only explain to us, by the small concentrations involved, the failure of the earlier work but show us that for the lower plants vanadium at relatively high concentrations not only is not toxic but achieves, by means of a process which has not been elucidated, a new increase in growth, with nevertheless a maximum beyond which it always becomes toxic.

In addition, from all these experiments we may not only conclude that vanadium is useful to life but also that it is necessary, as it is not replaceable by any other element. In fact up

to now there is no known case in which one metal having a physiological action may be replaced by another. Thus sodium and potassium, which are so similar chemically, may not replace each other in living matter.

Finally, let us cite the recent work of Sam-path (1944), who showed that the sporulation of yeast cells was inhibited by 280 p.p.m. V, but was favored with 140 p.p.m. As the yeast sporulate only in a medium that is unfavor-

able to them, we may conclude from this that 140 p.p.m. V is a dose well above the optimum for the growth of yeast, which is in accord, in terms of order of magnitude, with the earlier experiments.

In conclusion, vanadium plays a definite physiological role in relation to plants, and although definite proof is lacking for the higher plants, we do have it for at least two lower groups of organisms.

VANADIUM IN ANIMALS

STATICS

AS IN THE CASE OF THE PLANTS, a considerable amount of investigation has resulted in demonstrating that the elementary composition and the physiological functioning of animals are dependent upon a relatively large number of metalloids and metals, certain of which, called oligoelements, exist in only very small amounts. With regard to the majority of these elements, these ideas have been proved correct by the experiments carried on for such animal species as have been studied and have thus an entirely general character. With regard to the others, they rest only on partial discoveries, and new research is necessary to determine if their presence is limited to certain groups of species or is as general as the former. This question has been posed for a long time with regard to vanadium in animals.

Henze (1911) found 42,000 p.p.m. V in the blood of an ascidian and was the first to indicate the presence of the element in an animal. Since then, with the exception of the work done on the ascidians that we shall discuss further, there has been little investigation of this metal in animals.

Phillips (1918) was fortunate enough to find a holothurian, *Stichopus mobii*, in Tortugas which contained 1235 p.p.m. V in the dry animal, and this figure was not due to a printer's error as it was confirmed by the author elsewhere in the text.

Thereafter Wright and Papish (1929) found it in milk, using, it is true, carbon-arc spectrography, and we know the limitations imposed thereby (see above, Vanadium in Plants). Using the same method, Zbinden (1931) "confirmed" its presence in human and cow's milk. Again, spectrographically, Newell and McCollum (1931) considered its presence as only very doubtful in marine animals, following the examination of this metal in the graphite electrodes that they used. Then, again using spectrography, Blumberg and Rask (1933) stated correctly that the presence of vanadium in milk cannot be established definitely because of its presence in the graphite electrodes that were used.

However, Boyd and De (1933) under the

same conditions thought that they had found it in several human organs: in the pancreas, liver, and kidney, but not in the brain.

Then, as we have said regarding some plants, Vinogradov (1934), without giving any new protocols, considered it as distributed in small quantities not only in plants but in animals.

Drea (1934, 1935) continuing to base his results on analysis using carbon-arc spectrography, as did his predecessors, thought that he found some in milk, eggs, and chicken. Lowater and Murray (1937) always found it present in teeth, which is to be expected as they always used carbon electrodes.

We must wait for Webb and Fearon (1937) to see a more serious study. These investigators, after having shown that carbon electrodes cannot be used to demonstrate the presence of vanadium by spectrography, showed that metallic electrodes did not produce this phenomenon, and found vanadium in "large quantities" in a mollusk, *Pleurobranchus plumula*.

The work of Webb and Fearon does not seem to have been known by Bell (1938) who by spectrography "determined" 240 to 650 p.p.m. V in the ash of egg white, and then, relative to the chicken: 1400 in the food, 150 in the blood, and 150 in the liver (ash in each case).

Daniel and Hewston (1942), under conditions that they set forth, thought that they were able to detect spectrographically a minimum of 1 to 5 p.p.m. V in some ash of biological material, but they did not consider vanadium to be well distributed in living organisms. They estimated that vanadium, if it exists in normal tissues of the rat and in egg yolk, is found at concentrations lower than 1 to 5 p.p.m.

Thus 31 years after the discovery by Henze of vanadium in the ascidians, with the exception of the above, it was not known absolutely whether vanadium was or was not present in animals in general. The question had to be taken up again and with much more interest, as by that time it was known that vanadium was not only normally present in plants but that it played a physiological role.

Employing an elaborate technique for ashing without loss of volatile material and a carefully controlled spectrographic technique, Noddack and Noddack (1940) found:

	MILLIGRAMS OF V PER KILOGRAM, DRY
Porifera	
<i>Halichondria</i> sp. (whole)	30
Coelenterata	
<i>Cyanea capillata</i> (whole)	5
<i>Metridium dianthus</i> (whole)	40
Echinodermata	
<i>Stichopus tremulus</i> (eviscerated)	57
<i>Brissopsis lynfera</i> (test)	5
<i>Asterias rubens</i> (eviscerated)	9
Tunicata	
<i>Ciona intestinalis</i> (whole)	620
Pisces	
<i>Ctenolabrus rupestris</i> (whole)	1.6
<i>Squalus acanthius</i> (eviscerated)	1.8

Using the chemical method that he had employed for plants, D. Bertrand (1943a) attacked the problem after having tested the validity of the method and having verified that it was possible to determine as little as 0.02 p.p.m. V in the dry animal. The quantities found were so small, especially for the vertebrates, that the analyses were made difficult by the need for very frequent checks of the reactants, in order to be absolutely certain of the origin and quantities of vanadium. This study should be considerably extended, as only 35 samples were analyzed (see table 3).

Vanadium was always found, except in one organ, the kidney of the dog, and in white mice. Perhaps there was vanadium in these two cases, but there may have been too little to be detected by the method used. It is well to call attention to the fact that the mice, during the six weeks preceding their analysis, received only foods that were found to be very poor in this metal. (Due to wartime circumstances, the Pasteur Institute of Paris which provided these animals had considerable difficulty in feeding their experimental animals.) Three of the seven were born of parents that had been fed the same diet. The small number of offspring recorded in breeding (despite the presence of a sufficient quantity of vitamin C) indicates moreover that

this diet, imposed by circumstances, was far from being complete.

It seems possible, however, to conclude that vanadium is normally present in animals. The two samples of insects were relatively poor. The holothurian analyzed does not seem to confirm the statement that holothurians in general are rich in vanadium, as in this analysis only 1/1235th the amount found by Phillips 24 years previously was found.

On the contrary one invertebrate, *Plumatella fungosa*, was revealed to be much the richest in vanadium of all the animals studied.

The figures are on the whole considerably higher for the invertebrates, being of the order of 1.2 p.p.m. as a mean for the dry samples and 0.28 p.p.m. for the fresh samples, while for the vertebrates the means were 0.1 p.p.m. and 0.026 p.p.m., respectively. This last figure enables us to understand the analytical failures of the first investigators, and if that difference is confirmed it will constitute a particularly significant fact.

For the vertebrates, the liver is among the tissues that contain the most vanadium; thus one again encounters for this metal the facts already known for the other oligoelements and for this organ. If the number of atoms corresponding to this figure is calculated per liver cell, the result is approximately 3.15×10^{11} . If this is compared with the table given by Hutchinson (1943) in his excellent monograph on the biogeochemistry of aluminum and certain related elements, we see that vanadium comes in the sixteenth place, just after nickel, being approximately two times less.

Molybdenum gives according to my analyses $30,500,000 \times 10^7$ instead of the figure given by Ter Meulen and used by Hutchinson, which is a bit high, but this new value does not change the position of this element. On the contrary, cobalt passes to $40,000,000 \times 10^6$, well in excess of biotin, and nickel to $14,300,000 \times 10^6$ or approximately twice what is indicated in the table.

The figures found for vanadium in animals are much lower than those that Henze had found for the blood of an ascidian, *Phallusia mamillata*. Also, although up to very recently almost nothing was known for animals, this

TABLE 3

	Dry Matter, Per Cent of Wet Weight	Milligrams of V per Kilogram, Dry
Porifera		
<i>Ficulina ficus</i>	20.0%	1.7
Coelenterata		
<i>Anemonia sulcata</i> (31 ex.)	20.2	2.3
Echinodermata		
<i>Asterias</i> (<i>Marthasterias</i>) <i>glacialis</i> (1 ex.), body wall only	34.2	2.8
<i>Asterias</i> (<i>Marthasterias</i>) <i>glacialis</i> , without body wall	25.0	3.0
<i>Paracentrocus lividus</i> (24 ex.), without skeleton	12.5	0.8
<i>Cucumaria lefevrei</i>	18.2	1.0
Bryozoa		
<i>Plumatella fungosa</i>	—	16.8
Annelida		
<i>Perinereis cultrifera</i> (145 ex.)	24.6	0.7
<i>Arenicola marina</i> (38 ex.)	13.5	1.8
Mollusca		
<i>Patella vulgata</i> (20 ex.), without shell	26.1	0.1
<i>Helix</i> sp. (31 ex.), without shell	13.4	0.5
<i>Loligo</i> sp. (1 ex.)	22.8	0.4
<i>Mytilus</i> sp. (276 ex.), without shell	24.9	1.2
<i>Gryphea</i> sp. (50 ex.), without shell	9.7	1.3
Arthropoda		
<i>Carcinus moenas</i> , entire (10 ex.)	32.8	0.4
<i>Lepas anatifera</i> , entire	42.8	1.2
<i>Bombyx mori</i> , pupa (150 ex.)	—	0.14
<i>Apis mellifera</i> (2160 ex.)	31.2	0.16
Vertebrata		
<i>Cyprinus carpio</i> , ovary	—	0.19
Dogfish (1 ex.)	23.6	0.04
Red gurnet (1 ex.)	28.0	0.10
<i>Merlangus merlangus</i> (5 ex.)	22.5	0.14
Swordfish (1 ex.)	24.2	0.22
<i>Bufo bufo</i> (6 ex.)	21.6	0.20
Ringed adder (1 ex.)	23.4	0.30
<i>Pelecanus onocrotalus</i> , pectoral muscle	22.2	0.03
<i>Phalacrocorax carbo</i> , pectoral muscle	25.0	0.04
<i>Phalacrocorax carbo</i> , liver (2 ex.)	22.7	0.2
<i>Canis familiaris</i> , kidney (4 ex.)	24.8	0.00 (0.02)
Pancreas (3 ex.)	38.2	0.008
Blood	18.9	0.086
Liver	18.9	0.20
Siberian deer, liver	27.8	0.14
<i>Mus domesticus</i> , white (7 ex.)	33.4	0.00 (0.02)
<i>Rattus norvegicus</i> (1 ex.)	36.4	0.15

was not true for the tunicates.

The discovery of Henze was immediately verified by G. Bertrand. Henze himself in 1912, studying the form in which vanadium might exist in the blood of *Phallusia mamillata*, concluded that this metal was bound in the form of a vanadium-protein, the degree

of oxidation corresponding to a trioxide. Then in 1913, extending his research, he also found vanadium in the blood of four other ascidians, but on the contrary was not certain of its presence in that of *Cynthia papillosa*.

Hecht (1918) studied the blood of *Ascidia aetra* and noted that the green blood cells con-

tained a vanadium compound, but he was content to refer to Henze, without giving any new evidence. However, he supposed that this vanadium played a catalytic role and not that corresponding to the iron in hemoglobin.

After Portes and Benoit (1923) confirmed the presence of vanadium in *Ascidia*, Pied and Azéma (1930) by spectrographic analysis (valid, as enormous quantities of vanadium were involved) found it in *Phallusia mamillata*, *P. fumigata*, *Ascidia mentula*, and (a new fact although in lesser quantities, it is true) in two *Botrylloides*, *rubrum* and *schlosseri*.

Cantacuzène and Tchekirian (1932) determined it in the ash of nine ascidians. Vinogradov (1930) after having demonstrated for the first time that *Phallusia obliqua* contained 302 p.p.m. V for the live animal and having found some vanadium in *Ciona intestinalis*, found more in *Molpadia intestinalis*. He took up this work again (1932) and just barely found vanadium in 3 grams of ash of *Sarcomotrylloides aurea*, but none in *Molpadia affinis* or *Cucumaria frondosa*. In 1934 he gave a table summarizing the known results comprising the 13 ascidians that he had analyzed to date, which was the first attempt at a systematic study.

After 1932, Henze, returning with the aid of Stohr and Müller to the vanadium chromogen of the blood of the ascidians, demonstrated that the fixation of oxygen by this pigment is not analogous with that of its combination with hemoglobin, as the gas is not liberated when its tension is lowered in the atmosphere put in contact with this pigment. He isolated the pigment, analyzed it chemically, and then after various experiments concluded that the vanadic combination is certainly not a respiratory pigment but a substance capable of participating *in vivo* in reduction processes. As the blood of the ascidians is poorer in carbonic acid than sea water (2–3 cm., 3% instead of 6), Henze wondered if marine carbonic acid might not be used by these animals to form the cells of their tunic, in which case the reduction of the CO₂ necessary for this synthesis might call the vanadium chromogen into play.

Webb (1939), in a more systematic study, found vanadium in the blood of nine ascidians and showed that the chromogen that

contains vanadium is always found in a special type of cell, which he called vanadocyte. This vanadium chromogen has no respiratory function and no known physiological function. In addition, according to him, it is not a protein or porphyrin complex but only an association of vanadium with a rectilinear chain of pyrrole rings, comparable to a bile pigment.

Didier Bertrand (1943c) determined vanadium in nine families of the 11 that it is possible to find on the coasts of France. The Correllidae were not studied, as they were considered as not digressing sufficiently from the type of the Ascidiidae to constitute a separate family, nor were the Perophoridae, of which it was not possible to find a sufficient quantity; these animals, very rare in France, are represented only by individuals of a very small size.

All the known results are collected in table 4, in which the classification of Harant (1931) has been adopted.

The concentration of vanadium in the animal without its tunic is much greater than in the entire animal. Webb had already wondered whether all the metal did not come from the blood: his analyses of *Phallusia mamillata* seemed to prove that in that species 80% of the vanadium is contained in the blood. The analyses of D. Bertrand on *Ascidia mentula* did not confirm the generalization of this hypothesis. Indeed in the cells contained in 390 milligrams of the blood (representing more than 50% of the total blood of an animal weighing 25 grams fresh), there was only 89.5 gamma V (and only 1.8 for the rest of the blood), while in the animal without its tunic there was more than 560 and in the entire animal more than 1600.

Vanadium was always found by D. Bertrand in the samples that he studied; an insufficiently sensitive technique may have prevented finding the element in certain cases by other investigators. We may thus consider that vanadium exists normally in all the ascidians, but it does not seem that vanadium of the blood (chiefly contained in the blood cells, as demonstrated, but a small quantity which this writer did not observe also in the serum), may in the long run represent the major part of the vanadium in the animal.

The phlebobranchs, in which may be found

TABLE 4

	Vanadium in Milligrams per Kilogram, Dry	Wet	Author
STOLIDOBRANCHIA			
Hexastigmatales			
Molgulidae			
<i>Molgula manhattensis</i> (DeKay)	31.8	0	D. Bertrand, 1942a-1942e
<i>Eugyra arenosa</i> Alder and Hancock	—	0	Vinogradov, 1934
Polystygmatales			
Pyuridae			
<i>Microcosmus glacialis</i> (Sars)	0.0	0	Vinogradov, 1934
<i>Microcosmus sulcata</i> (Cliquebert)	0.0 (20)	—	Portes and Benoit, 1923
<i>Tethyum partitum</i> (Stimpson)	0.0 (20)	—	Webb, 1939 ^a
<i>Pyura savignyi</i> (Phillipi)	7.1	—	D. Bertrand, 1942a-1942e
<i>Pyura pyriformis</i> (Rathke)	—	0	Vinogradov, 1934
Styelidae			
<i>Dendrodoa grossularia</i> (Van Beneden)	48	—	Webb, 1939
<i>Dendrodoa grossularia</i> (Van Beneden)	10.2	—	D. Bertrand, 1942a-1942e
<i>Dendrodoa grossularia</i> (Van Beneden)	—	68	Vinogradov, 1934
<i>Distomus varialosus</i> (Gartner)	6.4	—	D. Bertrand, 1942a-1942e
<i>Distomus varialosus</i> (Gartner), without tunic	131	—	D. Bertrand, 1942a-1942e
<i>Styela rustica</i> (Linnaeus)	—	0	Vinogradov, 1934
Botryllidae			
<i>Botryllus leachi</i> Savigny	+ ^{b,c}	—	Pied and Azéma, 1930
<i>Botryllus leachi</i> Savigny	0.0 (20)	—	Webb, 1939
<i>Botryllus schlosseri</i> (Pallas)	+ ^c	—	Pied and Azéma, 1930
<i>Botryllus schlosseri</i> (Pallas)	+	—	Cantacuzène and Tchekirian, 1932
<i>Botryllus schlosseri</i> (Pallas)	0.0 (20)	—	Webb, 1939
<i>Botryllus schlosseri</i> (Pallas)	7.8	—	D. Bertrand, 1942a-1942e
<i>Botryllus schlosseri</i> f. <i>aurea</i> Sars	+	92	Vinogradov, 1932, 1934
PHLEBOBRANCHIA			
Distigmatales			
Perophoridae			
	0.0	—	—
Tetrastigmatales			
Hemigones			
Cionidae			
<i>Diazona violacea</i> Savigny	+++	—	Vinogradov, 1932
<i>Diazona violacea</i> Savigny	+++	—	Henze, 1913
<i>Ciona intestinalis</i> Fleming	+++	—	Henze, 1913
<i>Ciona intestinalis</i> Fleming	+++	—	Cantacuzène and Tchekirian, 1932
<i>Ciona intestinalis</i> Fleming	1300	13	Vinogradov, 1932, 1934
<i>Ciona intestinalis</i> Fleming	400	—	Webb, 1939
<i>Ciona intestinalis</i> Fleming	166	—	D. Bertrand, 1942a-1942e

^a Webb's figures pertain to animals without tunic.

^b The plus signs indicate the presence of more or less large amounts of vanadium.

^c This indicates that the results were obtained exclusively by carbon-arc spectroscopy and are therefore doubtful.

TABLE 4—Continued

	Vanadium in Milligrams per Kilogram, Dry	Wet	Author
Pleurogones			
Asciidiidae			
<i>Ascidia mentula</i> Müller	+++	—	Henze, 1913
<i>Ascidia mentula</i> Müller	+++ ^e	—	Pied and Azéma, 1930
<i>Ascidia mentula</i> Müller	+++	—	Cantacuzène and Tchekirian, 1932
<i>Ascidia mentula</i> Müller	1860	—	Webb, 1939
<i>Ascidia mentula</i> Müller	982	—	D. Bertrand, 1942a-1942e
<i>Ascidia mentula</i> Müller, without tunic	6520	—	D. Bertrand, 1942a-1942e
<i>Ascidia mentula</i> Müller, tunic alone	741	—	D. Bertrand, 1942a-1942e
<i>Ascidia mentula</i> Müller, in 390 mg. of blood serum	1.2	—	D. Bertrand, 1942a-1942e
<i>Ascidia mentula</i> Müller, blood cells	89.5	—	D. Bertrand, 1942a-1942e
<i>Ascidia obliqua</i> Alder	—	302	Vinogradov, 1934
<i>Phallusia mamillata</i> Cuvier	+++	—	Henze, 1911
<i>Phallusia mamillata</i> Cuvier	1.0	—	Portes and Benoit, 1923
<i>Phallusia mamillata</i> Cuvier	+++ ^e	—	Pied and Azéma, 1930
<i>Phallusia mamillata</i> Cuvier	1700	—	Webb, 1939
<i>Phallusia fumigata</i> Grube	+++	—	Pied and Azéma, 1930
<i>Phallusia fumigata</i> Grube	+++	—	Cantacuzène and Tchekirian, 1932
<i>Phallusia fumigata</i> Grube	+++	—	Webb, 1939
<i>Ascidia atra</i> Lesueur	+++	—	Hecht, 1918
<i>Ascidia aspersa</i> Müller	++	—	Cantacuzène and Tchekirian, 1932
<i>Ascidia aspersa</i> Müller	++	50	Vinogradov, 1932, 1934
<i>Ascidia aspersa</i> Müller	1450	—	Webb, 1939
<i>Ascidia aspersa scabra</i>	1120.0	—	Webb, 1939
Corellidae	0.0	—	—
APLOUSOBRANCHIA			
Hemigones			
Polycitoridae			
<i>Polycitor vitreus</i> (Sars)	—	35	Vinogradov, 1934
<i>Clavelina lepadiformis</i> (Müller)	7.0	—	D. Bertrand, 1942a-1942e
Didemnidae			
<i>Didemnum candidum</i> Savigny	2.6	—	D. Bertrand, 1942a-1942e
<i>Didemnum albidum</i> (Verrill)	—	0	Vinogradov, 1934
<i>Didemnum (Leptoclinum) maculosum</i> (Milne Edwards)	+(?)	—	Cantacuzène and Tchekirian, 1932
<i>Didemnum (Leptoclinum) maculosum</i> (Milne Edwards)	30.8	—	D. Bertrand, 1942a-1942e
<i>Didemnum (Polysyncrator) lacazei</i> (Giard)	+	—	Cantacuzène and Tchekirian, 1932
Hypogones			
Polyclinidae			
<i>Morchellium argus</i> (Milne Edwards)	0.0	—	Cantacuzène and Tchekirian, 1932
<i>Morchellium argus</i> (Milne Edwards)	3.7	—	D. Bertrand, 1942a-1942e
<i>Parascidia turbinata</i> (Savigny)	0.0	—	Cantacuzène and Tchekirian, 1932
<i>Parascidia turbinata</i> (Savigny)	8.3	—	D. Bertrand, 1942a-1942e
<i>Parascidia areolata</i> (Chiaje)	3.9	—	D. Bertrand, 1942a-1941e
<i>Synoicum elegans</i> (Giard)	0.0	—	Cantacuzène and Tchekirian, 1932
<i>Aplidium pallidum</i> (Verrill)	4.3	—	D. Bertrand, 1942a-1942e
<i>Amaroucium mutabile</i> (Sars)	—	0	Vinogradov, 1934

concentrations of the order of 1 gram per kilogram of whole dry animal, are much richer than the stolidobranchs and the aplousobranchs, wherein there is at the most a concentration of the order of 40 milligrams. Nevertheless, even for these last two orders, the quantities of vanadium found are considerably greater than the mean of those found in the invertebrates, which is already higher than that of the vertebrates, demonstrating that the ascidians form a very special group, as much from the biochemical point of view as from the morphological and anatomical points of view.

The comparison of these results with those found by Phillips and by D. Bertrand for two holothurians suggests that it may well be the same for these animals, but only a special study will permit us to know if it is really so or if the example of *Holothuria* analyzed by Phillips was only especially rich as a consequence of its diet (in which case vanadium would be very slightly toxic to this invertebrate). This leads us to a grave problem which for the moment remains a complete mystery. Where does the vanadium come from that is found in such great quantities in

the phlebobranch ascidians? Certainly not from the sea water, which, as we have seen, is very poor in the element; even assuming that the ascidian succeeded in removing all the vanadium contained in the water passing into its siphon, the duration of the life of the animal would be far too short to explain the accumulation. Moreover, the marine muds in the environment of these ascidians are by no means rich in vanadium, and we must conclude that it is the food of these animals that serves as the source from which they obtain the metal, the physiological role of which is completely unknown. We thus find ourselves confronted again by the problem of the origin of vanadium in the petroleum, as it is probable that if this comes from a particular plankton organism, the same organisms or others closely related serve as the food of the Ascidians.

It would be necessary to prove, and this has not yet been done, that some plankton organism is very rich in vanadium. Such a discovery would naturally lead to the investigation of all the animals that feed on the organism, in order to determine if they too are capable of accumulating this element.

DYNAMICS

PHYSIOLOGICAL ROLE OF VANADIUM IN ANIMALS

We have already seen regarding the ascidians that we know absolutely nothing of the role played by vanadium in living organisms. Few attempts have been made with a view to finding out its possible role in the other animals. We shall not concern ourselves with all of the research, much of which clearly has an exclusively pharmacodynamic character.

Roffo and Calcagno (1931) investigated the action of small amounts of vanadium salts on the development *in vivo* of tissue cells and tumor cells. According to these investigators, all the vanadates studied prevented the development of these cells, and that action was established for very weak concentrations, going in certain cases up to 6.5×10^{-8} , or 0.65 p.p.m. V.

Beard with various collaborators (Myers and Beard, 1931; Myers, Beard, and Barnes, 1931; Beard, Baker, and Myers, 1931; Beard, 1931) in a very thorough study of anemia of

the rat demonstrated that iron alone as a metal was insufficient, and that it was necessary to add other elements to it, including vanadium in the rather large doses of 0.05 milligrams per day (for 0.5 milligrams of iron). In another paper he diminished somewhat the importance of this vanadium, retaining for it a slight positive action. Keil and Nelson (1933) found no increase in hemoglobin in rats that had received intraperitoneal injections of vanadium salts.

Ranzi (1935) has pointed out that vanadium, like magnesium, increased during the development of the embryo of *Sepia officinalis*, and that the latter must take up the vanadium from sea water.

Franke and Moxon (1936) studied the minimum lethal doses of vanadium for the young rat. These were fixed at 4 to 5 milligrams Na VO_3 per kilogram injected intraperitoneally, an amount that killed at least 75% of the experimental animals, and at 50 p.p.m. in the food, a concentration of 25

p.p.m. being injurious. These amounts are known to be much greater than the rat normally finds in its diet.

The work of Bernheim and Bernheim (1938) is much more interesting with respect to the physiology of vanadium. These investigators showed that if vanadium in the form of vanadium metavanadate or acetate is added at the rate of 10 to 20 gamma (of vanadium for the entire liver) to suspensions of rat liver or guinea-pig liver at pH 6.7, there is an increase in the rate of oxidation, which is probably due to the catalysis of an enzymatic action on a phospholipid. The authors conclude that if vanadium is found in small quantities in all tissues, these results solve the question as to whether vanadium has a normal catalytic function and whether it is an essential element. If it is admitted that their process of preparation of the liver suspension eliminated the vanadium contained therein the quantities of the element employed were considerably in excess of the normal content of the liver. These experiments must be taken up again in the light of present-day knowledge, in order to sustain the conclusion drawn by Bernheim and Bernheim (1939), who elsewhere demonstrated that the oxidation of phospholipids by the vanadium-protein system of washed liver was inhibited by manganese and cobalt.

Bowen (1940), studying the effect of vanadium, manganese, and iron on the synthesis of the protoplasm of *Chilomonas paramecium*, made some very interesting discoveries. Na_3VO_4 or VOCl_2 were added to the culture solution to observe their effect on the rate of reproduction and the synthesis of fats and polysides ("starch") in this animal. The action of VOCl_2 provoked a definite, but

slight, rise in the frequency of division, which increased with the quantity of VOCl_2 up to a maximum of approximately 10^{-5} (51 p.p.m. V), and then decreased. This increase in the frequency of division did not take place with Na_3VO_4 . Consequently tetravalent vanadium at a certain concentration promotes the division of *Chilomonas*, while pentavalent vanadium does not. It is easily understood that such a phenomenon could not be observed in plants where the optimal concentration is so low that the valency of the vanadium is regulated by the medium. But it must be noted that in this work on *Chilomonas paramecium* it is quite possible that the effect observed under the influence of vanadium does not correspond to the normal unknown concentration in the organism or its natural environment, which concentration may be considerably lower, according to the analyses published on the normal content of vanadium in animals, unless *Chilomonas* forms an exceptional category analogous to that of the ascidians. By analogy with what happens, as we have seen, with *Aspergillus niger*, this effect is probably pharmacodynamic and not normal physiologically.

Finally McCollum (1941), in a general survey of nutrition, discussed the role of inorganic elements, including vanadium, for which he stated that it has no biological importance, either for plants or animals.

In conclusion, in contrast to plants about which we are better informed, we are completely ignorant of the physiological role of vanadium in animals, where its presence is constant. The problem remains untouched, even for the ascidians, which are the richest in vanadium.

GENERAL CONCLUSIONS

VANADIUM IS A REMARKABLE ELEMENT from the chemical point of view. It acts in fact like a transitional element between the subdivisions A and B of group V of the periodic classification of the elements, its pentoxide placing it near nitrogen and, above all, near phosphorus.

The isomorphism of the vanadates with pyromorphite, apatite, and even mimetite emphasizes further this resemblance and in addition relates vanadium to arsenic. The formation of complex anions, on the contrary, places it nearer to chromium and molybdenum. The tetrachloride relates the element to the carbon group. Vanadium in the trivalent state gives, as do chromium and iron, double salts such as the thiocyanate. Finally the isomorphic relations between the sulfates place vanadium closer to the magnesium series of elements.

The chemistry of vanadium, which may act as a bivalent, trivalent, tetravalent, or pentavalent element, especially its physical chemistry, is far from being definitively established. However, it is known that the salts of vanadium possess very marked catalytic properties, which are profitably used in the laboratory and in various industries.

The metal is extremely widely distributed in nature, in a very dispersed fashion, at a concentration in the lithosphere of approximately 100 p.p.m., which is by no means negligible.

From the general study that we have seen, two categories of facts arise: the first is related to the biochemistry of vanadium, while the second is in a more general fashion related to its biogeochemistry. The results achieved with regard to the first category are by far the more important.

Since 1942-1943 it has been well established that vanadium is one of the normal constituents of living matter, both animal and vegetable, terrestrial or marine. Its concentration in living matter varies considerably in the groups of organisms in which it has been reported, and on this subject it might be well to remark that the quantities of vanadium may be reported as related to fresh or dry material or to the ash as well as in terms of the percentage of atoms instead of in terms of weight relationships.

It is generally useful in publications to report quantities in terms of dry matter, that is to say, the constant weight obtained by desiccation at 105° C., and that in most cases is very near to the dry weight, but it is necessary that the author indicate the percentage of fresh weight of *his* sample. It is thus possible to calculate the mean value of the reported content for a fresh sample collected under ideal conditions. This fresh weight varies considerably according to the conditions of collection and the time interval between the collection and the weighing.

Too few analyses have so far been done for either plants or animals to permit us to draw many conclusions. Nevertheless it may be noted that with reference to the percentages in living organisms, if the insects which seem very poor (0.045 p.p.m.) and the ascidians, some of which are very rich (more than 200 p.p.m.), are excepted, vanadium is slightly more abundant in the invertebrates (0.28 p.p.m.) than in plants (0.16 p.p.m.), while the vertebrates are approximately one-tenth as rich as the former (0.026 p.p.m.).

In both plants and animals, the only cases in which it is certain that there is normally a vanadium content well above the mean are in organisms of archaic origin, *Amanita muscaria* and the phlebobranch ascidians. Moreover, in these two cases, this amazing richness in vanadium is found only in very narrowly defined groups, since the stolidobranchs and the aplousobranchs, although very close to the phlebobranchs, are incomparably poorer in vanadium. Again *A. muscaria* is the only amanite possessing this peculiarity. Neither *A. pantherina*, extremely closely related in all its other characters, nor any of the other European amanites are vanadium accumulators. As a matter of fact, all the other amanites differ little, as regards their vanadium content, from the mean value for plants.

As these abnormal amounts are constant, we are faced with a biological enigma. Are we to see therein the last descendants of series going back to the most remote geological epochs or, on the contrary, the most recent outlines of new series? The first hypothesis seems to be justified by the existence of the petroleums and coals which are rich in vana-

TABLE 5
COMPARATIVE ELEMENTARY COMPOSITION BY WEIGHT

Earth's Crust			Lucerne ^a			Man ^b		
O	49.2		O	77.90		O	62.81	
Si	26		C	11.34		C	19.37	
Al	7.4	n. 10 ⁰	H	8.72	n. 10 ⁰	H	9.31	n. 10 ⁰
Fe	4.2		N	8.25		N	5.14	
Ca	3.5					Ca	1.38	
Mg	2.35							
Na	2.4							
K	2.35							
H	1.0							
Ti	5	n. 10 ⁻¹	P	7.06	n. 10 ⁻¹	S	6.4	n. 10 ⁻¹
C	4		Ca	5.80		P	6.3	
Cl	2		K	1.70		Na	2.6	
S	1.5		S	1.037		K	2.2	
P	1					Cl	1.8	
F	1							
Mn	1							
N, Ba, Bi, V, Li,		n. 10 ⁻²	Mg	8.2	n. 10 ⁻²	Mg	4	n. 10 ⁻²
Ni, Sr, Cr, Zr,			Cl	7.0				
Br, Ce, Cu			Na	3.16				
Be, I, Sn, Co, Th,		n. 10 ⁻³	Si	9.3	n. 10 ⁻³	Fe	5	n. 10 ⁻³
V, Zn, Pb, Mo, Rb, Y			Fe	2.7		F	4.1	
			Al	2.5		Si	4	
						Zn	2.5	
Ar, W, Ta, Cs,		n. 10 ⁻⁴						
Bi, Cd, Hg, Ct								
			B	7.0	n. 10 ⁻⁴	Rb ^c	9	n. 10 ⁻⁴
			Rb	4.6		Cu	4	
			Mn	3.6		Br	2	
			Zn	3.5		Sn	2	
			Cu	2.5		Mn	1	
			F	1.5		I	1	
			Mo ^d	1				
La, As, Nd, Nb,		n. 10 ⁻⁵	Ti	9	n. 10 ⁻⁵	Al	5	n. 10 ⁻⁵
Sb, Ag, Se, Sc			Ni	5		Pb	5	
			Br	5		Mo ^c	2	
			Ti	4.6		B	2	
			V	1.6				
Tl, Te, Pr, Au, Pt		n. 10 ⁻⁶						
			I	2.5	n. 10 ⁻⁶	As	5	n. 10 ⁻⁶
			Co	2		Co	4	
						Li ^e	3	
						V ^c	2.6	
						Ni	2.5	
			As, Sn, Pb, Sr, Ba	n. 10 ⁻⁷		Sr, Ba		n. 10 ⁻⁷

^a Water, 75.1%; org., 22.45%; ash, 2.45%.

^b Water, 60%; org., 35.7%; ash, 4.3%.

^c Mean figure for mammals.

^d Normally in plants, 3.10⁻⁶.

dium. But then it must be admitted that of the amanites only *A. muscaria* sprang from those ancient epochs and that the other toadstools of the group appeared more recently. In the same way in the ascidians, the stolidobranchs and the aplousobranchs would appear to be more recent than the phlebo-branchs.

Let us compare the vanadium content as well as that of other elements in the earth's crust (according to the work of Clarke; see Clarke and Washington, 1924), with two living organisms: a plant (the lucerne) and an animal (man). The figures for the last two of these are taken from the course given by Gabriel Bertrand, 1905-1938, Professor at the Faculté des Sciences of Paris and are corrected from the most recent analyses done in his laboratory.

We see that in plants as well as in animals vanadium is one of the elements, among those that have been determined, that appear in very small amounts, as it just precedes iodine and cobalt in plants in the twenty-ninth place, while in the earth's crust it appears in the twentieth place with a concentration 1000 times greater than in plants and 10,000 times greater than in man.

Even if we are totally ignorant of the physiological role (if there is one) of vanadium in animals, we are not so as regards plants, because for them it is indispensable in certain cases but at such low concentrations that this category of living organisms is always sure to find its need in nature, while because of its low toxicity there is no fear of encountering excess quantities. If we may believe certain analyses of American soils, some higher plants, such as barley, do grow badly, or not at all, on soils that are too rich in vanadium.

The vanadium concentrations of living organisms are so low that it might well be asked whether they could be concerned in the vanadium cycle in nature.

Taking up a part of the subject taught by Gabriel Bertrand since 1905, Vernadsky (1923), whose work on living matter had begun in 1917, set forth in 1922 his ideas on the chemical composition of living matter and the chemistry of the earth's crust at the Petrograd Society of Naturalists. He estimated the quantity of vanadium existing in living matter, both animal and vegetable, to

be approximately $10^{-4}\%$ (1 p.p.m.), a figure which appears in his book on geochemistry (1924a) as between $10^{-3}\%$ and $10^{-4}\%$ (1 to 10 p.p.m.). Such amounts could not have escaped the numerous investigators who have studied the elementary chemical composition of the biosphere. Indeed, with such contents the hydrochloric solution of the ash is colored blue, as Vernadsky (1924b) himself realized. It was this blue coloration that attracted the attention of Ter Meulen to *A. muscaria*. However, it is very comprehensible that Vernadsky might at the time have extended the conceptions of Lindgren on the cycle of vanadium to the greater part of the migration of the element, and he even wrote, "its history can be represented by a primary reversible geochemical cycle." Given the considerable disproportion that now exists between the quantities of vanadium found on one hand in the lithosphere or even simply in the arable soils derived from the lithosphere partly through the action of plants and on the other hand in living organisms, it is very improbable that the majority of living organisms could be involved in such a cycle or even, more simply, in dispersing vanadium in the lithosphere. At most it may be said with some chance of truth that plants intervene to diminish the vanadium content of the superficial terrestrial stratum. Even in the ash, the vanadium content of plants is incomparably lower than that of the medium in which they grow. Consequently with time the most superficial strata of the arable soil must become less rich in vanadium than the deep strata. The few rare analytical profiles seem to justify this conclusion, which permits us moreover to understand why on the whole the arable soils, at least the older European ones, are less rich in vanadium than the lithosphere.

Moreover Vernadsky himself recognized that "Juvenile minerals of pure vanadium do not exist and isomorphic mixtures are, in general, very poor in vanadium. The great mass of these atoms (of vanadium) is in the state of dispersion. . . . It seems that the phreatic regions have vanadium concentrations which exist nowhere else in the crust."

His remarks concerning the sediments discovered in North America, in Colorado and

Utah, in which vanadium is found in the form of uranyl and potassium vanadates, again bring to light an exciting problem, that of the variation of the elementary composition of living organisms through the course of the geological ages. In fact these minerals "are found as cement of Mesozoic sandstone, sometimes very rich in organic matter, in wood, in remains of carbonized plants, in bone. It was deposited in shallow aquatic basins, near beaches. Some ores are known to cover an area of several thousand square kilometers. . . . The genesis of these beds is not very well known." Up to now, uranium has not been found as a normal constituent of living organisms. Consequently, if it does really exist in them, it can only be in a very small proportion.

Vernadsky thought that the mean elementary composition of living organisms should not have varied through the course of the geological epochs. Profound morphological evolution and the increasing tendency to pass from autotrophy to heterotrophy make us think, on the contrary, that this evolution must have been accompanied by a profound modification of the chemical functions of living organisms and consequently of their elementary composition. It seems on this hypothesis that the variety of early attempts to elaborate organic matter, starting from various types of chemical processes which involved for certain lines a relative accumulation of one or more elements, was more and more limited in the course of the aging of the earth, leaving, save for rare examples in the process of extinction, only organisms with possibilities that are multiple in one sense, restricted in another, and strictly dependent upon one another, and in which the synthesis of basic substances was realized. In them, such synthesis is performed not by a small number of independent, varied individuals, but in a cycle including a considerable number of organisms. In the restricted field of vanadium, *A. muscaria* and the ascidians have already given us a confirmation of this point of view. The coals will permit us to extend such a viewpoint somewhat.

Only the analysis of organisms of remote geological ages would allow us to demonstrate this proposition effectively, but in the absence of such material, analysis of coals,

taken as representative of ancient plants, can give us some information. Let us recall that the coal flora comprised various gigantic species of *Cordaites*, of tree ferns, *Calamites*, *Sigillaria*, *Lepidodendron*, and *Stenophyllum*, growing in a rather different environment from that of those modern plants whose chemical composition we are beginning to understand. We may in fact admit that they lived under conditions of intense illumination, in a uniform climate, hot and humid, without great contrasts in the season. In the sea, as in fresh waters, algae multiplied and have been classified as *Solenopora*, *Neurathophycus*, *Pila*, or *Reinschia* (Piveteau, *vide* Baud, 1942). As for the compact, elastic, "boghead" coals we must see in them planktonogenic deposits especially rich in gelosic algae. They correspond to the "sapropel" of Potonie discussed above under petroleum in which beside plant debris may be found remains of higher animals, fish or Crustacea.

Without an estimate of the part played by the waters of streams or rivers and running waters in the formation of detrital or complex rocks, the various types of coal could not have been individualized, according to Duparque, from a single continuous deposit but must have been derived from accumulations of different sorts in distinct geographical divisions. From this we may conclude that the analysis of coals in general cannot tell us the elementary composition of an archaic type of plant but can only give us a mean for various types.

According to a current opinion, the mineral substances that furnish the ash may have not only a primary origin from substances contained in the plants themselves from concretions laid down during deposition and argillaceous material brought in with the organic debris, but also a secondary origin, namely, the filling of the concealed crevices of the coals and of their diaclasses by rhombohedral carbonates, by bisulphides, or, later, by sulphates.

Such a view is surely very exaggerated and is based on a complete lack of appreciation of the elementary composition of living organisms. It seemed to be supported particularly by the analysis of the ash of English coals, which are very rich in silica, alumina, and iron, the argillaceous origin of which was de-

cided upon too hastily. Lessing (1920), studying two of the four constituents of thick coal, Harnstead Colliery, demonstrated that the aluminum of its ash could not exist in the form of silicate. Hutchinson (1943), in his monograph on the biogeochemistry of aluminum, reviewed to date the work on this question, which can only be answered with certainty by new researches. Nevertheless the known facts are in favor of the hypothesis that I have adopted.

Instead of studying the composition of the ash, we should accept a catalytic role and not only a plastic one for the elements whose concentration we are comparing in the plants making up the coals and in modern plants, and it is preferable to relate the quantities of elements present to the carbon. This procedure permits us to eliminate the evident quantitative variations resulting from the profound transformations undergone by the plant in the process of coal formation. We may assume as a first approximation that all of the carbon in the primitive plant is retained in the coal. If a mean figure for per cent of ash and for per cent of carbon be taken, the coals that contain from 30 to 300 p.p.m. V in the ash contain from 0.8 to 8 p.p.m. V relative to carbon, while in modern plants this figure is, on an average, 1.4 p.p.m. The relatively slight difference exhibited by modern plant ash and coal is thus almost completely eliminated. This indicates that in the plants that contributed to the formation of the coals low in vanadium the element played a catalytic role equivalent to that in modern plants. For the coals that yield ash containing from 1500 to 6000 p.p.m. V, these figures related to carbon are 42 to 170 p.p.m., which are lower than those given by *A. muscaria*. The high vanadium coals therefore appear less extraordinary on the hypothesis that *A. muscaria* is the only known surviving cryptogam that retains some ancestral chemical characteristics.

Thanks to the analyses of Fuchs (1935), we can make such comparisons for coal-producing plants and modern plants. The following figures are converted to percentages of carbon content and represent average approximations; they are given only for elements determined in both coals and plants.

	GERMAN COAL	LUCERNE	COAL FROM WALES
Ca	0.99	5.15	0.218
Fe	0.30	0.024	1.42
Mg	0.16	0.725	0.122
Mn	0.010	0.0032	0.015
Si	0.092	0.082	2.16
Al	0.045	0.022	1.64
Na	0.064	0.27	} 0.364
K	0.005	1.5	
P	traces	6.25	0.0225
Cu*	10^{-6} to 10^{-5}	2.2×10^{-3}	
Ni*	10^{-7} to 10^{-6}	4.5×10^{-4}	
Zn*	10^{-7} to 10^{-6}	3.1×10^{-3}	
Ti*	10^{-7} to 10^{-6}	8×10^{-4}	

* As these were determined spectrographically, there may be substantial errors in exact amounts, and the figures are given as only a rough indication.

For the oligoelements, Goldschmidt (1935), using a more precise technique, gives additional figures for some coals. These are also converted to percentages of the carbon content.

	COAL	MODERN PLANTS
Ni	2×10^{-4}	4.5×10^{-4}
Co	8.5×10^{-5}	1.8×10^{-5}
Mo	5.6×10^{-5}	2.7×10^{-4}
B	1.7×10^{-4}	6×10^{-3}

Examination of these figure immediately shows very good agreement for the oligoelements, less for molybdenum and boron, but in view of the analytical technique used the determinations on coal need to be repeated. We may conclude that the synergic catalytic role of the minor elements was already determined in broad outline when the plants forming the coal were growing. The fact that archaic plants are richer in sodium than in potassium does not surprise us, because at the present time this is still true of aquatic plants.

Magnesium is more abundant now, but it is involved almost exclusively in the formation of chlorophyll. We find here again an easily explicable fact; the conditions of temperature, humidity, and illumination completely justify the hypothesis that plants of remote geological epochs, which were the raw material of coal, had chlorophyll needs well below those of modern plants. Great differences on the other hand are manifested in the cases of phosphorus, calcium, and iron. Differences relative to silica exist only in the

case of certain coals, and we may recall that at the present time many plants are still very rich in silicon, notably horsetails and grasses, to give only two examples of very dissimilar modern plants.

These facts entirely justify the hypotheses that we have put forward on the inconstancy of the elementary chemical composition in the course of the geological epochs. Certain groups of living organisms, some descendants of which still exist, have been able to orient their biochemical activity in such a direction that they became "rich" in one or more elements, these characteristics having eventually disappeared in the course of evolution for reasons that we shall not discuss here as they would be considerably beyond the scope of the present work. We do, however, find justification for the very high vanadium contents of certain coals, and even of petroleums in such hypothesis for it is not certain that modern plankton organisms are still rich in this metal.

But it is still only a question of hypothesis, as the experimental demonstration is not yet perfect, owing to the lack of coordinated

analytical results. I should like, therefore, in closing, to recall a statement made by Pasteur, which is too often forgotten: "At the beginning of experimental research on any subject whatsoever, the imagination must give wings to the idea, [but it is necessary] to institute decisive experiments leaving no place for the imagination, [and] at the moment of concluding and interpreting the facts which the observations have assembled, the imagination must be dominated and enslaved by the material results of the experiments."

[NOTE ADDED IN PAGE PROOF: As this work goes to press a summary of a conference on "X-disease in cattle (hyperkeratosis)" (Contribution No. 1 of the McCollum-Pratt Institute, the Johns Hopkins University, Baltimore; n.d., ?1949) records that recent analyses made during a United States Government survey of the minor constituents of 60 plant samples, including alfalfa, bluegrass, lespedeza, Dallis grass, Johnson grass, ladino clover, kudzu, oat hay, peanut hay, and sorghum, gave a small and quite uniform content of from 0.3 to 1.1 p.p.m. V. This presumably refers to the dry plant and is in good agreement with the results presented on pages 423 to 434.—G.E.H.]

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