A Review of Research on the Biological Transmutation of Chemical Elements

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Based on an unfinished paper by Prof. L.W.J. Holleman;

Translated and completed with criticism by David Cuthbertson.

We can state as an indisputable axiom that under all conditions, artificial or natural, nothing is created; an equal quantity of matter exists before and after the experiment and nothing occurs outside the changes and modifications in the combinations of the elements.

Lavoisier (1789)

Abstract

Professor Holleman reviewed evidence which suggests that Lavoisier's law does not always hold true for plants and animals. However, he also showed that none of this evidence is good enough to be considered as definitive, either for or against his belief in the biological transmutation of chemical elements. His own experiments, showing the disappearance and subsequent reappearance of the chemical element potassium in closed cultures of the green alga *Chlorella*, have therefore been presented in detail to help and encourage others who may wish to conduct further such research.

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Foreword to the Present Version of this Review

The aim of this critical review is (and was) to present the work of Professor Dr. L.W.J. Holleman on a series of experiments on the possible biological transmutation of chemical elements in cultures of the alga *Chlorella vulgaris* to as wide an audience as possible. It is hoped by this means that further research may be encouraged. This present work was commissioned by the Professor L.W.J. Holleman Stichting [Trust].

At the centre of this review lies an experiment that was for Holleman the culmination of a life long search to prove the possible existence of the biological transmutation of elements. He took his inspiration from the agriculture-course, a number of lectures given by Rudolf Steiner in June 1924 to a group of farmers in Koberwitz, Poland. The experiment was written up (in German) and privately published and circulated by Holleman in 1981. He reported the provisional results of an experiment involving a series of closed *Chlorella* cultures and which demonstrated the disappearance (and subsequent reappearance) of the chemical element potassium. This report referred to a critical review article on the biological transmutation of chemical elements that was shortly to be published, covering both historical and modern experiments, including details of his own work. Sadly, this review article was never completed.

The present provisional review article is an attempt to translate [from his native Dutch] and complete that unfinished article. The first 5 and parts of the 6th and 7th sections of this present review article are direct translations of the surviving drafts of Holleman's manuscript. Unfortunately these drafts were not extensive enough to make it possible to complete the article as Holleman presumably intended. The final form of the last half of the review was never fully sketched out. The final sections that I have written to complete this work are therefore mostly based on his surviving laboratory notebooks, notes and letters, as well as on his privately circulated report of his initial, intriguing potassium results that were obtained in experiment II of his experimental series with the alga *Chlorella*.

It is likely that Holleman wrote his surviving drafts over an extended period of time, certainly up to 1982. That he intended to write up his *Chlorella* work fully once the potential transmutation results of experiment II had been confirmed might help explain why his fourth main experiment, which was designed to confirm these results, was hardly written up at all. It was as if he were holding his breath for the analytical results of experiment IV, which were completed by the end of 1982, before writing anything down. The results were, sadly, negative. The blank pages in his laboratory notebook, which he had left ready for a description of the methods, materials and the full results, were never completed. Something had gone terribly wrong which he could not explain. Experiment V led to experiment VI and still no replication of the unexplained results from experiment II. Eventually, some 14 years after starting his *Chlorella* work, he lost his laboratory. However that did not stop him from wishing to continue. Old age and infirmity played a part, but I believe it to have been disappointment that stopped him in the end.

Nevertheless he made several things clear. Firstly that he had not lost his faith that biological transmutations may be possible. Secondly that his belief was not enough. It had

to be proved under rigorous scientific conditions, and not once but many times. To this end he intended that this and related work must be written up so as to invite the criticism of others. This he felt to be essential so that the future research that he wished for might have the benefit of the wisdom of others.

In February 1995 I was invited by Wim Holleman's daughter, Sophia Holleman, to continue her father's research. This I tentatively accepted. It led, in May 1996, to the presentation of my initial studies of the literature and other sources relating to this subject in a colloquium to the Louis Bolk Institute of the Netherlands. Despite the colloquium's positive reception I found myself unable to take on the tremendous burden of responsibility involved in conducting such revolutionary work [literally so for the conventional scientific community: Of note is that Holleman described this subject as precarious, even treacherous; the 19th century German transmutation researcher Herzeele, who provided Holleman with much of his inspiration, described it as a hopeless task. If the phenomenon of the biological transmutation of chemical elements was an easy one to prove, it would already be a generally accepted fact]. My wish here is to facilitate the possibility that others may continue Wim Holleman's pioneering work. This I hope to do by means of a critical translation and review of Holleman's notes and writings relating to his *Chlorella* research.

A few words may be needed on the style and content of this review. As was previously mentioned, the first 5 sections and parts of the 6th and 7th are direct translations of Holleman's rough drafts of his intended review. Only occasionally [though more so in the later sections] did I find it necessary to rewrite individual sentences, though only where I believed that their comprehension might otherwise have proved difficult. Where I felt the need to comment directly on Holleman's text [which I have freely done] I have chosen not to use footnotes. To distinguish his commentary from that of my own, I have identified mine by writing it in the first person and the present tense. I hope that this may prove acceptable to the reader. The final sections, covering Holleman's work from 1982-1989, are entirely my own, though wherever possible I have drawn directly on the thought processes recorded by Holleman in his numerous scattered notes and letters. This has proved to be extremely challenging, though highly rewarding. This present review makes no claim to be complete; given more time and financial resources, many references quoted by Holleman could have been checked and further relevant literature referred to; during the writing many questions were raised for which answers might have been obtained. Comments would however, be gratefully received, either by myself or the Stichting.

I have taken quite literally Holleman's request, in his own draft Foreword that is to follow, to bring forth any criticisms that I have had during my studies of this work. This is a highly controversial area of science that I believe must be approached in a direct, open, but rigorous manner. The main criticism that both Holleman and myself have for other previous research, is not that the experimental results were right or wrong, but rather that the experimental methods were rarely published in sufficient detail to enable an independent assessment. I hope that this review of Holleman's work goes some way in enabling both the lay reader and the scientific specialist to be able to assess much of the large volume of work conducted by Holleman on this subject.

Since writing this report, further notes and papers relating to Holleman's work have been discovered. It is intended some time in the next few months, once funds become available, to review these in the hopes of shedding more light on Holleman's as yet unexplained, intriguing, and challenging results.

David Cuthbertson, February 1999.

Foreword

This report relates to path finding research on the possible existence of a biological transmutation of elements; i.e. a transmutation brought about by means of organic life.

These first results - bearing in mind that there is still much lacking in the conducting of the experiments - submitted to a small group of colleagues in the hope thereby to awaken interest in the subject and at the same time to draw out criticism that will undoubtedly be of advantage to the author.

Seeing their - possibly apparent - incompatibility with the prevailing understanding of this subject matter, the results may only be ripe for publication after further confirmation.

Meanwhile, thanks are given here for the kindness shown by the management and fellow workers of the Pathology Institute of the R. U. Utrecht (considering the somewhat precarious nature of this undertaking).

Special recognition is given to Mrs. W. Terpstra of the Biophysics Laboratory of the R. U. Utrecht for her valuable advice and provision of pure *Chlorella* cultures.

[Holleman, ca.1982]

1 Introduction: Transmutation in Inorganic Nature

For an effective account of the subject handled in this report, a short consideration of the historical background of this subject is necessary.

1.1 The Chemical Laws

The development of present day chemistry depends on the recognition of a number of quantitative rules, of which the first and most fundamental is the law of the indestructibility of matter [otherwise known as the law of the conservation of matter]. Its first formulation is attributed to *Lavoisier (1789)* who considered it to be an axiom. One may probably assume that the idea already lived in a less finished form with a number of earlier thinkers.

A second, already earlier generally considered fundamental truth, concerned the existence of a limited number of raw materials, the *chemical elements* (Boyle, 1661). The idea of the indestructibility of matter, *in general*, was directly linked by Lavoisier to the idea of elements. In his original formulation of the first law two statements are combined:

1. In a closed system wherein chemical processes take place, the total weight of *matter* (thus of all substances together) is invariable ("nothing is created")

2. The total weight of *each of the elements*, from which the substances are composed, is equally unchangeable ("nothing takes place other than changes and modifications in the combinations of the elements").

It is worth noting that the experimental proof for Lavoisier's law only refers to the first part of his formulation: he established from a number of inorganic and organic processes that the *total* weight remained constant. This limited number of experiments became the only evidence for the entire law during the whole of the nineteenth century. By the end of this period Landolt (1897) with the utmost accurate weighing confirmed the invariability of chemical reactions, once again only for the total weight: he excluded errors to more than $1 partin 10^6$. Later Manly (1913) improved the accuracy to $1in10^8$.

Meanwhile conceptions about the structure of matter were conceived whereby the aforementioned researches could almost be viewed as superfluous. Originating from Dalton, the representation of *atoms* as the building blocks of matter took the subject by storm. Whilst scientists had no clear image of what happened with the formation of a chemical bond, the now familiar proposition was developed of a number of unchangeable particles, of a different nature for each element, which arrange themselves in space in a specified manner. The atoms became thought of as extremely small and, for each atom, identical to each other. It was possible with the aid of this extremely simple proposition to give a reasonable explanation of the quantitative chemical laws.

In relation to the following it is important to point out that, however important the atomic proposal is for the quantitative handling of the chemical processes, the *qualities* of the elements and their bondings are pushed strongly into the background, so much so that our attention has become removed from the empirically observable, remarkable infinite multitude of chemical compounds.

1.2 Transmutation in Inorganic Nature

In the beginning of the 20th century facts became known such that the conception of the absolute unchangeability of matter, and thus also the immutability of the atom, was thrown to the ground. The discovery found it's origin in the borderland between physics and chemistry: it was determined that with some, mostly heavy elements, radiation was emitted which was linked to a change in the chemical character of the studied element. For these so called radioactive elements the term *transmutation* first became established. In the course of time it was found that these elements go through a disintegration process which, via a number of intermediaries (elements whose identity could be demonstrated), ends with a stable element, normally lead. These processes occur with a loss of mass, partly in the form of so called radioactive particles (alpha and beta-particles) and also as pure electromagnetic radiation.

From this discovery it is to be concluded that the law of the conservation of mass in Lavoisier's original formulation only applies to non radioactive elements. Because mass is now viewed as a form of energy [Einstein's famous $E = mc^2$], the fundamental law remains as the conservation of (total) energy for all material changes, including those of a radioactive nature.

The character of the radioactive phenomena - their independence of temperature and other outer circumstances and the fact that they remain uninfluenced by the chemical transitions that the respective elements undergo - leads one, for their further understanding, towards a deeper layer of matter from that in which chemistry normally plays. The atomic conception arises once again from the pressures of explanation; there were (besides radioactivity) many other facts that lead to the acceptance of a structure for the atom with a *nucleus* as the seat of the radioactive disintegration and an orbital shell which plays the main role in it's normal chemical behaviour.

In a following phase of research it appeared that radioactivity is not limited in nature to elements that are apparent exceptions but that they are also capable of being induced in the most stable elements by artificial means, by bombarding them with radioactive particles.

Thus a new chemistry has been developed, atomic chemistry, whereby enormous quantities of energy are given into human hands; the atomic era has been created.

1.3 Older Transmutation Conceptions: Alchemy and Vitalism in Old Agricultural Science

The previous rough overview of the development of the materialistic viewpoint, developed since about 1800, led to the conclusion that the present-day conception of the atom has reached a point where no more principle changes can be made.

This conception appears particularly to have persisted in the didactic of chemistry. Presently the atomic view is simply taken as the primary given and it thereby completely ignores the means, through the chemical laws, by which this conception was reached. An open consideration of the attained endpoint, that still permits another way of thinking, appears impossible because the thought up image is already fixed in the mind of the student after even a basic introduction to chemistry.

Nevertheless there is evidence of a vague and basic realisation that our current knowledge is derived from older conceptions of matter; that there were once individuals who worked with sources of knowledge that have since been lost. Thus, shortly after the discovery of radioactivity the associated transmutations were mostly greeted as the "realisation of the dreams of the alchemists".

This refers to older ideas that are no longer taken seriously because they are not the results of experiments in the present-day meaning of the word. If one ignores the period of rampant charlatanism in alchemy [involving material gain through the attempt to convert base metals into gold] there follows, from the little that is known about alchemistic transmutations, the greatest of contrasts between the alchemy of then and now. Nowadays it is - at least in principle - a clear process with the input of extremely great energy and an apparatus of overwhelming dimensions, whilst the human involvement with the process is as insignificant as possible - at that time it was a method of working only accessible for particular, prepared people and which required exceptional intensive human input, with a minimum of external means of help [see section 10 and Appendix I].

2 Transmutation in Organic Nature

[Holleman's use of the word organic differs from that of modern chemistry; nowadays the term refers to chemical compounds containing the element carbon, independent of the source of that carbon; Holleman's use dates back to a time when all carbon compounds were considered to have originated - directly or indirectly - from living organisms. Organic nature thus refers here to the living, biological world.]

2.1 Vitalism in Agricultural Science at the Beginning of the 19th Century

Towards the end of the 18th century the alchemistic way of viewing matter was mostly extinct. Certain ways of thinking that can broadly be considered as alchemistic have, however, survived for considerably longer. Here belongs the idea that in organic nature transmutations of elements and even the creation of matter can occur spontaneously. The former process may be called *biological transmutation*; there the origin of these transmutations has mostly become ascribed to a strange organically acting "life force", which one may speak of as vital energy. Thus it was the opinion of the leading agriculturalist *Thaer* that under certain circumstances calcium in the plant became changed into silicon, whilst this substance may itself, according to him, be formed from potassium. *Lampadius (1832)* accepted that silicon existed in plants due to its new creation.

2.2 Biological Transmutation Experiments to 1842

Although voices against the vitalistic ideas were not lacking, no reliable evidence was put forward, so vitalism remained in the beginning of the nineteenth century the main, ruling concept. To this end agriculturalists such as *Thaer* and *Einhof*, who apparently enjoyed the greatest fame, contributed considerably. There was no lack of researchers who applied themselves to find experimental evidence for the vitalistic concept.

Work was stimulated in this respect during the years 1795, '96 and '97 when the Berlin Academy of Science announced a competition with the following aim (by means of which was considered the possible role of a "life force", and in the broadest sense the creation and destruction of elements, under the influence of the organic process, belong here as well):

> Of which type are the earthly materials which are encountered by means of chemical analysis of native grain species? Do they come into the grains as they are found, or do they come into being by means of the life force and brought into growth by the workings of the plant?

The award fell to *Schrader* for experiments regarding the formation of minerals in grains. He allowed seeds of wheat, barley and rye, amongst others, to germinate in an artificial medium of flowers of sulphur (that was shown to be completely ash free) and watered them with distilled water. Contamination from dust was guarded for. From analyses of the

developed seedlings compared with the seeds used he concluded that mineral matter had indeed been created.

Similar experiments were conducted by *Braconnot* (1807) who enjoyed considerable reputation as a chemist. He allowed plants from seed to grow on different artificial media (flowers of sulphur, red lead oxide, granulated lead, pure river sand and even an organic product; decomposed wood that was extracted with hot water). Although he knew of the importance of an analysis of the growth medium he presented no results there from; he demonstrated the growth media as completely insoluble in water. An analysis of the seed was also missing. Nevertheless he concluded that considerable formation of the mineral components, especially potassium in experiments with mustard seed and radish, had taken place.

Braconnot was, in his vitalistic opinion, even more radical than some of his peers and prophesied a return to the science of the *theory of Thales*, that everything originates from water. The mineral kingdom should then be considered as;

> the immense residuum, the big storehouse, in which the organic realm unceasingly disposes of matter.

Matter, in all its diversity, should, according to Braconnot, originate by means of fabrication from the proto-water. He supposed that not only water (that in ancient times was considered to be an "element") but also all the other elements, such as potassium, are formed from hydrogen and oxygen. The occurrence of fossils, according to him, proved this. Moreover any salts added to the soil should be deleterious to plant growth.

Against the above described research stand replications by Lessaigne (1821) and *Jablonski* (1836) who found not the slightest increase in the amount of ash in the plant above that in the seed. Therewith they joined the critics of vitalism, of which *de Saussure* (1767 - 1845) may be seen as the most important representative.

De Saussure had already in 1804 let a first summary of his work appear under the title "Chemical Researches on Vegetation". It is an example of careful scientific work in which he was beyond his time. A large part of his opinions about plant physiology, which are still valid today, were already placed and supported in this work by quantitative experiments. As a follower of Lavoisier, Saussure stood strongly with the standpoint of the conservation of matter and referred all transmutation and creation to the realm of fables. He put special emphasis on the necessity in this field to be absolutely certain, with experiments, that the so called created matter was not already present in the environment. So he demonstrated, for example, that the presence of silicates in the plant, which were attributed to the life-force by Lampadius, were in reality determined by the amount of silicon in the soil.

The work of Saussure later turned out not to be completely fruitful, chiefly because of his imperfect chemical analyses. This did not however take away his contribution to an understanding of the life processes in the plant, especially his putting exact research methods to the fore, which has been shown to be of fundamental significance.

2.3 An Attempt at an Historical Justification of the Vitalistic Idea

A modern writer of this period of agricultural science (Browne 1944) was surprised by the tenacity with which the vitalistic way of thinking was maintained until c.1840. He viewed this phase as a backward step in the development of science and ascribed it in part to external factors such as a lack of communication between scholars whereby either previous researches such as that by Saussure were overlooked or the uncritical adoption of others such as Braconnot. This applied especially to the changing understanding of the influences of soil and fertilizers on plant growth, which was still at a primitive stage.

The uncertainty that prevailed in this area during the first decades of the previous century can be more or less understood when taken within the framework of the then existing conception of nature. One must place oneself into ways of thinking that included the immediately given macroscopic image of nature and which were totally free from an atomic image of the world that was later to rule physics. Suppose that an agriculturalist determined that during its growth a plant changed its chemical composition. This process happened in front of direct observation in a manner which differed strongly from a chemical process and which is beyond the nature of an organism; it appears to be taken up and subjected to some other, higher power than exists in the inorganic world. With the changing insights into the assimilation and taking up of salts by the root system, etc. still in an initial phase, it is therefore not so astonishing that parallel with the external metamorphosis of the plant, a metamorphosis of the matter from which it is built could be accepted, which itself extends to the transmutation of the elements present in the plant. [The existing laws of physics can explain how, due to the gravitational force, an apple falls down out of a tree; was it the "vital force" that got it up there in the first place?!]

First and foremost, the strong development of inorganic chemistry, bound with the atomic conception, took the upper hand, apparently banishing this vitalistic, holistic thinking. Indeed there is in the atomic world conception no room for the formation of an idea of living substance [i.e. there is no "scientific" difference between, say, biologically fixed nitrogen and nitrogen of an inorganic origin; see however the publications of the *Arbeidsbericht* of the Institut fur Biologisch-Dynamische Forschung E. V., Darmstadt, Germany]. What it comes down to is that the immutable elements are only able, in this image, to change places. Above all, they are thought of exclusively, as being independent of the universe in which they exist; either of an organism or the inorganic world. There is, in this world, no possible transformation that could carry a "life-characteristic".

Therefore a situation has gradually arisen wherein, on the one side, the workings of life are not recognised [i.e. the "vitalistic force"] but on the other side, the inorganic [physical] side of the researched, living being, is exclusively taken. This was an essential development that cannot be valued highly enough. The inorganic could also be viewed in living beings, separately, as it were, in artificial cultures and experiments, which is indispensable to an objective, scientific handling of living nature.

This does not preclude that, in the future, ways shall be found to be able to study life in a manner that will literally enable life's own intimate character to be seen to its own, full, advantage. It is also certain that, from new observations in this direction [not stated,

but presumably those of "Goethean observation", in biology in general, but especially in the fields of biodynamic agriculture and anthroposophical medicine; though Holleman's own transmutation studies reported here were (outwardly) conducted along purely conventional lines; see section 10], new insights can be expected.

The idea of a "life force" that temporarily played a role in the transition period leading to the atomic conception was, before long, shown to be without content, and was only of use to cover a lack of insight. It marks a point in time wherein the living had to be removed from human consciousness in order to make way for the non-living.

2.4 The End of the Vitalistic Period: Wiegmann and Polstorff's Experiments

The uncertainty that continued into the third decade of the 19th century regarding the possible role of a life force in plant development led, at the end of this period, to a competition, this time written anonymously and published in the "Royal Goettingen Society of Science". Drawn up for the then already strongly altered scientific climate so that, in opposition to the 1806 competition, this time the word "life force" was not used. The question was:

> Are the inorganic elements which are found in plants such essential components of living plants that they are needed for their complete development and are they externally provided?

The answer considered for award came from Wiegmann (professor of soil science in Brunswijk) and Polstorff (pharmacist also from Brunswijk). It was based on experiments that for the first time were conducted according to rules that could at present still be valid.

Wiegmann, from the literature of previous researchers, came to the following conclusions:

1. That plants need for their complete development a certain quantity of inorganic constituents, that later turn up as ash components.

2. That these mineral components are primarily taken up from the soil and for a very much smaller proportion from the atmosphere (falling dust, rainwater or snow).

For a proof of these two propositions the authors set up a series of pot experiments with a synthetic soil, composed of a mixture of cleaned quartz sand with the, as then understood, most important inorganic soil components (added to by a number of humic salts that at that time were considered to be indispensable for plant growth). Control experiments were set up with only the afore mentioned quartz sand for soil. An analysis of it demonstrated that apart from silicon dioxide it contained 2% other soil components. The experimental plants were tobacco, vetch, clover, barley, oats and buckwheat. The cultures were protected against dust and regularly watered with distilled water.

After the plants had come to the end of their development, they were harvested (whereby the roots were washed with distilled water to remove attached soil), dried, ashed, and each ash sample weighed and analysed. After correction for the seed's ash content, it was found

that the experimental plants had taken up 3 times more mineral substance than the controls. The development of the latter was strongly behind those of the experimental plants. Tobacco had taken up the greatest quantity of ash components, the other plants, in their afore named order, less.

With this result the first part of the competition was answered. The last question posed;

> ... and are they (the inorganic elements) provided externally?

led to many different interpretations, but was presumably aimed at evidence for the conservation of elements as according to Lavoisier. It was this interpretation that Wiegmann and Polstorff took. They decided to undertake an experiment that once and for all must bring to a conclusion the fought over problem of biological transmutation and respectively, creation.

2.5 The "Experimentum Crucis" of Wiegmann and Polstorff

To come to a definite conclusion here, the authors followed the technique of their predecessors, but with a "soil" that consisted of the most inert material known to them at that time. They let 28 seeds of *Lepidium sativum* germinate in distilled water in a platinum crucible that was filled with fine platinum wire. The crucible was placed under a glass bell jar through which circulated a mixture of 1% carbon dioxide. The seeds germinated and grew into small plants until, after 26 days, they began to die. After drying the crucible and its contents, ashing and weighing obtained 0.0025 grams of ash. The weight of ash obtained from 28 seeds was likewise 0.0025 grams.

Wiegmann and Polstorff made from this experiment the following conclusions:

1. Plants can for a period of time continue living on the reserves of inorganic materials present in the seeds from which they came, but that growth stops once these reserves are insufficient for their further development.

2. The inorganic components of plants can in no way be considered as products of their life process, nor as results of unknown elements, nor exceptional derivatives of the four elements that are known as the building blocks of organic matter.

3. As the plants were isolated from all unwanted sources of inorganic matter, the quantity that they contained cannot be greater than the original amount that was present in the seed.

It was as if the world had been waiting impatiently for Wiegmann and Polstorff's results so radical was the change brought about in the publications of scientific circles. The last remnants of the vitalistic conception were thus swept away and from here on, in the rest of the century there is (with a single exception, see section 4.3) no more talk of the possibility of biological transmutation.

Should the experiment of Wiegmann and Polstorff be compared with the work of the previous century, then it is the technical advancement that is indeed noteworthy. On further

consideration, apart from appreciation for the exact experimental method, there are some doubts whether the publication of 1842 is as decisive as it first appears. Remarkably criticism was never expressed regarding such an important experiment and the negative conclusions were blindly taken up by the scientific world. One cannot thus wholly withdraw from the impression that not only the researchers but also the scientific community harboured the not completely conscious wish to promote the negative decision, to undermine the possibility of biological transmutation so as to force this problem to be shown as being forever settled.

In order to give this assertion content experiments have to meet conditions that must firstly be formulated in order to count as sufficiently conclusive.

3 Directions for Transmutation Experiments

3.1 General Viewpoints

To prove the existence of biological transmutation, experiments must be bound by spatial and temporal considerations.

3.1.1 Spatial Considerations: the Closed System

Living organisms are in constant exchange with their surroundings. To record the changes in chemical composition that occur in a particular amount of material which is under the influence of a living organism, one is committed to a quantitative determination of all the elements inside a closed space (hereafter known as the *system*), that encloses both the living material and a piece of its natural environment. This environment includes, apart from light as an unweighable component [though the mass of light is practically unweighable, it's energy is measurable, and since it is critical for the development of all green plants, full details of the intensity and nature of any light source must always be recorded (see for example Tipnis and Pratt, 1960) - a point neglected in his German language article], the air gasses, water and salts [; the system should also include any solid surfaces, such as the bounding walls of the experimental system which, though chemically non-reactive, may ab- or adsorb a proportion of one or more of the above]. A continuous provision of the gaseous component is necessary for the maintenance of life, thus the quantities of the gasses consumed and formed must be determined and ideally one should work in an air-tight system. Although one such attempt has been made (see section 4.3) an experiment conducted in such a way would not be easy, so that, despite working towards perfection, one would generally limit oneself to non-volatile elements and those which do not volatilise during *ashing*. [Ashing is a process here involving the heating of a sample to about 500 degrees in an oven, thus burning off all organic matter leaving only an inorganic, mineral residue]. Such non-volatiles can be studied, in principle, in open containers without losses. For the further enclosure of the system, clear (glass or plastic) lids can be used with openings for the entry and release of gasses.

By such a choice of system, one loses very many possibilities that an experiment may have to offer. Should it be possible for a transmutation to occur with one of the four "*organic*" [my

emphasis] elements (carbon, hydrogen, oxygen and nitrogen) [all of which may be volatile as element or compound], this could not be determined by such a limited experimental design.

[Holleman notes in the margin here an experiment by Picket on the measurement of argon gas production by yeast.]

3.1.2 The Need for Control Experiments and the Statistical Handling of Results

For an absolute proof of a change in the composition of a system an analysis must be conducted of this system in at least two stages (a beginning and an end stage), over a period in time during which a development in the organic material may occur. This is in practice only to be used as a method when an arbitrarily formed change in the composition of an, in part, living system can be followed without profound interference in the structure of its matter. Existing methods for this ([left blank but NMR spectroscopy might be one such method]) have not yet been worked out in a form that lends itself to the research that is being considered here. The application of the standard methods of plant analysis, which result in the destruction of the plant material, are totally unsuitable to the research described above.

One is therefore forced to conduct this research on the beginning and end stages of two or more independent systems [that are in all relevant aspects identical]. From a part of the plant material that is as homogeneous as possible, in the initial stages (seeds, roots, etc.) one can put a number of systems of the same type together; some of which (the control systems) are directly analysed [see however section 8.1.5], the remaining (the experimental system) only after the development of the plant.

This method has been, until now, generally used in biological transmutation proofs, often with the neglect of the statistical problem that arises here. The composition of different plant structures, e.g. leaf or stalk, also that of a particular quantity of a determined weight of seeds from a single lot, is in general fairly variable [Holleman speaks here from personal experience based on his early unsuccessful work to repeat Herzeele's extensive transmutation researches]; with experiments involving cuttings, variations of 10% or more are no exception. Such variations even occur when each half of a longitudinally divided stem is tested. The question as to whether an experimentally determined difference in composition between experiment and control is significant can nowadays be answered by well known statistical methods. Because no such account was given with older research, the results obtained were often illusory [Holleman refers the reader here to an intended appendix which was never written, describing his earlier Herzeele research].

Significant results [positive or negative] can in these cases always be obtained by increasing the number of replicates. Should the starting material exist in smaller units (e.g. small seeds) it is to be expected that it will be more homogeneous, so that significant results may be obtained with fewer replicate experiments.

The statistical problem is totally lost with the use of single celled organisms, e.g. algal suspensions of a microplankton type (average cross-section of the cell a few hundredths of a millimetre) [provided that the cell population is sufficiently homogeneous]. When sinking is avoided, variations in the average composition of such suspensions apparently fall within

the errors of the experimental method.

3.1.3 The Duration of the Experiment

The question as to which point in time such an experiment can be shown to have ended is only to be answered in relation to the degree of development that the organisms attain during the experiment. An exploratory piece of research on biological transmutation should in the first instance be directed towards the tracing of an effect within the *whole* course of development of the organisms. Now, in the case of working with larger plants, the development of the different individuals in a system is not uniform; only some (in the most favourable, the majority) go through the whole development expected under the given conditions. Thus chemical analysis of the system *as a whole* always delivers an average of all the attained development stages. This applies probably just as much for a culture of micro-organisms.

So long as the development of the system is clearly in an on going direction and the finishing material is of reasonable homogeneity, it may be pretty well assumed, that a large majority of organisms are associated in a comparable stage. Under these preconditions the best chance of reproducing the outcomes also exists.

The best moment for the ending of the experiment may be provisionally chosen when it is best estimated that the maximum unfolding of the organic life is attained, with the proviso that also in the break-down and decomposition stage, effects can still occur.

3.1.4 Step-wise Analysis of Parallel, Identical Systems

So long as one is unable by any means to determine in which development stage it is possible for an organism to produce a transmutation effect, it is necessary not only to arrange an analysis at the begin and end points of the system, but also at different points in time during the experiment. Accepting that at each point in time a majority of the individuals of a system occur in approximately the same stage of development, one can hereby in any case get an impression of the progress of the process. Things that occur, for example, in a particular stage of the process that become compensated for by their opposite in a later stage, can only, by this means, come to light.

For the practical conducting of this research one is driven to the setting up of a number of parallel experiments with systems of identical composition, that each may be broken off at a different point of development.

3.1.5 Extent of the Organic Process

Since, for the research described here, the *specific* influence of the organism's life process is at issue, the *extent* of the organic development must, within the accepted time limit, be promoted as much as possible.

With an eye to this the following measures are put forward.

3.1.5.1 Composition of the Nutrient Solution

The supply of the essential mineral food components in optimal quantities is probably an important factor for the correct unfolding of the organic process. It is noteworthy that earlier researchers paid relatively little attention to this. The material used was allowed to germinate either in distilled water or supplied with extremely one sided nutrient solutions. The reasons, which were given earlier, shall only quickly be gone into in the following ( )[sic]. In principle it is not to be discounted that only in deficient nutrient solutions does a plant use a transmutation process as a way out.

For an exploratory piece of research the choice should really be given to the optimal feeding of the experimental organism by means of the organism's most suitable nutrient solution. Hereby the material transmutations during the progress of the organic process can be studied and all phases of the development will be obtained. [See however section 10.1.3].

3.1.5.2 The Cumulative Method

[This section was compiled from two rough pencil written drafts of Holleman's; I am therefore responsible for a minor amount of editing to provide a single, hopefully coherent text. The principle here described, though essentially a simple one, has not proved easy to explain; it is however of crucial significance to Holleman's experimental design. Nevertheless I have refrained from rewriting Holleman's original text. The reader is thus referred not only to the last paragraph of this section, but also to section 6.1 and section 7.1.1 for practical examples of how the cumulative method was conducted.]

The experiment shall generally be set up so as to attain the largest possible effect. An as yet untested means towards this end is the single or multiple replication of the growth experiment in one and the same system [see section 3.1.1 for a reminder of the definition of a system as used here]. This means of working appears specially suited to experiments with a small amount of homogeneous culture material in which a noticeable development can be expected, i.e. for example, micro-organisms. A possible transmutation becomes, in the first instance, just as many times increased as the number of part experiments. The accumulation in effects possibly limits a natural growth when, by this method of working, an essential element is removed from [or added to] the nutrient solution in too great a measure.

At the end of the experimental period in the first experiment, one ashes the content of the dish system and brings these back again [by dissolving the ashes] into the original form of the nutrient solution that was chosen [I have expressed doubts, in section 10.1.5, as to whether the exact original chemical composition of the nutrient solution was in fact reconstituted]. This should generally be possible without adding, or losing, measurable amounts of ash components (cations) [I do not understand why anions are not also considered important; of note here is that Holleman found it necessary to add extra nitric acid to adjust the pH of the reconstituted nutrient solution back to it's original value]. The reconstituted nutrient solution obtained, can be used again for a growth experiment in the same system. [These guidelines should obviously be considered as just that; the results of any modifications should always be measurable and should not affect the health of the cultured organisms.]

(The following text only in key words; to be elaborated later.) [Sic].

3.2 Practical Measures

The avoidance of positive contamination [causing artificially high results] and negative [causing artificially low results] contamination. Culture containers must be completely inert over a long period; resistant to long term action of neutral watery solutions, also against corrosive chemicals (nitric acid, perchloric acid) and against heating to ca.500 degrees (ashing). Quartz glass is the best. Protection against infalling dust. For the growth of algae, agitation of the solution is necessary: shaking machine at constant temperature [water-bath]. Supply of air carrying 5% carbon dioxide through flexible tubing and, by means of a splitting point, equally divided over the culture dishes.

Parallel working of control and experiment; the handling must be identical, with the exception of the addition of an excess of nitric acid, with the *experimental* cultures at the end of the growth period, with the *controls* directly at the setting up of the experiment, so that the organic development is prevented [this was the unsuccessful practice used only in experiment III; see section 6.1].

Working in as sterile conditions as possible.

4 Critical Review of Earlier Experiments

4.1 Research Before 1840

Generally insufficient information to judge the experiments.

4.2 Wiegmann and Polstorff's Experiment

Criticism regarding the following points:

1. The experiment only consisted of the development of *Lepidium* seeds to seedlings; because no nutrient solution was given, development was unable to proceed, no noticeable assimilation.

2. Only the *total* weight of ash from the seedlings was compared with that of the same number of seeds. Therefore the existence of transmutation in the narrowest sense is not excluded [for example, if an element of a certain atomic weight transmutes into half the quantity of an element with twice its atomic weight the total weights of the system, before and after, would remain unchanged].

3. The precision of the weighing is presumably of the order of 0.1mg. For a total weight of 2.5mg this is a possible variation of 4 to 8%. A creation of this order of magnitude is thus not completely excluded.

4.3 Later Research

Von Herzeele (1876 - '83). His work was rediscovered by accident in about ca.1930 (the philosopher W. H. Preuss had dedicated an article to him; Preuss defended the idea that inorganic nature was a product of the organic; Herzeele was in agreement, apparently inspired by Goethe).

Herzeele experimented with seeds and even roots which were allowed to develop under glass bell jars. His analyses give the impression of professionalism, despite the lack of details. Of a more unfortunate nature, he gave no consideration for the variability of the plant material. The occasional large effects that he found were therefore not significant. This was clearly shown to be true by a replication [by Holleman] of his most spectacular "transmutations" ([Holleman again refers the reader here to the unwritten appendix describing his earlier Herzeele work]).

Spessard (1940). This was the only published experiment in which an organic process was studied in a hermetically sealed container. The bottles were weighed after some years. At the end, living protozoa were still to be seen through the glass walls. Presumably plant assimilation and animal respiration followed each other more or less in balance [plants produce oxygen and consume carbon dioxide, animals take in oxygen and produce carbon dioxide]. There was a weight *increase* of a few tenths of a milligram (with a balance accuracy of 0.02mg). Sources of error, so far as they were known, were carefully eliminated. The predicted continuation of this work did not appear. The increase in weight that was found was far too big to be considered as a "materialisation" of the received light rays. [I am curious as to why, of all the possible critique of this work (insufficient controls, no information on whether steps were taken to prevent the settling of dust on the outside of the vessel or other external contamination), Holleman chose an argument from within the paradigm of atomic theory; see section 1.2 and section 3.1.1].

Kervran (1966, 1972a, b, 1976). A researcher who, just as Goldfein (see below), at the level of nuclear chemistry, made biological transmutation plausible. [I consider that Holleman was being either generous or naive here; Kervran's model is a simple and potentially useful one - if proved to be true - but it is highly simplistic and has been severely criticised by nuclear physicists (see de Gee, 1973; Zvirblis, 1977)]. Practical researches that were undertaken at his instigation in Switzerland and Japan were lacking with regards criterion 3.1.4. [Kervran's practical experiments have in my opinion and I believe that of Holleman, been rightly criticised for their generally unscientific nature; evidence given was often circumstantial or anecdotal; controls were generally absent or inadequate (3.1.2); raw data and its statistical handling, if any, were not published (3.1.2); the studied organisms were not always kept in any sort of closed system (3.1.1). See de Gee (1973), Paster(1980). Nevertheless, a large volume of work relating to this subject was brought together by Kervran that cannot be entirely be ignored].

Goldfein (1978) developed a model on a microscopic scale, whereby biological transmutation may be possible. The DNA spiral in the cell's nucleus [sic] could work as a cyclotron at a molecular scale, the thus developed electron stream could transmute specific atoms. [The postulated cell structure involved was in fact the mitochondria and not the nucleus.] The

author had assumed that the light energy taken up in the form of matter can be re-released as energy. The weight increase was actually ca.3 orders of magnitude larger than can be calculated from the hypothesis. [See section 5].

5 Arguments For and Against Further Research

It may have become apparent from the proceeding critical discussion of earlier research that the problem of biological transmutation has not been solved, either in a positive or in a negative sense.

The main motivating force for renewed research is undoubtedly the conviction that the earlier work had not completely exploited all the experimental possibilities in this area. In itself this need not be a reason to start such new research. There are also a number of arguments of a theoretical nature that on the whole argue against biological transmutation and which may make further research seem pointless. Digressions from the classical laws of conservation would have paradoxical or even absurd consequences for the whole system of nature [as constructed by theoretical physics, the predictions of which are increasingly being experimentally confirmed].

Two arguments push themselves to the fore:

1. It is to be considered as an incontrovertible fact that the plant for its growth and development depends on the supply of mineral components from outside [so believe conventional scientists]. On the basis of this it is hard to understand what sense can be attributed to the further creation of elements already supplied [an argument independently used by Michel Haring regarding algae (personal communication)].

2. A consequence of a continuous transmutation by means of the living plant is that the whole soil could change through plant growth alone. [This they are well known to do, in conjunction with other soil organisms; but they are only known to be able to alter the distribution and composition of chemical elements and their compounds. Consider, for example, the alleged consequences of the evolution of the first green plants and their production of free, gaseous, oxygen where none had existed before; the present day fixation of nitrogen, by plants, from the air; and the soil formative powers of plant roots, earthworms, and innumerable other soil fungi and micro-organisms in general, without which our soils as we know them today would not exist. What Holleman refers to here though, are the consequences of hypothesised transmutation, or even creation (and/or destruction) of chemical elements, leading to fundamental, irreversible changes in the elemental composition of the soil and thereby of the Earth itself; see section 6.3.3 for further details]. Although one could imagine such a shift in the composition of the earth in its earliest days of existence when the relative proportions of everything were different, for the present age such a process is hard to imagine and is probably in contradiction to all existing experience in the field of agriculture [and biology in general; see however, Kervran(1972)].

Now the question is whether within the overall tendencies of plant physiology, which seem to meet the laws of the inorganic world, finer sub-processes occur which escape direct observation [see section 10]. In spite of everything that can be argued against a deviation from the usual way of thinking, it is not unthinkable that within the framework of the normal physiological laws, sub-processes happen in which transmutations occur. These could have a meaningful function in the life process, for instance by means of the formation of an as yet unknown isotope of an element [indication from Steiner (1924) and Koenig (1982); see also section 10.1.5 that is needed for the development of an organism. The first counter argument is therefore less pressing than it initially appears. When in addition such sub-processes may be considered as being reversible, so that a conversion in a particular stage of the organic development process is reversed in another stage, then the weight of the second counter argument also diminishes; there is no need to fear a geo-chemical catastrophe[!].

3. A much weightier objection relates to the energy changes in the case of transmutations. In the inorganic world transmutations usually only take place through the supply of considerable energy in the form of radiation of various sorts [or phenomenally high temperatures such as are found in the centres of stars or supernovae]. It is assumed that for such processes a very high energy threshold has to be crossed. Yet also in this case the possibility is given of a transmutation under much smaller energy changes by means of the so called tunnel effect. [Though theoretically possible, it is, according to the same theory, very, very, highly improbable (see Zvirblis, 1977). Nevertheless, in an interview with the professor and director of organic chemistry Pierre Barranger for the journal *Science et Vie* in 1959, who apparently had conducted extensive replications of the transmutation research of Herzeele, the analogy of the nucleus of an atom as a strong box, or safe was used; difficult to break open using blind violence, but easily opened by skilful manipulation (quoted from Tomkins and Bird, 1989)].

An attempt at a rational explanation of transmutations in the living cell was made by Goldfein (1978). He based his deductions on the transmutations which Kervran postulated and which Komaki is supposed to have experimentally proved. These transformations, for example ${}^{39}_{19}K$ into ${}^{40}_{20}Ca$, are supposed to be able to happen totally under energy gain and therefore spontaneously[!]. The localisation of these processes is to be found, according to Goldfein, in the mitochondria. He supposed that within these organelles a magnesium compound of adenosinetriphosphate functions as the microscopic model for a cyclotron within which there is an acceleration of ions which is sufficient to cause a transmutation. The author concluded that hereby a new source of energy was in reach and actually of unlimited capacity!

The model has not been worked out in detail by Goldfein; it is also very much a question whether, along the lines of his very daring hypothesis, a correct idea of biological transmutation can ever be found. [Whether Holleman here considers that any hypothesis based on nuclear chemistry is doomed to failure, or merely that this hypothesis fails to answer a number of fundamental questions posed by conventional nuclear physics (e.g. de Gee, 1973; Zvirblis, 1977), is not clear.] However, the article had the merit that it focused attention on

the possibilities which can hide behind the enormous multiplicity of biochemical reactions that occur in a living plant.

When one reviews the whole of biological transmutation research (which has been going on for more than a century), one has no other possibility than either to put the whole problem aside as pointless, or to devote oneself - removed from theoretical considerations - to renewed research, using modern techniques, on the *factual* [empirical] background to this as yet unsolved problem.

6 Description of Holleman's Transmutation Experiments

6.1 Summary of the Methods Used

The experiments that are to be described were as far as possible conducted according to the guidelines stated in section 3.1.5. The organism used was *Chlorella vulgaris*. The cumulative method given in section 3.1.5 was followed; nutrient solution was inoculated with a small amount of an algal culture in the same medium. Under the conditions chosen, this culture became fully developed in 10 - 14 days. At the end of this growth period the entire contents of the dish used for the experiment was evaporated, ashed and returned to the original condition of the initial nutrient solution as described in section 7.1.5. For a second growth period this new nutrient solution was inoculated by a culture that had during this time been made ready. This culture was again brought to development, etc... By this means it was possible under the given conditions to repeat the periods of growth six times in, most importantly, the one volume of feeding solution in one and the same vessel.

Since it is important to be able to determine the size of a possible effect after one, two, etc., to six growth periods, the whole experiment was split up into six parallel part experiments, of which the first was broken off after one growth period, the following after two, etc., through to the sixth growth period.

At the same time, six control experiments were set up with entirely the same composition as the experimental group that went with them and for which only the handling after each inoculation differed; they were evaporated and ashed immediately after inoculation. It must be pointed out that this difference in handling does not entirely comply with the demands formulated in section 3.2 for the utmost maintenance of the identity of experiment and control. In later experiments, described below, this difficulty was attempted to be met by the control dishes being at all times during the entire growth period allowed to run together in the apparatus with the actual growth experiments. The algal growth was prevented by means of an artificially brought about acid reaction (experiment III), the exclusion of light (experiment 8508-1), or by heating for one hour at 100 degrees centigrade (experiment V).

6.2 Essentials of the Set Ups

[This unnumbered section should perhaps belong elsewhere as it, for example, covers the intended analysis of a number of non volatile chemical elements which, in fact, Holleman

did not succeed in doing. It is however, included here because of it's direct relevance to his Chlorella research. See section 6.3, section 7.1.5, section 7.2.3 and section 9.1.4].

1. Quantitative composition measured before and after.
2. The experiment to extend as far as possible to the whole life process. Assimilation facilitated by means of (a) air carrying CO_2, (b) extra light, (c) agitation.
3. Study only the non volatile elements: *K, Ca, Mg,* P, (C, H, N, [potentially volatile as elements or compounds]), S(?), Fe(?). Especially study *magnesium* with regards to the important role of this element in assimilation.
4. [Illegible].
5. Increase of the effect obtained by (a) repetition of culture growth in a solution composed of the same minerals; (b) stimulation of the growth by supply of air - carbon dioxide.
6. Limiting sources of error by; [illegible, incomplete].

6.3 Composition of the Experimental Series; Experiments I - III

Experiments I-III (1975-1982) are given below, as described by Holleman. His later experiments (1982-1989) are described by myself (D.R.C.) based on his laboratory notebooks and other loose notes; these were often very sketchy and difficult to follow and understand.

6.3.1 Experiments I - III (1975 - 1982)

There were in total 3 series of experiments set up (I-III). The first two consisted of 6 quartz dishes with inoculated nutrient solution and 6 control dishes with similarly handled and inoculated nutrient solution, which were immediately evaporated as described in section 7.1.5, ashed and redissolved to produce a solution of the ashes. [See figure 1, section 7.1.1 for an overview of the experimental set-up].

I. *The first experiment* came no further than the first growth period; a fault in the thermostat of the shaking bath towards the end of this period raised the water temperature to close to 100 degrees. The experimental cultures, in parallel with the controls, were quantitatively worked through to ash solutions. The development of the algae, during nine days of the experiment, was negligible. The likely reason was the small quantity (0.1ml) of inoculation material used. In the following experiments (II and III) the inoculation was thus increased to 2ml of the powerfully developed stock culture. This amount was derived from a paper of van Hille (1930).

II. *The second experiment* progressed right through to completion, although the development of the algae in the later six stages (a, b, ..., f) was extremely variable.

The ashing caused a considerable alkalisation of the mixture of salts obtained. Therefore, in order to ensure the viability of the algae in the following stages a generous quantity of

nitric acid was added to the ash and the excess removed by careful evaporation. For further information see section 7.1.5 [Holleman originally referred here to a section which has not been found; it may have shed light on a technical problem encountered during the ashing process that probably affected the results of the experiment enormously - see sections 6.3.2, 10.1.5, 10.1.5, 10.2.3 for further details]. It appeared during the progress of the experiments that the pH of the newly reconstituted solution depended strongly on the amount of nitric acid added and the method of evaporation. From pre-experiments it was initially concluded that an addition of 0.1m.eq. nitric acid was sufficient. It later turned out that 0.5 to 1.0m.eq. was needed to ensure a pH of 4-6 for the solution attained.

III. *The third experiment* was in part combined with the second. A place for a culture dish in the shaking bath became free after each stage of experiment II which could be utilised by a new culture. This was made use of in experiment III such that a number of culture and control dishes could go through a number of stages together. The control dish of this experiment was *beforehand* endowed with the same quantity of nitric acid that the experimental culture received *after* completion of growth. The idea was to overcome the imperfect parallel treatment of experimental and control cultures and to place both groups open to entirely the same external conditions. Unfortunately it turned out that this quantity of nitric acid (5ml 0.01N) was not sufficient to completely prevent the development of the algae.

A number of intermediary experiments were conducted during this time on the difficulties of analysing for calcium and magnesium. See section 7.2.3 for further details.

6.3.2 Experiments IV - V (1982 - 1987)

Following the unexplained results of experiment II (see section 7.2.1), the experimental procedures were improved and refined. Special attention was given to the prevention of contamination; culture maintenance; ashing and hydrolysis procedures; and the number of experimental replicates (i.e. parallel running identical cultures). [See figures 1 and 7 in sections 7.1.1 and 8.1.1 for details of the experimental set-ups].

Experiment IV cannot be described in any detail because of a severe lack of information recorded in Holleman's laboratory notebook; blank pages were left to be completed at a later date, but this was never done. Whilst the initial set up and most of the results were recorded on loose notes and in his laboratory notebook, neither the aims, nor procedure, nor conclusions were given. It was presumably intended to replicate, or compliment the results of Experiment II. Chlorophyll content, phosphate and the elements potassium and sodium (the latter two analysed by flame photometry) were measured for the 30 (or 24?) cultures. It is not clear whether the number of cycles was 4 or - more logically, given that 6 sets of 4 culture beakers were used - 6. The indications are that they were probably 6 sets of 2 experimental cultures, 1 control and 1 culture for the taking of samples, thus the experiment was presumably run for 6 cycles. Holleman states elsewhere that the results were not positive; i.e. they followed the conservation laws. The accuracy of the polyphosphate determinations was not thought to be very good.

A large number of relatively small experiments and tests were conducted between experiments IV and V (August '82 -December '85):

- The size and shape of possible culture vessels were examined with the aim, not only of improving agitation, but also with the intention of decreasing the size of the culture vessels to be able to increase the number of vessels that might fit in the shaking bath.

- It was considered that the ashing process, following the Middleton-Stuckey method [presumably the method used so far] may not be sufficient for a complete oxidation of all organic matter. Instead two culture tubes were treated with both nitric and sulphuric acids. In fact [according to Holleman] the sulphate ions would be likely to replace all other anions (except phosphate), which was not acceptable [- would the sulphate affect the algae?]. Therefore further tests on different ashing and hydrolysis procedures (both for the reconstitution of a new culture solution and also for phosphate hydrolysis for analysis) were conducted.

- The consequences of trace element impurities present in the "suprapure" nitric acid were considered to be significant. With each dose of nitric acid, traces of copper would accumulate in the culture solutions. Toxic levels would potentially be reached after only 4 cycles. Whether any practical action was taken was not stated.

- Phosphate, chlorophyll and organic matter determination was investigated to varying degrees.

- The loss of the Labline shaking bath resulted in a major change in apparatus and, to a lesser extent, procedure.

- Agitation of the algae to keep them in suspension was attained by the bubbling of carbon dioxide carrying air directly into the culture fluid. The most suitable culture vessels were test tubes. Temperature was maintained inside a terrarium (an aquarium without water) and was lit by fluorescent tubes designed for use with an aquarium.

- Evaporation and partial ashing of completed algal growth by means of an aluminium block heater for the test tubes was the subject of extensive testing.

- The deficit of potassium measured in experiment II was attempted by Holleman to be brought down to what he called "trivial reasons" [see section 10.2.3 for a critical discussion on this]. According to the literature, polyphosphates, which are formed by heating the ash to 500 degrees, under certain conditions, can form insoluble sodium salts. Holleman put forward the hypothesis that this may also be true for potassium. An analysis of the ash solution by flame photometry requires the absence of any such precipitates. Thus the necessary removal of any precipitates by filtration may result in the measurement of an apparent reduction in the potassium content of a *Chlorella* culture. The acid treatment of the ash is important in the hydrolysis of the polyphosphates, thus converting them into soluble phosphate ions.

The experimental procedure was to treat the ashed *Chlorella* culture with insufficient acid. The unexpected result was that the ash was completely soluble in pure water (at ca.70 degrees). The highly alkaline solution was more so than was expected. The pH was investigated with the titration of hydrochloric acid.

- Another potential cause of the potassium loss recorded in experiment II and considered by Holleman was the involvement of the algal organic matter in the ashing process. The oxidation during the heating to 500 degrees of this organic matter could result in the for-

mation of localised hot spots of a significantly higher temperature. This could lead to the melting, evaporation and loss of some potassium compounds. The [circumstantial] evidence for such an hypothesis was that the potassium loss occurred only with the experimental growth cultures and not in the control dishes in which algal growth did not occur. These former cultures would contain significantly greater amounts of organic matter [this would not, however, explain the potassium's subsequent reappearance].

- The alkalinity of the ash solutions cannot, apparently, be exclusively explained by a removal of water from the phosphates [polyphosphate formation]. Most likely is the removal of nitrates from solution. The nitrates are taken up by the algae out of solution, but there is also the possibility of nitrite formation. Therefore preliminary experiments involving the determination of nitrates were conducted. Unfortunately the results were unreliable due to a suspect spectrophotometer.

- [By way of light relief??] 10 dandelion (*Taraxacum*) seeds were germinated and subsequently ashed, along with a further 10 control (ungerminated) seeds. Unfortunately there is no record of the ashes ever having been analysed.

- During (and after) the sprouting of the dandelion seeds, *Chlorella* experiments continued as before. Growth experiments were conducted involving the measurement of algal growth and the monitoring/regulation of the gas supply. The overall gas pressure was able to be better controlled by the addition of a more accurate regulator and a pressure gauge. The supply to each culture tube was able to be controlled by means of adjustable pressure clips attached to the silicon rubber tubing connected to every gas inlet glass capillary tube. The algal growth was monitored every couple of days by measuring the optical density of specially diluted, very small samples of well mixed *Chlorella* culture solution.

- A proposal to determine the loss of culture fluid due to the possibility of the gas bubbles, bubbling through the cultures, creating spray (micro droplets) was considered, but no clear details or results were given.

- The weight loss of the system was measured so that distilled water could be added to replace that lost by evaporation. This was crucial to the measurements of algal concentration. The necessary uncoupling of the culture tubes from the gas supply was, however, a very delicate operation. Thus it was decided, instead of weighing, to mark the original fluid level on the side of the tube and at the end of the algal growth period, to top up the tube to its original level.

Further modifications to the culture apparatus were made and tested:

- The position of the lights was improved.

- To improve the mixing of the algal suspensions, quartz capillary tubes were compared with very fine PVC and also polythene tubing. The gas pressure, inlet tube internal diameter and also the height of the inlet tube from the bottom of the culture tube all affected the size and rate of bubbles produced. This in turn had an affect on the agitation of the *Chlorella* which is essential to prevent it's sinking and the formation of clumps.

Foaming of the solutions was another problem. Tests involved:

- Floating a loosely fitting piece of plastic on top of the culture liquid;

- Siliconising the tubes;

- Use of the proprietary solution “Span 85”;

- Adding a tiny amount of liquid paraffin to the culture solution;

- Smearing a very small amount of “Vaseline” over the inlet tubes. This latter treatment proved to be the best.

- The evaporative loss of water from the growth cultures was attempted to be compensated for by the warming of the gas wash flask. This successfully increased the water content of the gas supply. In fact when the gas wash flask was heated to 55 degrees the algal cultures actually gained in volume. A “Tecam” thermostatically controlled water bath set to 27 degrees, in which the gas wash flask sat, provided the chosen compromise temperature. The water loss was found to depend on the temperature difference between the laboratory and the cultures.

- The tubing, through which the gas was supplied, was shortened to help reduce water loss by condensation inside the tubes.

Experiment 8508-1 formed a preliminary part of experiment V. Six duplicate culture tubes (i.e. 12 in total) were inoculated with *Chlorella*, but with one of these pairs used as a control. The control pair was identical to the others except that light was excluded, thus preventing algal growth. The pairs were stopped after 2, 4, 7, 9, and 11 days. The control pair also underwent the full culture conditions for 11 days. The chlorophyll concentration of each of these tubes was measured by two different techniques. The ash solutions of the two control tubes were later used as part of experiment V.

Before starting experiment V, an experiment was started with the aim of determining that potassium in ionic form is completely detected by flame photometry, independent of the presence of other ions and polyphosphate ions in particular. Holleman here states that these polyphosphates are not only produced during ashing, but also during the physiological process (Kuhl, [?]). The two pairs of culture tubes differed only in the treatment of their ashes. One pair was hydrolysed in the same manner as experiment II with $HClO_4$, the other pair was hydrolysed by the preferred method with HCl. The first pair, treated as in experiment II, possessed a slight precipitate possibly of calcium phosphates. As required for flame photometry the solutions were filtered, thus removing any precipitate from the solutions to be analysed. The ash solutions were [unfortunately] never analysed.

Rather than complete his investigations to test for possible trivial reasons for the temporary loss of potassium from the experimental series of experiment II, Holleman set up experiment V, which was designed to be a full repeat of experiment II. All the improvements in apparatus and procedure that had been developed were incorporated (see section 8 for details). The procedure involved maximising the number of replicates and cycles given the constraint that there was room for only 12 tubes in the culture container. The control cultures were killed by a heating to approximately 100 degrees. The growth cultures were then placed alongside the controls in the culture container, both sets of tubes being treated equally. Due to constraints of space the experimental cultures and the controls with which they were paired [i.e. the experimental and control cultures which were to undergo the same number of experimental cycles] were not able to run at the same time together however. (See section 8.1.5 for details).

The observation of strong algal growth in a control tube (originally heated for 2 hours at 100 degrees) led to the immediate setting up of a small experiment. The idea was to test the effectiveness of heat killing the algal cultures. Four culture tubes (using the same stem culture as before) were heated; one for 15 minutes; one for 30 minutes; one tube for 60 minutes and the fourth tube was evaporated till dry then redissolved with distilled water. The first 3 tubes after 14 days showed a certain degree of development. A second experiment was also set up: Holleman had noticed from the literature (Kuhl [?], Walker [?]) that autoclaving, rather than the ultrafiltration used by Holleman, was normally used for the sterilisation of culture media. He considered whether the replacement of this method by ultrafiltration could be the reason for his variable results. Another 4 culture tubes were set up; one pair with autoclaved nutrient solution, the other was not [ultrafiltration was in fact not mentioned here, so I can only assume that it was]. After 4 days hardly any differences were to be observed between them.

Experiment 8611-1 was described as a preliminary experiment to a replication of experiment V. Two parallel experiments with 4 identical tubes in each. The 2 parallel experiments were inoculated each with a different stem culture. The optical density of the cultures was measured at regular intervals. Evaporation of the culture fluid was observed to be considerable. To compensate, a calibrated measuring stick was used to measure the volume of the individual tubes and thus the amount of water lost from each. Approximately 2% of the volume was lost per day. The possibility of the raising of the temperature of the wash flask by a few degrees to compensate for evaporative losses had been tried before and was not reliable. The only practical alternative considered was a return to the weighing, before and after each growth period, of each culture tube and its accompanying quartz glass capillary gas inlet tube.

Four of these tubes were ashed, in 2 stages, in preparation for the next experiment. The tubes were divided into pairs; one set received 0.5ml of 0.1N HNO_3 the other set twice as much (2.5x and 5x that used in experiment V, respectively). Both were neutralised using NaOH and topped up with water to 5ml. Their optical density over a period of 12 days was monitored. The first pair showed very good growth, from start to finish. The second pair showed extremely poor growth to begin with, but by the end of the growth period they were very close to having caught up.

Experiment 8712-1 involved the ashing in 2 stages of 5ml each of 5 different stem cultures, of different ages and storage histories, left over from previous cycles of experiment V. These ashes were apparently hydrolysed, as they were recorded amongst the flame photometry potassium and sodium analysis results of experiment V. Nutrient solution, made 3.5 years previously was also analysed and showed lower potassium values than might be expected. This nutrient solution was presumably that used in Experiment V. [Some or all?] of the tubes containing the ashed nutrient solution did however also contain the small rings of silicon rubber tubing used to hold the quartz capillary inlet tubes in place, which had presumably fallen in by accident. Thus the ash was contaminated by silicates. What effect they may have had, if any, was not given. The promised comment on these flame photometry results was never made.

The quartz test tubes showed signs of corrosion from the algal ashing. Also, with the leading of hot air through aqueous solutions which were at boiling point, corrosion was clearly to be seen. The suspension, which presumably consists of pure silicon dioxide, may possibly, by adsorption, have an affect on the chemical composition of the culture solution. The cause of this corrosion may well be (overheated?) steam. By heating too quickly, such steam could be produced by the ashing of the algal carbohydrates and also if such oxidation (burning) occurs before the tubes were completely dry. By means of a phased ashing process, involving the heating being conducted in stages, the water can escape before the burning of the carbon residues. An exploratory experiment was conducted with this in mind: Two quartz test tubes were used, one well used and partly corroded, the other pretty well transparent. Equal amounts of the same algal culture were added, then evaporated till dry (at ca.100 degrees) in the usual manner. On being placed in the oven, detailed observations were made on the ashing process as the temperature increased with time. As usual, the temperature was held for a little over an hour at 500 degrees. The next day again normal procedure was followed for the second ashing at 500 degrees. After half an hour the light colour of the carbon deposits were clearly reduced. After an Easter break [!] the ashing was repeated until all signs of carbon deposits had disappeared. The ash was redissolved and inoculated. *Chlorella* development was not particularly good. The cultures were then stored in the fridge and the experiment was not taken further. The observations made during the initial heating were potentially significant however. Most of the breakdown (charring) of the organic matter occurred during the heating from 200-300 degrees. Thus a first phase of ashing could take place at 250 degrees for about half an hour. The evaporation procedure was also considered.

A test of the vitality of two stem cultures, one 5 months and the other one year old gave the surprising result that whilst both were still active, the oldest was most so!

Holleman's last laboratory notebook ("*Chlorella* Research Oct. 1984 - [May 1987] Book V") ended with a consideration of further research involving synchronised *Chlorella* cultures, though he was somewhat sceptical of the literature on the subject. [The following section shows that this scepticism proved to be unfounded.]

6.3.3 Experiment VI (1987 - 1989)

[This last phase of Holleman's work was written on loose notes. My lack of a full understanding of many of the methods and procedures in this section was mostly due to an absence of any explanation by him of these proceedings. In fact, no significant results were recorded during this period which helps explain the enigmatic results of experiment II. Nevertheless, I do not wish to imply that Holleman's synchronisation experiments were not of importance. These last experiments break the mould of much of his previous work. I therefore feel able to take the potentially controversial step of interpreting, rather than directly reporting what I can of that which Holleman wished to be the starting point for further research.]

This section differs from all previous sections in as much as the emphasis is almost entirely on *Chlorella* itself. Because very little information was recorded on the procedures

used, let us start with the principles given in section 3, etc. which have been the basis for all his previous research. Here he states the importance of promoting the conditions necessary for the maximum unfolding of organic life, or development [See section 10.2.2 for a consideration of the complexities involved in such a simple statement]. This was facilitated by examining *Chlorella* samples from cultures under a microscope. Thus the development of the individual cells was able to be followed. Quantitative cell counts gave an exact measurement of cell density over time. This, coupled with qualitative observations, enabled the progress of external cell development and reproduction to be observed. As usual, the optical densities were also taken; a few times they were compared with total dry weight production. Carbohydrate and protein production were also considered for measurement.

The chemical processes were not entirely neglected; pH as well as nitrate, potassium and sodium were at various times on the programme for analysis.

The reason for Holleman embarking on a journey towards a deeper understanding of *Chlorella* was certainly his wish to find an explanation for the unexplained appearance and subsequent reappearance of potassium in experiment II. In a letter written in 1991 he stated that for a long time after the results of experiment II [and probably even more so after experiment IV failed to replicate them] he was frightened that he had made a terrible experimental error. It was only later that he developed the idea that a transmutation process may be part of a rhythmic, reversible process [see section 5 and also 11]. His earliest recorded ideas on this involved the processes of assimilation and dissimulation [the latter term is not a biological term; at first I considered it to refer the process of respiration which it may well for Holleman have actually become, however in the form in which it was first considered it almost certainly referred to the process of decomposition - see section 10.2.2 for further consideration]. The processes of assimilation and growth occur in the light; respiration and reproduction in the dark. It was the processes of growth and reproduction that Holleman was to focus on by the beginning of this section. The means to this end was the use of synchronous cultures. The method chosen to obtain a homogeneous, synchronised algal culture, with the phases of growth and reproduction in step was fortunately an extremely simple one. It merely involved the implementation of a fixed (regular), light/dark regime of, say, 10 hours light and 14 hours dark.

The literature states that parallel to the light/dark cycle the synchronous cultures should be maintained at a constant cell density by means of a dilution procedure that is in phase with the light/dark regime. This is normally done by counting the number algal cells per millilitre on a daily basis. From this count a dilution of the culture can be calculated to give a fixed cell density. The figure chosen from the literature by Holleman was 1.6×10^6 cells/ml. There is no evidence from his notes that such a regular dilution was ever made. Despite the cell count being made fairly regularly, the dilution itself was only recorded as being done at the start of every new culture. In experiment VI however, a dilution may have been conducted at the end of the first growth cycle, immediately before ashing. There is no evidence that these ashes were used though, either to start a new culture or that they were analysed.

Now it is that I wish to speculate on Holleman's intentions. From the guidelines in sec-

tion 3, and supported in practice as evidenced by his laboratory notebooks and elsewhere, it is clear that the cumulative method was at the centre of Holleman's transmutation research with *Chlorella*. Therefore it is probable that his intention was to combine the cumulative method with the production of synchronous cultures. A continuous dilution, in these circumstances, though desirable in maximising the growth of the algae, would involve the continual addition, to the original mineral content, of new nutrient solution; thus any accumulation of the results of a potential transmutation would also be diluted. In cycle one of experiment VI there would have had to have been a dilution of 300 times! (In a later experiment a cell multiplication of over 10,000 times was recorded!!) With the determination of the *Chlorella* concentration at the end of the first growth period, the required volume of algae required for the inoculation of the next culture cycle may be calculated, removed and temporarily stored. The remaining culture solution may be ashed and redissolved in the usual manner, before being re-inoculated by the *Chlorella* sample taken earlier. If the timing of the ashing always occurs at the same point in the light/dark regime, any transmutation that may occur during a particular physiological (growth/reproduction, assimilation/respiration) stage of the algae would be accumulated.

The Kalignost quantitative chemical test for the element potassium was re-examined in preparation for ash analyses, but was stopped, presumably because of the moving of his department and his subsequent loss of laboratory space.

7 Holleman's Experiments 1975 - 1982

7.1 Materials and Methods

7.1.1 Overview

The experimental set up is shown in figure 1. The culture methods used were those of standard laboratory practice. Thus bottled carbon dioxide gas was used to provide an air mixture containing 5% carbon dioxide. This was bubbled through a wash bottle that doubled up to grow the inoculation culture. This inoculation culture was used for the following cycle of the experiment. The gas was filtered by passing it through wads of cotton wool on both sides of the wash bottle and then split equally to pass over all six experimental cultures. The cultures were inoculated by adding 2ml of the previously grown inoculation culture to 50ml of a slightly modified standard chemical nutrient solution developed for the culture of the alga *Chlorella vulgaris*. The six experimental culture dishes were agitated and kept at a constant 27 degrees in a thermostatically controlled shaking water bath. The water bath contained twice distilled water and was provided with a loosely fitting lid, as were the culture dishes. Extra illumination was provided by a standard household 150W tungsten lamp suspended above the shaking bath.

The procedure is sketched out in figure 2 for the six experimental culture dishes. As stated above, all six experimental culture dishes began with 50ml of new nutrient solution and were inoculated with 2ml of a previously prepared culture solution. They were allowed to

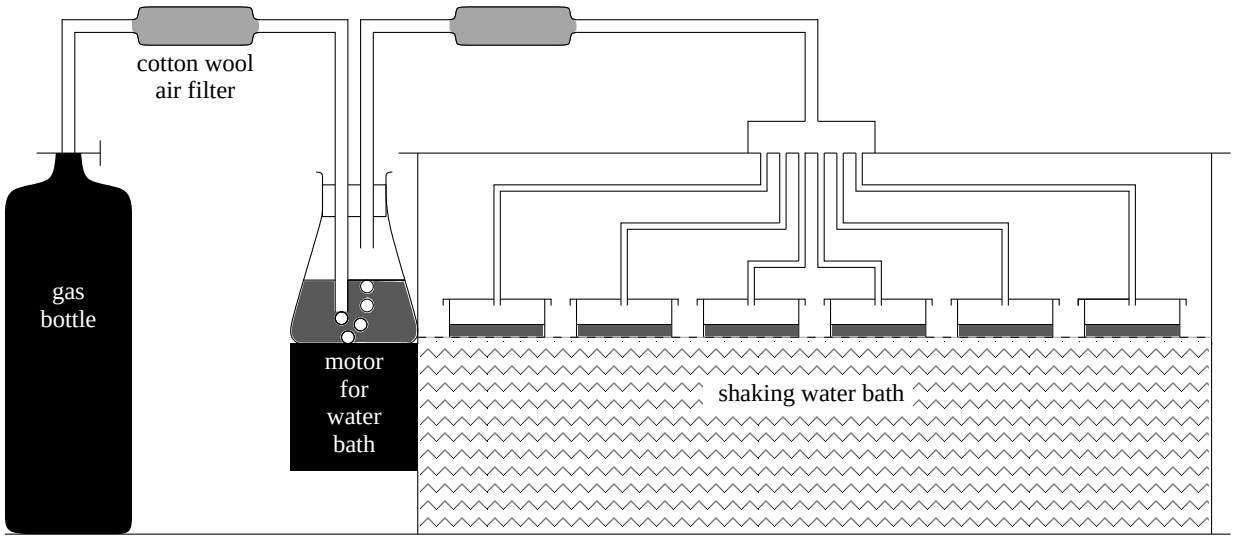

Figure 1: Apparatus for Experiment II

grow until the algae appeared to have visibly reached the full extent of their normal growth (or shortly after). This was about 12 days. The dishes, with their algae, were removed, evaporated and fully ashed in a furnace at 500 degrees. The ashes, still in their original dishes, were redissolved in distilled water to form the nutrient solution for the next cycle of the experiment. This was inoculated with 2ml of the culture grown in the gas wash bottle during the previous cycle.

The amounts of the chemical elements potassium and sodium were measured during the course of the experiment by the removal, at each cycle, of one of the experimental dishes after the ashing of its contents. Thus the first cycle started with six growth cultures, the second with five, through to the sixth and last cycle which consisted only of the one remaining dish.

The control cultures differed from the experimental ones in the first significant experiment of Holleman's (experiment II) on two points: 1. After adding the 2ml inoculation culture they were immediately ashed: 2. The dishes containing the ashes were kept in a desiccating chamber separate from the experimental cultures during the twelve days or so that the latter were growing. Only once the experimental dishes were themselves ashed were they and the controls that went with them kept together.

The controls of Holleman's third experiment (III) were modified to reduce the difference between the control and experimental dishes to just one point: the timing of an addition of 10ml of 0.01N nitric acid (HNO_3). Rather than adding the acid during the ashing process, it was added for the controls as a component of the nutrient solution with the intention of making the nutrient solution unsuitable for the growth of *Chlorella.* This proved, however, to be unsuccessful.

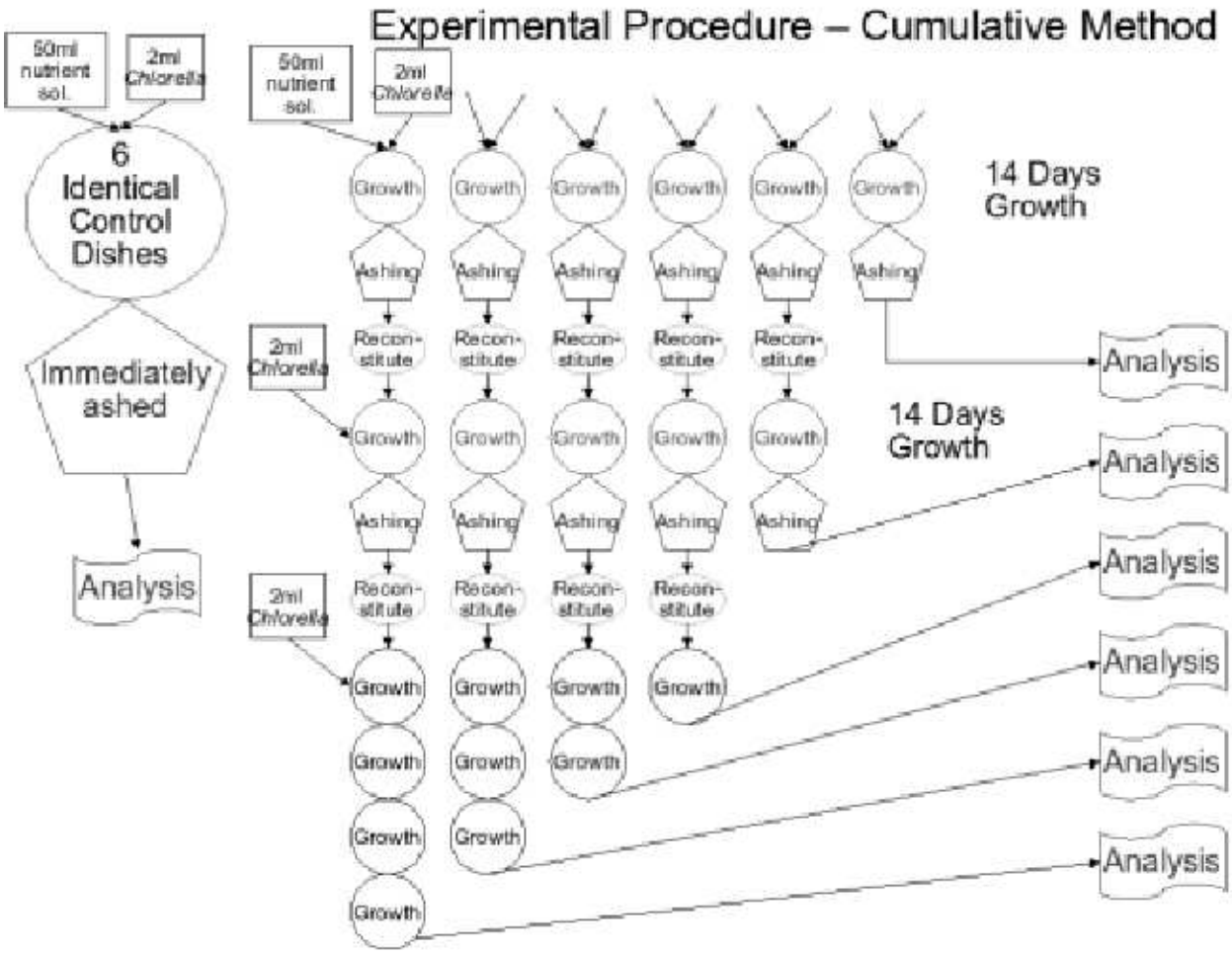

Figure 2: Experimental Proceedure for Experiment II

7.1.2 Apparatus and Materials

7.1.2.1 Shaking Bath

For the homogeneous development of the algae an agitation of the surrounding fluid is necessary. This prevents clumping and maintains a suspension of single cells, thus ensuring an even supply of nutrient solution to all cells. This was attained by the culture dishes being shaken. The apparatus used for this purpose (Dubnoff Incu-Shaker, Labline model 61) was thermostatically controlled and was held at 27 degrees throughout the growth stages of the experiments. It shook to and fro at a frequency of 1 beat per second and an amplitude of 3.5cm. The dishes stood on a perforated plate and were held in place by screws fixed (with difficulty [!]) into the plate. The bath was filled to the bottom of the dishes with distilled water. The whole machine was completely covered by a Perspex lid made with a hole for the bringing in of carbon dioxide rich air. Although during the experiment water condensed out onto the lid, it appeared that evaporation from the experimental dishes was mostly acceptable as their volume by the end of a growth period was practically unchanged.

7.1.2.2 Containment Materials

The algal suspension was contained in round quartz glass dishes with straight vertical sides (diameter 10cm, height 1.5cm, thickness 0.3cm). The material, Vitreosil, withstands treatment with acids and heating to 500 degrees extremely well. Before being used the dishes were thoroughly cleaned with an alkali scouring material (Vim) and rinsed for a prolonged period of time in deionised water, twice distilled.

7.1.2.3 Other Glassware

The provision of solutions and the culture of algae occurred in standard, cleaned laboratory glassware (Jena G... [?]).

The measuring glassware used (pipettes, measuring cylinders) were calibrated. Moreover, for each specific handling, one and the same item (pipette, measuring cylinder) was used with special consideration for the identical handling of proof and control.

The stock nutrient solution and the ash solutions that were obtained were stored in good closing polythene bottles.

7.1.2.4 Chemicals

With all work, Merk analytical chemicals were used. In particular, nitric acid, which was used in fairly large quantities in the ashing and reconstituting of the nutrient medium, was in this regard, critical. A proportion was isolated for direct use from a measured quantity (1 litre) of concentrated HNO_3 intended for this work.

7.1.2.5 Gas Supply

Algal growth was promoted by the provision of a carbon dioxide rich atmosphere. The CO_2 was lead from a gas cylinder, through a control valve and then passed through a wash flask (held at 27 degrees) which contained nutrient solution and which was inoculated with an algal suspension with the same content as the experimental cultures. This solution acted as inoculation material for the following growth period. The gas stream on either side of the wash flask was filtered by a wad of cotton wool.

The CO_2 enriched air passed on through a connecting tube of silicon rubber into a dividing head. This was situated in the Perspex lid of the shaking bath and served to distribute the gas stream equally, via 6 identical lengths of rubber tubing to each of the six culture dishes, exiting through their Perspex lids into the air space above the culture liquid. The underside of these lids was furnished with three ribs whereby a space of approximately 1mm above the walls of the dishes allowed the gas mixture to escape.

7.1.2.6 Lighting

The necessary provision of radiant energy [light!!] was met by use of a 150 Watt Philips lamp [almost certainly a household tungsten bulb], 50cm above the experimental set up. A rough estimate of the light intensity was 1000-1500 Lux.

Lighting, gas flow and shaking bath were generally only switched on during working hours [10am-5pm Monday to Friday]. On only a few occasions did this handling continue through the night.

7.1.2.7 Sterilisation

The growth of the algae progressed under sterile conditions. The glass work used was sterilised by heating at 120 degrees for 1 hour; the polythene bottles and the quartz dishes by UV irradiation. The nutrient solution was filtered through a ceramic candle, which was designed as a bacterial filter. All open ends of containers, etc., were either covered or suitably sealed, even when not in use.

7.1.3 Nutrient Solution

The composition given by Kuhl (1962a) was followed with one difference. The *magnesium* content was reduced to 1/10 of the original stated amount. The missing Mg was replaced by an equimolar solution of Na_2SO_4. The reason for this reduction was the hypothesis that should a transmutation occur, it would intervene in the first place in the assimilation process, with an increase in the amount of magnesium that is so essential for the production and functioning of chlorophyll. A small increase [supposed Holleman] in the magnesium content could only be determined when the initially given magnesium was as small as possible [see section 10.1.3 for further details].

Otherwise it was demonstrated that the nutrient solution composition made excellent algal growth possible.

The solution was prepared from a few stem solutions (A1-A4, B and C). This was presumably to prevent chemical reactions resulting in the formation of insoluble precipitates. The composition of the stem solutions is given in the table below:

Kuhl solution [as modified by Holleman]		
A1	KNO_3	$5.5\text{x}10^{-3}$
	KH_2PO_4	$4.5\text{x}10^{-3}$
	$NaNO_3$	$4.5\text{x}10^{-3}$
	$Na_2HPO_4.2H_2O$	$0.5\text{x}10^{-3}$
A2	$MgSO_4.4H_2O$	$1.0\text{x}10^{-4}$
A3	Na_2SO_4	$0.9\text{x}10^{-3}$
A4	$CaSO_4.2H_2O$	$1.0\text{x}10^{-4}$
B	$FeSO_4/EDTA\ complex$	$2.5\text{x}10^{-5}$
C	H_3BO_3	$1.0\text{x}10^{-6}$
	$MnSO_4.4H_2O$	$1.0\text{x}10^{-6}$
	$ZnSO_4.7H_2O$	$1.0\text{x}10^{-6}$
	$CuSO_4.5H_2O$	$1.0\text{x}10^{-8}$
	$(NH_4)6Mo_7O_{24}.4H_2O$	$1.0\text{x}10^{-8}$

7.1.4 Algal Cultures

7.1.4.1 Production of Cultures

Cultures of *Chlorella vulgaris*, originating from pure stock cultures from the Culture Centre of Algae and Protozoa, Cambridge, UK, were kindly made available by the Experimental Physics Laboratory, Section for Spectroscopic Biology, of the Utrecht University.

With the aim of keeping the stock cultures alive for the duration of the whole experiment, they were kept in a dimly lit environment at about 17 degrees. Fresh algal cultures were needed later to prepare each of the six growth periods of the experiment. This was attained, in advance, by means of the gas wash flask, which contained 50ml of nutrient solution inoculated by 2ml of a previously grown inoculation culture, being bubbled through with CO_2 rich air and kept at a temperature of approximately 27 degrees. In the inevitable periods of time between the end of growth and its usage for the following cycle, the suspension was stored as stated earlier in this section.

7.1.4.2 Meassurement of Cell Number

The number of cells per cm^3 in the algal suspension was determined according to the methods of Boney (1974) and Hille (1938). Two millilitres of the algal suspension was centrifuged for 5 minutes at 4000 rpm. The residue was heated with 3-4ml of methanol in a water bath for 5 minutes. After cooling it was centrifuged again and decanted into a 10ml measuring cylinder. The extraction was repeated and decanted after centrifuging into the same measuring cylinder, which was filled at room temperature with methanol to 10ml. The optical density

was measured in a spectrophotometer at a wavelength of 660 microns and compared with pure methanol. The reference solution for all following measurements was a chlorophyll extract of the original *Chlorella* culture obtained following the above prescribed methods. The linear relationship between optical density and concentration of the extract was checked by measuring a number of dilutions of this extract. [This method in fact gives a determination of chlorophyll content rather than relative cell density per se.]

7.1.5 Procedure

7.1.5.1 Growth of Cultures

Fifty millilitres of the sterile nutrient solution was pipetted into each dish, as was 2ml of algal suspension (grown in the same nutrient medium). The suspension was brought over with a 'Stas pipette' or so called 'Swift pipette' to overcome sedimentation during pipetting. The dishes were covered with sterile plastic lids and placed in the shaking bath. To the gas wash flask a culture of the same composition was added. After this was completed, the lids were replaced by the previously described Perspex covers (see 7.1.2) which were connected to the gas supply apparatus. The shaking bath was covered and the gas valve opened. The speed of the gas stream (air with 5% CO_2) was roughly read from the rate of the bubbles through the gas wash flask.

At the end of a growth period the mineralization of the cultures and control dishes followed.

7.1.5.2 Shaking Bath

The first step was to adjust the acidity (pH) of the culture solution back to its original value using 10ml of 0.01N nitric acid. This quantity was determined from earlier experiments. The dishes were then placed under an infra red lamp for 5 minutes. During this process the dry remnant was already carbonized and partly ashed. A short heating for 10 minutes in a furnace at 500-520 degrees was necessary for complete ashing. Because of a chemical reaction between the acid phosphates and the nitrates a second quantity (10ml, 0.01N) of nitric acid was added to the dishes, after ashing, at a moderate temperature to neutralise this reaction. Finally the ash was dissolved in twice distilled water to produce 50ml of a new more or less neutral feeding solution. Again, the acidity was checked and determined by means of earlier parallel experiments.

7.1.5.3 Controls

As previously mentioned in 7, the controls of Holleman's first significant experiment (II) differed from the experimental cultures only in so far as that they were acid treated, evaporated and ashed (7.1.5) immediately after the inoculation of the nutrient solution. They were then stored in their original dishes in a desiccating chamber, for the duration of the growth of the experimental cultures, in order to prevent water vapour from affecting the dry ashes. Once the ashing of the experimental cultures was complete, the control dishes were

able to be brought out and placed alongside their equivalent experimental dishes for the rest of their parallel treatment and handling.

The treatment of the controls of Holleman's third experiment was a potential improvement on his earlier experiments. The inoculated nutrient solution was treated immediately with the 10ml of 0.01N nitric acid, which for the experimental cultures was added at the end of the growth period prior to evaporation. The idea was that this premature acidification would prevent algal growth. The control dishes were able to remain alongside their experimental counterparts at all times. [Unfortunately the acid proved not to be strong enough to prevent algal growth].

7.1.5.4 Chemical Analysis

The ashes of one each of the experimental and control dishes were removed in preparation for chemical analysis at the end of each growth cycle. The neutralised ash was dissolved in 5ml 0.01N perchloric acid and briefly (10 minutes) heated over a steam bath. After cooling it was filtered through an ash free filter and/or decanted into a measuring cylinder where it was topped up with twice distilled water to 50ml. The solutions were kept in well closed polythene bottles in the fridge.

Potassium was the first element to be determined. This was done according to a method by Flaschka (1953) by titrimetric measurement of the insoluble potassium salt of tetraphenylborate (Kalignost). The determination was conducted using 5 separate samples taken from each of the 6 experimental and the 6 control ash solutions. Each parallel experimental and control solution was analysed during the same work period. In most cases it was also conducted at the same time as an analysis of the original feeding solution and a calibration solution of an exactly known concentration of 0.01N KCl. (Measured value averaged $1.000x10^{-2}M$, n=16, $s = 0.018x10^{-2}$).

Potassium was further determined by flame photometry, as was the other element measured, sodium. The ash solutions were needed to be diluted by 1/100 before analysis.

The fact that sodium was included in the analysis was due to the expectation that it is an unimportant element in the [algal] organic process. It would therefore be a purely neutral part of the feeding solution and should thus strictly follow the conservation law. Assuming this to be true, it was used as an internal standard. Any change in the volume of the culture liquid as a whole, be it through evaporation or pipetting errors, would have no influence on the ratio of the concentration of any element relative to the sodium. Changes in these concentration ratios are therefore a more trustworthy sign of the growth or diminution of an element than would be the concentration itself. [The determinations of sulphate, phosphate, calcium and magnesium proved unsuccessful, the latter pair despite extensive experiments. See section 7.2.3 for details].

7.2 Results

The results for the first significant experiment (II) are given in figure 3, figure 4, figure 5 and figure 6.

7.2.1 Analytical Results

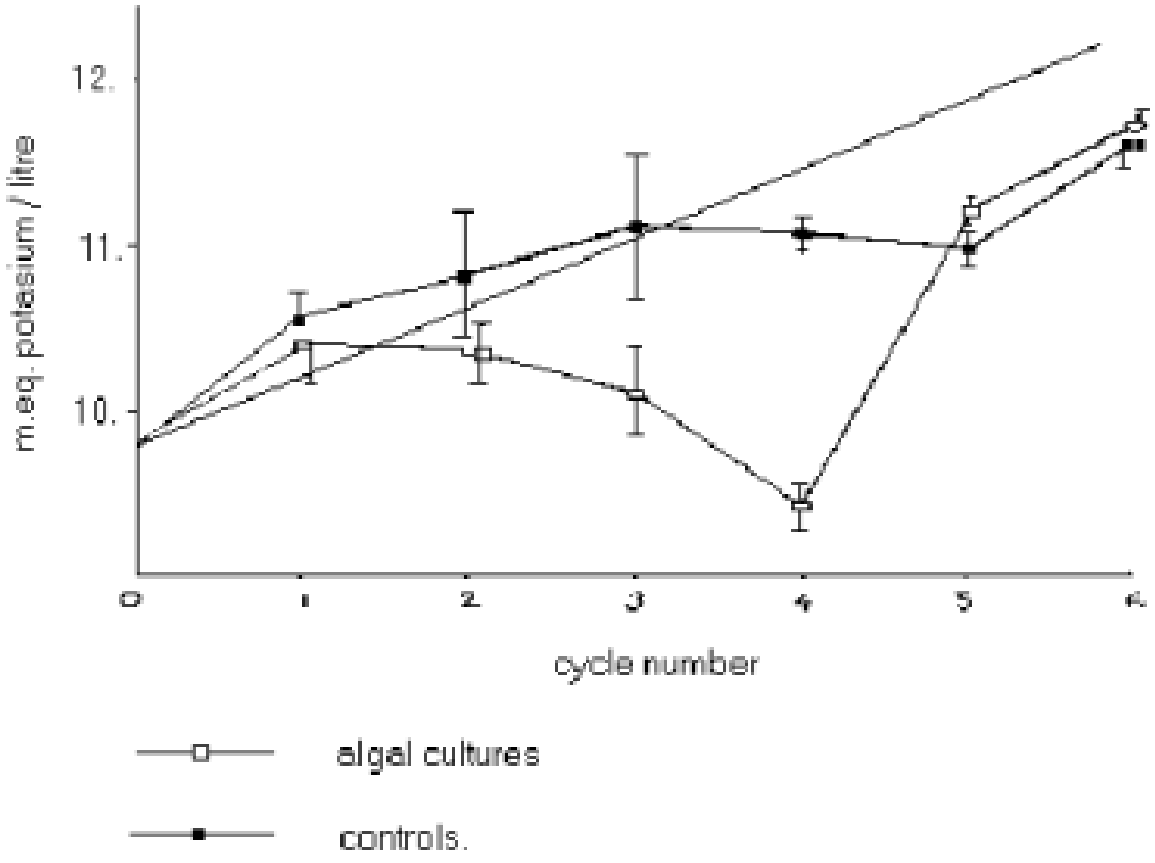

Figure 3: Potasium Content of Ash Solutions by Kalignost Method

Figure 3 shows the potassium content of each of the 6 control (solid circles) and 6 experimental (open circles) dishes as measured by the Kalignost method at the end of each of the 6 growth cycles. The error bars associated with each of these values records the range of results obtained from the 5 separate samples taken from the analysed dishes. The dashed line shows the theoretically expected results. The 4% slope of this line is due to the addition, at the beginning of each growth cycle, of an extra 2ml of chemically identical inoculation culture to each 50ml of nutrient solution. The starting point of this line used the experimentally measured potassium content of the pure nutrient solution (average = $0.985\text{x}10^{-2}M$, n=15, s=$0.018\text{x}10^{-2}$).

Figure 4 and figure 5 show the results of the potassium and sodium analyses as determined using flame photometry. There are no error bars here as only one sample was taken from each dish for the potassium and sodium analyses.

Figure 6 shows the ratio of potassium to sodium as measured by flame photometry for

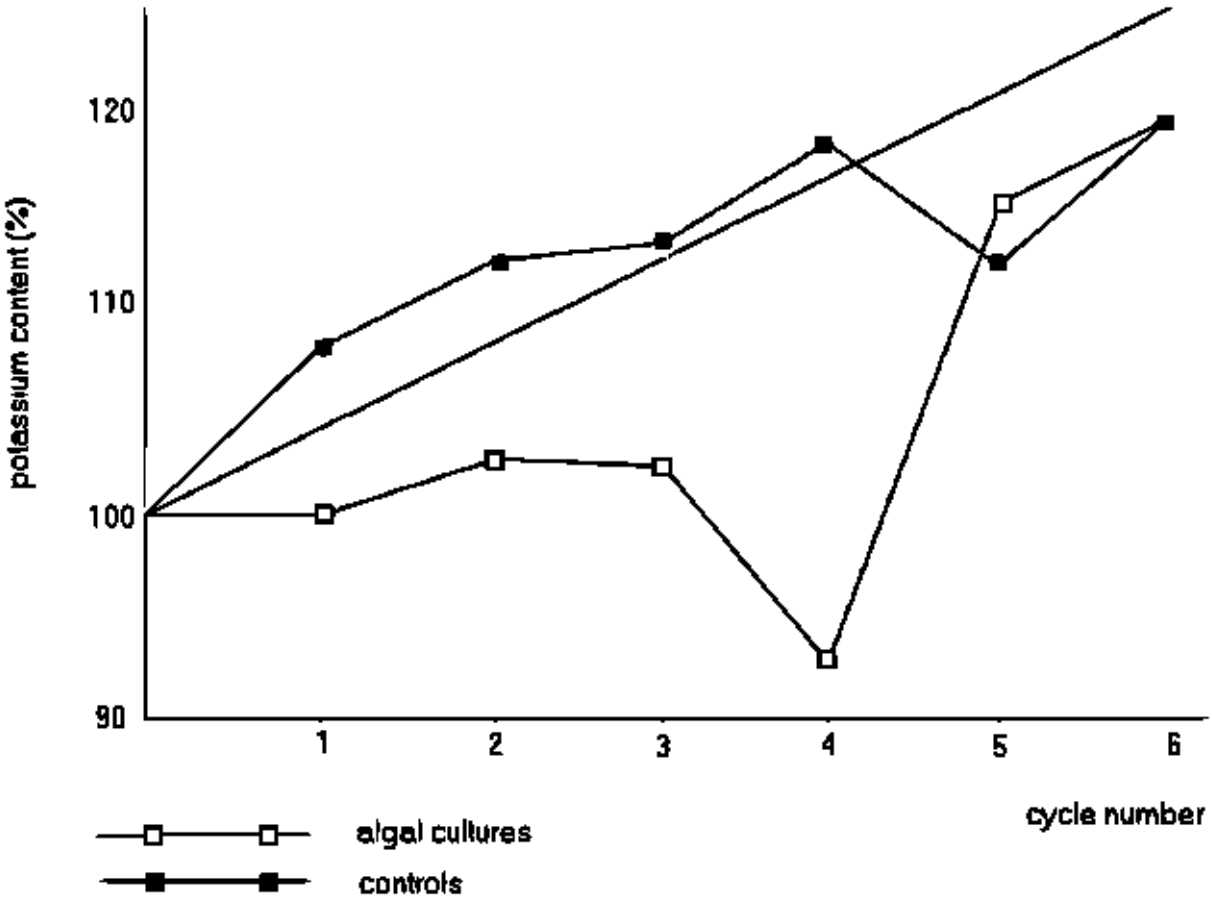

Figure 4: Potassium Content of Ash Solutions by Flame Photometry

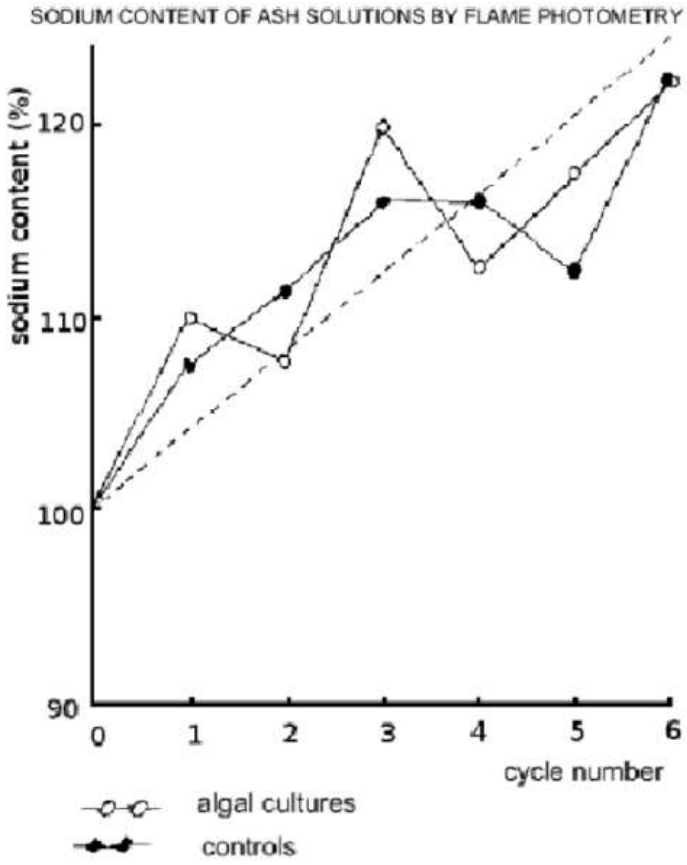

Figure 5: Sodium Content of Ash Solutions by Flame Photometry

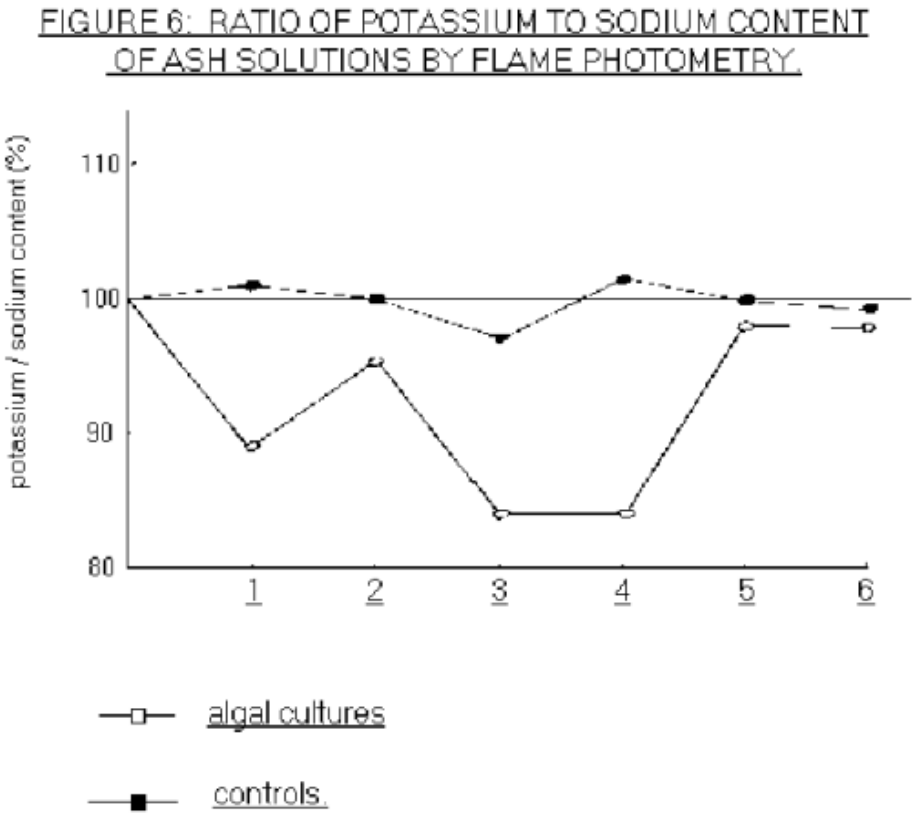

Figure 6: Ratio of Potassium to Sodium Content of Ash Solutions by Flame Photometry

each of the control and experimental dishes analysed at the end of each of the 6 growth periods.

The first thing to note is that in comparing figure 3 and figure 4, there is relatively little variation between the two totally independent methods of potassium analysis. Thus any differences observed between controls and experiment must be attributed to factors independent of the final analysis method itself.

A second noteworthy feature is the similarity observed between controls and experiment, as predicted, in the sodium contents, as shown in figure 5.

This contrasts with the relatively large reduction in the potassium content in the experimental cultures, especially for the 4th growth period. This is clearly demonstrated with the potassium/sodium ratios as shown in figure 6. The control results have a range of about 4%; the experimental results drop down to a statistically significant 17%.

The return of the potassium content back to expected values for the 5th and 6th growth periods of the experimental dishes remains equally intriguing.

7.2.2 Health of the Cultures

The growth of the algae could be visibly followed in the part experiments by comparing them with the original culture. In the first 3 growth periods it was completely satisfactory, in the last 3 it became less so. Apart from this the appearance of the suspension also changed. Lumps of cells were found and the colour became darker. The same was observed with the inoculation cultures that went with them. The relative quantity [and/or health] of the algae in the inoculation cultures was determined by means of the measurement of their chlorophyll content in comparison with the original culture. It varied between 106-120% in the 6 successive growth periods of 10-14 days.

7.2.3 Sources of Error Identified by Holleman

Changes in the volume of the culture solution during the growth phase of the experiment resulting in later analytical errors would include pipetting errors. Of note here is that during the 5th growth phase a reduction in the shaking bath's oscillating frequency resulted in an unexpectedly strong wave in the relevant dishes. Such a loss, Holleman believed, could account, at least in part, for the low analytical values recorded for the part experiments 5 and 6 (figure 3, figure 4 and figure 5). A third source of error was the evaporation which occurred with the ashed solutions being stored awaiting analysis. This was despite their being stored in a fridge in good closing polythene bottles. A small evaporation could not be avoided due to the long storage times determined by circumstance. This could account for the generally slightly high values recorded for the earlier part experiments. [I feel it important to point out that an evaporative loss at any other stage of the experimental procedure other than during ash solution storage would not be detected in the analytical results].

Whilst making the initial nutrient solution a small precipitate was found to occur (despite precautions) which may account for the slightly reduced starting point for the potassium content given in figure 3.

The original plan to determine the mineral content for all cations (see section 6.2) of the nutrient solution could not be realised. The analysis of the next two most important elements, calcium and magnesium failed because of the formation of a precipitate in the ash solutions that did not, or only very slowly, dissolve in mineral acids. It occurred mostly in the experiments in which algae had grown more than three times in one and the same quartz dish. Despite the absence of a definitive identification, on the basis of an article by Hattingberg, et al (1966) it was suspected to be a calcium and/or magnesium polyphosphate salt that accumulated due to the cumulative working method. Possible further references quoted in Holleman's laboratory notebooks include Pascal (?); Karbe and Jander (1942); Ullman (?); v. Wazner (1950, '52, '53, '56); Griffith (1956).

8 Experiment V

[This section covers the 5th experiment in Holleman's numbered series. The sources available for this section were the laboratory notebooks and various loose notes. Where relevant, details are given here of some of the other experiments and tests leading up to this experiment. An overview of them may be found in section 6.3.2].

8.1 Materials and Methods

8.1.1 Overview

The experimental set up is shown in figure 7. The gas supply was essentially the same as experiment II, but with a few minor improvements. A reduction valve and a rotameter provided extra control of the overall gas pressure. The filtered air passed into a gas wash bottle which was no longer used to grow an inoculation culture. The gas wash bottle was warmed in a thermostatically controlled water bath. This warming increased the water content of the gas which helped reduce evaporative losses in the culture tubes.

A three way tap enabled the gas system to be tested independently of the algal cultures. A second three way tap enabled a filtered air stream, supplied by an aquarium pump, to be switched into the system. This air stream was used, when needed, to aid the evaporation of the culture tube contents after growth. The inlet tubes, normally positioned near the bottom of the cultures, were raised above the liquid surface for this purpose.

After this second three way tap the gas stream was split 12 ways by a distributor head. Each of the silicon rubber tubes exiting the distributor head supplied the carbon dioxide enriched air to a rack of up to twelve culture tubes. Each of these rubber supply tubes possessed a "pinch tap" which gave individual pressure control to each test tube. The gas inlet tubes themselves were quartz glass capillary tubes which loosely fitted through a hole in the plastic test tube lid. The inlet tubes were held in place either side of the lid by small, closely fitting rings of silicon rubber tubing. The gas, which bubbled through the culture fluid, not only supplied carbon dioxide gas, it also supplied the necessary agitation

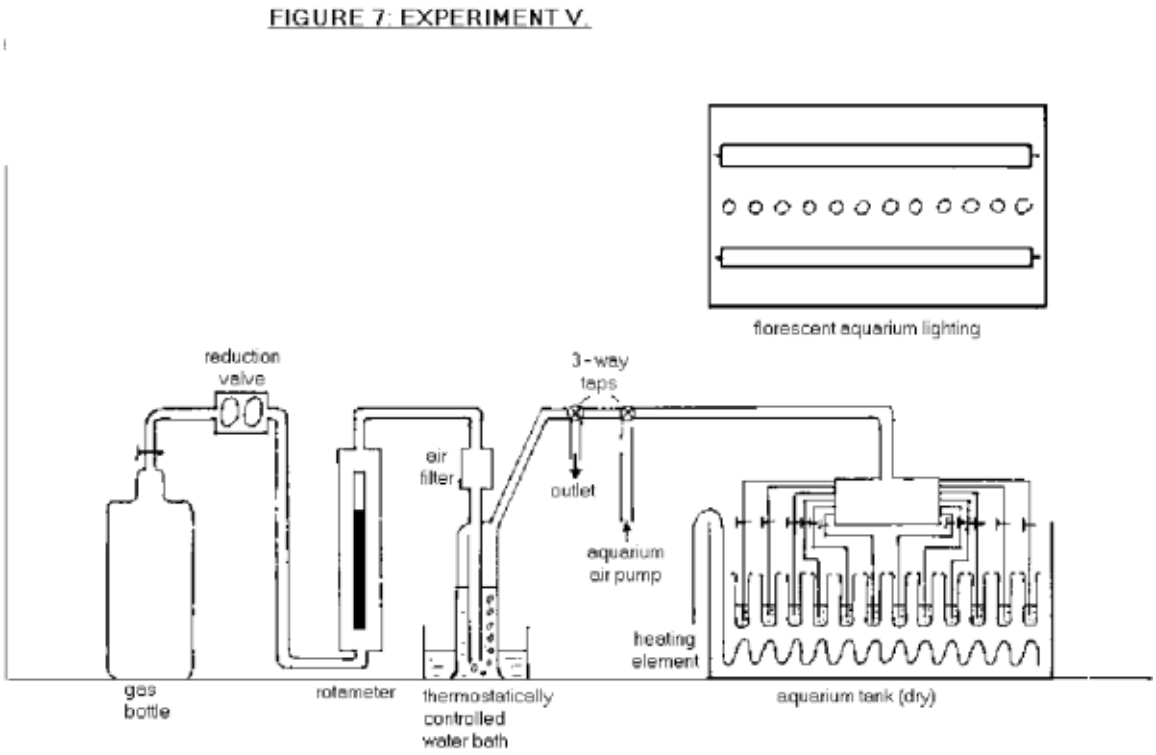

Figure 7: Experimental Set Up for Experiment V

to reduce the sinking and clumping of the algal cells, and thus promoted homogeneous growth throughout the culture.

The experimental procedure was essentially the same as that outlined in 7, etc.

The control cultures were different from experiment II. The inoculation procedure was identical to that of the experimental tubes. The controls were then heated for approximately 1.5 hours at 100 degrees to kill the algae. They were then (after cooling) replaced alongside the experimental tubes and were treated (including gas supply) identically to all other tubes, also including any additions of nitric acid.

8.1.2 Apparatus and Materials

8.1.2.1 Environmental Control

Because the previous shaking bath became unusable, an alternative method of maintaining the external environmental conditions conducive to maximal algal growth became necessary. A dry aquarium tank was chosen as the culture container. A thermostatically controlled heating element enabled the required temperature of 27 degrees inside the culture tubes to be maintained. The tubes themselves were held in a test tube rack which held 12 tubes in 2 rows of 6. Presumably the tank had a loosely fitting lid, though this was never recorded.

8.1.2.2 Containment Materials

The algal suspension was contained in quartz glass test tubes. Unfortunately the only further details given was that they were used to work with 5ml of liquid, and that there was 2 or more centimetres of space above that. The properties of these quartz tubes were presumably identical to those of the quartz Vitreosil dishes described in section 7.1.2

8.1.2.3 Other Glassware

The only recorded changes in glassware since those described in section 7.1.2 were the quartz glass capillary tubes used as gas inlet tubes, to bubble air through the culture solutions.

8.1.2.4 Chemicals

Presumably the same chemicals were used as described in section 7.1.2. It is not clear whether the "Suprapure" nitric acid recorded in this section was the same though. An examination of the published trace element impurities apparently showed copper levels of $0.2\text{x}10^{7}$M/l. In the nutrient solution it was at a level of $1\text{x}10^{7}$M/l. The toxicity of the copper was given by Holleman to be at a level of 1.5x107M/l and thus could easily be attained during normal experimental procedure. There was no evidence that Holleman took any measures to further assess the situation, or to take precautionary action.

8.1.2.5 Gas Supply

The essential details are given in section 8.1.1. The gas wash flask in this experiment again played a double role. This time, apart from helping to clean the gas, the water vapour picked up helped to compensate for evaporation in the culture tubes. The temperature of the thermostatically controlled water bath in which the flask sat was unfortunately only mentioned twice: once at 27 degrees when it was used for an earlier algal culture experiment; and once at 55 degrees when it was unsuccessfully used in a test to control the aforementioned evaporation problem of the cultures. At this latter temperature too much water vapour was carried over by the gas, leading to an increase in the volume of the cultures! I can only surmise that a temperature of 27 degrees was used, the same as that of the cultures. To reduce evaporation in the water bath itself, Holleman proposed using floating objects such as ping-pong balls.

Any gas leaks from poorly fitting connections were sealed using "Blue Tack". The foaming which occurred in the tubes was due to the bubbling of the gas through the cultures. It was successfully prevented by smearing a very fine layer of "Vaseline" over the outsides of the capillary inlet tubes.

8.1.2.6 Lighting

The lighting used here was supplied by 2[?] fluorescent lights (PL9, 9W-600lumen, Pope) [aquarium lights?], one on each side of the test tube rack and at a distance from the tubes of 8-9cm. They were, however, acknowledged to be insufficient in intensity for maximum algal growth.

Whilst not explicitly stated, one may assume that the gas supply and lighting were left continuously on for the duration of an experiment (except when interrupted by a holiday or period in hospital).

8.1.2.7 Sterilisation

The measures taken were essentially the same as in section 7.1.2. The following steps were introduced for experiment IV and presumably (where relevant) hold true for Experiment V:

1. Glass beakers sterile (inside).
2. Labline shaking bath cleaned out with 70-alcohol.
3. Ditto for Perspex lid.
4. Cotton wool filters autoclave.
5. Twice distilled water filtered by Millipore filter.
6. Ditto nutrient solution.
7. In Laminar Flow Cabinet:

(a) pipetting with sterile pipettes of sterile nutrient solution and sterile stem culture from Biophysics.

(b) -Tray of culture beakers brought over to shaking bath suitably covered.

8.1.3 Nutrient Solution

This remained the same as that described in section 7.1.3. The quantities of some of the individual elements present are given below:

	M/l
K	$10x10^{-3}$
Na	$7.3x10^{-3}$
Ca	$0.1x10^{-3}$
Mg	$0.1x10^{-3}$
Phosphate	$5.0x10^{-3}$
Sulphate	$0.9x10^{-3}$
Chloride	$0.1x10^{-3}$
Nitrate	$10x10^{-3}$

8.1.4 Algal Cultures

8.1.4.1 Production of Cultures

This was essentially the same as in section 7.1.4, except that individual inoculation cultures were no longer grown by Holleman himself in the wash flask, but were obtained directly from Biophysics. They were stored in tap water cooled flasks at about 17 degrees in dim daylight until needed.

8.1.4.2 Meassurement of Cell Number

For experiment V the Walker method of chlorophyll measurement was followed. It entailed the simple, direct measurement of the optical density of the (homogeneous - shaken -) culture at a wavelength of 600nm. Though normally undiluted, the recommended dilution of 1:26 was used for other tests and experiments when convenient.

For more accurate chlorophyll determination the van Hille method, described in section 7.1.4, was found to be preferable.

8.1.5 Procedure

8.1.5.1 Growth of Cultures

The procedure was basically the same as that described in section 7.1.5. The only difference was the volumes used: 5ml Kuhl (1/10Mg) inoculated by 0.2ml *Chlorella*. A fresh culture from Biophysics was used each time. All the tubes involved in the growth cycles were set up and subsequently ashed in pairs.

8.1.5.2 Ashing and Provision of Nutrient Solutions

Some changes were made since experiment II section (7.1.5). Firstly, after growth was completed (14 days) the gas supply was stopped and the supply tubes disconnected. The tap for the aquarium air pump was connected and the tubes transferred to the Aluminium Heating Block which was at approximately 100 degrees. The air was pumped over the surface of the culture medium (distance about 2cm) in the tubes. This enabled evaporation to take ca. 1.5 hours. The tubes were then placed in the furnace for 1 hour and heated to 500 degrees: The time spent at 500 degrees (or slightly above) was 10 minutes. After cooling each tube received 2ml twice distilled water and the drying and ashing procedure was repeated such that all carbon particles were fully burnt and driven off (this process was repeated a third time if necessary). The tubes then received 3ml of water and 2ml of 0.01N nitric acid to neutralise the newly made up nutrient solutions ready for the next cycle.

8.1.5.3 Controls

The first control pair of tubes for experiment V were identical to the growth cultures except that they developed (as near as possible) in total darkness. This was for the first cycle of the 6 cycles through which it passed. All other subsequent control tube pairs, as well as the first control pair (in all other subsequent cycles), were heated in the aluminium block heater at 100 degrees for 1.5 hours to kill the algae present (it was not stated to what extent evaporation had taken place during this heating). The experimental growth cycle was only started once the controls were placed alongside the experimental growth pairs. The timing of when the controls and their complimentary experimental pairs were "grown" with regards to the cycle number are easiest given in the form of a table:

\cycles number\	1	2	3	4	5	6	7
1	B+N	B+N	B+N	B+N	B+N	B+N*/	Cc+Rc*
2	C+R	C+R	C+R	C+R	C+R/	Dc+Qc	Dc+Qc*
3	D+Q	D+Q	D+Q	D+Q/	Ec+Tc	Ec+Tc	Ec+Tc*
4	E+T	E+T	E+T/	Fc+Sc	Fc+Sc	Fc+Sc	Fc+Sc*
5	F+S	F+S/	Vc+Wc	Vc+Wc	Vc+Wc	Vc+Wc	Vc+Wc*
6	V+W*/	Ac+Mc	Ac+Mc	Ac+Mc	Ac+Mc	Ac+Mc*	[Ac+Mc*]
	[Ac+Mc]						
N - growth tube; Nc - control; Nc* - control to be ashed.							

8.1.5.4 Chemical Analysis

This differed from that described in section 7.1.5 on a number of different points. The ashes for analysis were dissolved up in 4ml of distilled water to which 1ml 1.000N HCl was added. This was warmed for 1.5 hours in the aluminium block at ca.100 degrees. The Kalignost

method for potassium analysis was not used. Potassium and sodium, analysed by flame photometry, were the only two elements examined in experiment V.

8.2 Results

8.2.1 Analytical Results

Number of cycles	Potassium					Sodium				
	Calibration solution	Experiment		Control		Calibration solution	Experiment		Control	
		Tube	Result	Tube	Result		Tube	Result	Tube	Result
1	50	V	80	Cc	72.5	20	V	33.5	Cc	< 31
		W	79.5	Rc	79		W	34	Rc	34
2	50	F	82	Dc	74	20	F	35	Dc	31.5
		S	83	Qc	82		S	35	Qc	35
3	50	E	83	Ec	81.5	20	E	35.5	Ec	35
		T	84	Tc	82		T	36	Tc	35
4	50	D	86	Fc	83	20	D	37	Fc	35
		Q	84	Sc	88.5		Q	36.5	Sc	36.5
5	50	C	91	Vc	90	20	C	37	Vc	37
		R	87	Wc	87		R	36.5	Wc	36.5
6	40	B	94.5	Ac	93.5	40	B	51	Ac	48
		N	98	Mc	74.5		N	49	Mc	45.5

As before (see section 7.2.1), the ratio of Potassium:Sodium was calculated to help compensate for evaporative and other losses:

Cycle number	Growth			Control		
	Tube	Result	Average	Tube	Result	Average
1	V	2.39	2.37	Cc	> 2.34	2.33
	W	2.34		Rc	2.32	
2	F	2.34	2.36	Dc	2.26	2.30
	S	2.37		Qc	2.34	
3	E	2.34	2.34	Ec	2.33	2.34
	T	2.35		Tc	2.34	
4	D	2.32	2.31	Fc	2.37	2.40
	Q	2.30		Sc	2.42	
5	C	2.46	2.42	Vc	2.43	2.40
	R	2.38		Wc	2.38	
6		1.85	1.92	Ac	1.95	1.80
	N	2.00		Mc	1.64	
Experimental cycles 1 - 5:				Control average		2.354
				Growth average:		2.354

No significant difference in the potassium content was found between experimental and control cultures. This was a very different result to that of experiment II.

The potassium content of 5 of the inoculation solutions, that were at that time still available (it was 14 months since the first culture was obtained), were measured and all were in approximate in agreement. The potassium contents of four Kuhl solutions were measured; two were of 1/10Mg; the other two 1/1Mg; one of each were hydrolysed as normal, the other two were not hydrolysed [this treatment is not clear; I assume that this latter pair were neither dried nor ashed, i.e. that they were used without any treatment whatsoever]. The potassium concentrations of the 4 Kuhl solutions were in good agreement, but possessed significantly lower values than those of the cultures. All five algal cultures had been stored for 4-14 months in a variety of polythene and glass containers at a number of different temperatures and lighting levels. The results are given below:

Sample	Result (M/l)
Bf3	87.5
Bf4	91
Bf7(K)	80
Bf6	91
Bf7	84
1/10 with	75
1/1 with	72
1/10 without	74
1/1 without	71

See section 10.2.1 for a discussion of these results.

8.2.2 Health of the Cultures

Varying degrees of clumping and sinking occurred in all tubes. Development in general was found to be variable.

9 Holleman's Experiments, 1987 - 1989. (Experiment VI)

9.1 Materials and Methods

As stated in section 6.3.3 this final phase of Holleman's research led him to examine his algae in greater depth. Nevertheless developments were made in other areas as well.

9.1.1 Overview

Experiment VI, which was the only attempt at a replication of experiment II during this period, was incomplete and very few details were recorded. See section 6.3.3 for a somewhat speculative consideration of this experiment.

9.1.2 Apparatus and Materials

9.1.2.1 Lighting

The first lights used were fluorescent "double lamps"[?] of 9W and gave out 600 lumen, situated 7.5cm from the rack of culture tubes. A second lamp illuminated the other side of the rack.

On the basis of measurements by Sorokin and Myers (1950) the best lighting intensity for *Chlorella vulgaris* at 25 degrees was given as 2700lux (250f.c.). Accounting for losses due to the passage of light through the quartz wall, etc., the test tube rack was now 20[cm? from the lights??] so that the lighting was 2800Lux. No comparative lighting experiments were recorded.

9.1.3 Measurement of Cell Numbers

Cell numbers were counted directly under the microscope with the use of a Neubauer counting chamber [presumably such as is used in counting blood cells]. A sample of 0.1ml of the homogeneous culture was taken for this purpose. If necessary the culture was mixed using a rotor mixer. The counting chamber was delineated into a grid of squares. The number of cells present in each of sixteen of the grid squares was counted.

9.1.4 Procedure

As stated earlier, the information on experiment VI and other tests and experiments of this time were incomplete and details were limited or absent. See section 6.3.3 for details.

Any samples taken from cultures for chlorophyll measurement, etc. during growth were of 0.1ml in volume and were replaced by an equal amount of nutrient solution.

9.1.4.1 Light / Dark Regime

The regular implementation of a particular light/dark rhythm was proved able to induce a high degree of synchronicity of cell division. A number of light/dark rhythms were used, e.g. 8:12 hours light:dark (Ruppel 1962); 14:10 (Lorentzen 1963, Lorentzen and Schleif 1966); 9:15 (Sorokin [?]); 15:9 (Sorokin [?]); and 10:14 hours light:dark.

9.1.4.2 Ashing and Reconstitution of Nutrient Solution

The ashing procedure described in section 8.1.4 was further simplified. This prevented the potentially corrosive production of hot steam (6.3.2) and reduced the need for a second ashing. The culture was evaporated as normal in the aluminium block with filtered air blown over. It was then further dried in the oven at 120 degrees for one hour followed by one hour at 250-300 degrees and one hour at 480 degrees centigrade. After cooling the ashes were dissolved in distilled water and the above procedure repeated if necessary. Nitrate ions

were added in the form of nitric acid. The possibility of adding Na_2HPO_4 [instead/as well?] was also considered (see section 10.1.5 for a further consideration).

9.1.4.3 Chemical Analysis

Holleman stated his intention to measure pH, nitrate, carbohydrate, protein, potassium and nitrogen. It is not clear whether any were actually measured. The element potassium was to be measured by the Kalignost method. This, as Holleman demonstrated both here and elsewhere, was not an easy test to perform.

Ash hydrolysis for analysis differed slightly from section 8.1.5 in as much as 0.2N HCl was used [the volume was not recorded]. The ash solution was then topped up to 10 [or 25?]ml with distilled water.

9.2 Results

No analysis was conducted of the results recorded in this section. The recorded results concerned dry weight of *Chlorella* after growth; optical density at the Walker dilution of 1:26 measured at 600nm; and cell counts, often of the same diluted optical density samples.

9.2.1 Synchronicity of Chlorella Cultures

The synchronicity of *Chlorella* cultures is at its most simplest, based on a measure of the relative proportion of cells undergoing cell division at any one time. This was easily measured [by myself from Holleman's cell count/size data]. A cell shortly before division is very much larger than cells immediately after division. Two cultures were sampled shortly after the majority of cells had undergone cell division; the numbers of large cells and the total numbers of cells within each of the grids were counted. The degrees of synchronicity were calculated to be 95% and 98%.

9.2.2 Health of the Cultures

In general Holleman considered that the cultures showed less clumping and sinking, as well as a greater uniformity between culture tubes than in previous experiments. A number of quantitative as well as qualitative observations were made.

The qualitative observations were as stated in 6.3.3 and 9.2. Under the microscope Holleman was able, not only to count, but also to observe the general behaviour of individual living cells. Cell densities were observed to vary from starting cultures of a few million cells per millilitre to fully grown cultures of up to a few thousand million cells/ml. Cell division was observed. For *Chlorella* this involves the production of so called autospores. A single reproducing cell can generally produce 2 or 4 or 8 or 16 daughter cells [and numbers in between] in a single division. Holleman often observed clusters of 3 or 4 small [post reproduction] cells. On one occasion he believed he witnessed the explosive production, from a large mother cell, of 4 small daughter cells.

A small but important experiment involved the sampling, after mixing/stirring, of algal culture from the top, middle and bottom of the culture suspension. The counts were $5.6x10^6$, $17.7x10^6$ and 29.6x106 cells/ml respectively.

Another small experiment examined the effects of an increase in nitrate concentration. Whilst a moderate increase led to a higher cell concentration, a large increase led to the development of a lower than expected cell count.

10 Critical Discussion of Holleman's Chlorella Research

Professor Wim Holleman's approach to this work was inspired by the philosophy developed by Rudolf Steiner known as anthroposophy (see, for example, Lissau, 1987). In 1933, whilst studying the composting of manure, Holleman obtained a copy of Steiner's [Biodynamic] "Agriculture Course". In it were given a few details on biological transmutations of chemical elements by the plants used in the preparations recommended for the treatment of manure and compost heaps. The lecture course was given to a select group of farmers and others wishing to improve the quality of agriculture, who were already familiar with many of the more esoteric aspects of anthroposophy. The published transcript is therefore not easy to follow, and many have and are still continuing to interpret and work with it [one such interpretation is by Soper, 1976].

Thus Holleman's approach to this subject, i.e. in the first two introductory sections of this review article, differed from a more conventional approach. There was a greater emphasis on the qualities of the subject under consideration, and also of the mind-set of the scientific observer.

The Ancient Greek scientist and philosopher Aristotle divided the nature of matter into two parts, into "*Materia Prima*" and "*Materia Signata*", i.e. quality and quantity, or in other words, into idea and substance. An object only attains reality for a conscious observer when the idea, or concept of the object (quality), is combined with the observer's perception of the object (quantity). In medieval times the emphasis was on the spiritual representation - the qualities (taken in their broadest sense) - of the object and not the object itself. As Holleman indicates, in 2.3, such a one-sided viewpoint needed to be overcome. This was begun during the reformation and finished with the materialistic culture of the 19th century. Though this was a necessary development to counter the one-sided medieval world-view, a completely materialistic world-view is, according to Steiner and others, just as one-sided. For an ability to fully realise the reality of an object, both are required. Thus it was, in the first two sections and the fifth of this article, that Holleman made an attempt to reconcile materialistic science with an earlier alchemistic, or spiritual, way of thinking. The importance of this was the essence of Steiner's "Boundaries of Natural Science" (1920) and also his "Philosophy of Freedom (1894)", from which it was derived. [For those wishing to further explore the fundamentals of the natural world from an anthroposophical/scientific viewpoint, I can strongly recommend Lehrs (1985)].

The underlying methodological approach taken by Holleman was based on a Goethean, phenomenological approach, as interpreted and developed by Steiner(1897). This is essen-

tially an empirical approach, allowing the observations to speak for themselves. Holleman's work concentrated on the building up of observations such that the one led on to the next (see also Koepf and Jolly, 1978). Kervran (1972) - see 4.3 - by way of contrast collected together a wide range of often circumstantial observations in order to support a theoretical mechanism for biological transmutations. For further, practical insight into the Goethean phenomenological approach see, for example, Bockemuhl (1986) and Edelglass et al. (1992). In the Netherlands research based on such an approach is conducted by the Louis Bolk Institute, Driebergen.

The outer, material, directly perceivable nature of an object or organism under study (in this case chemical transmutations under the influence of *Chlorella vulgaris*) may be observed by a series of carefully conducted, essentially conventional experiments. In a letter to Mr De Vries of Leiden (unpublished, 1982), Holleman revealed that for him, what ... is perhaps the most important for this whole research: the mental transformation that we ourselves as scientists must make, should we wish to penetrate the processes of living nature with truth. This seems illusionary. We know almost nothing of the reality of living processes. It is yet another barrier that separates us from its understanding. Goethe has taken the first step, with a new possibility to cross this threshold. He remained entirely in the realm of morphology [external form, or structure]. Now the time has possibly come, that also for the chemical processes in living matter a step further may be made. The experimental work shall thereby go hand in hand with an inner development, such as is given in Anthroposophy. Whenever one omits to do this, one's work can fall into danger. It is not for nothing that Rudolf Steiner has repeatedly said,

> in order to penetrate the realm of the living, first the laboratory work-bench must become an altar.

Thus through what is essentially a meditation based on a living memory of the experimental observations, such as were made by Holleman, an insight may be attained into the quality, or essence, of the idea lying behind the phenomena under study.

Now, in Anthroposophical science consideration is often made of so-called higher processes or forces, observable only by means of our alleged higher senses [our lower senses being those of sight, touch, smell, etc.]. As an example, such higher senses are claimed to be used to "read" the blood crystallization plates used in Anthroposophical medicine in the diagnosis of a range of illnesses and conditions. Despite Wim Holleman's anthroposophical background, his own blood crystallization experiments conducted for a short time after a training at Dornach in Switzerland (probably some time around the late 50's or early 60's) did not follow Anthroposophical dogma. Until then (and still to the present day; personal communication from Machteld Huber, March 1996, Bolk Institute) such diagnoses are made by means of both our lower and higher senses, the last of which the majority of us are unaware (i.e. they are something the existence of which I feel unable either to confirm or deny). Conventional photography was considered unable to record any "super-sensible" images. Drawings, made by the highly specialised individuals able to "read" these plates, were the only means of recording and communicating their observations. Holleman considered such drawings not to

be accurate, or objective enough. Indeed he believed that the blood crystallization patterns could be photographed and that our lower senses alone, in this case sight, could be used in the diagnoses of a range of conditions, including cancer. He was, however, not sent the blood that was needed for such a research programme.

The above anecdote was included here to throw light on the experimental procedure used by Holleman in his transmutation research with *Chlorella*. Other researchers (e.g. Hauschka, 1983, and Kervran, 1972) believed that biological transmutations were best attained by maximising the "vital forces". Thus natural rainwater should be used rather than distilled tap water; the growth medium should be natural in origin rather than inorganic chemicals; lighting should be natural daylight, or direct sunlight rather than artificial, electric lighting; also the phases of the moon were implicated in the chances of a transmutation occurring. Holleman did not even acknowledge the possibility that such factors may have a role to play. His experimental laboratory procedure was (at least outwardly) entirely conventional.

Holleman was a slow [his family called him "Snail" because he was never known to run, but even so, he was always on time], careful, meticulous man, who was his own worst critic. He preferred to work alone (he allegedly had a previous bad experience with the scientific integrity of a fellow scientist on a previous biological transmutation project). Because of this, and his strong faith in the existence of biological transmutation, he appears to have become almost fanatical about his *Chlorella* research [one week after retirement, much to the disappointment of his wife, he was back to work on a regular basis, and continued to do so for the following 15 years].

At about the same time as, or shortly after, his first discovery of Steiner's Biodynamic Agriculture course, in 1933, he also obtained knowledge of and eventually a copy of the work of Herzeele (1876-1883). This large body of research had only just been rediscovered in 1930. Holleman briefly reviews this work in section 4.2. What Holleman didn't record was that he had attempted, on a number of occasions prior to his *Chlorella* research, to replicate some of the extraordinarily large gains and losses of chemical elements in germinated seeds and root cuttings. Holleman's obvious technical familiarity of both present-day and 19th-century chemical analysis led to his finding fault with all of Herzeele's significant results. In fact every one of the positive claims for transmutations recorded by other researchers that were investigated by Holleman [and also by myself, see section 4] proved to be inconclusive. (It is interesting that such literature reviewed by Kervran was accepted by him without criticism). Nevertheless Holleman wrote, in an undated copy of a letter of some 14-16 years after his first Herzeele replicate experiments (late 1940's?), that despite a whole catalogue of analytical sources of error that he had identified, he felt that Herzeele was clearly a technically competent chemist for his time and that he still felt able to believe his (qualitative rather than quantitative) results. Herzeele, apparently, had been inspired by Goethe, and the quote

> Der Boden wachst mit der Pflanze

[The soil develops with the plants] was, according to Wim Holleman's daughter, Sophia (personal communication, February 1995), often quoted by her father.

10.1 Materials and Methods

A detailed, critical review of Holleman's practical work is essential to a full understanding of the results attained and for their future replication.

10.1.1 Overview

The last four of the six main experiments that are described here were all essentially attempts to reproduce the apparent decrease, and subsequent return, in the potassium content of a closed culture of *Chlorella vulgaris* as observed in experiment II.

10.1.2 Apparatus and Materials

10.1.2.1 Shaking Bath and Temperature Controlled Chamber

The *Chlorella* cultures, in order to help ensure reproducibility of the results and comparability of parallel running cultures, were needed to be kept at a constant temperature. Whilst steps were taken to minimise temperature variations within the culture chambers, no comparative experiments were conducted to examine temperature effects on growth.

The agitation by the shaking bath in experiment II was also variable; either insufficient or, due to resonance, waves were created which resulted on one occasion in the loss of culture fluid from the dishes. It is also possible that water from the water bath entered the culture dishes.

10.1.2.2 Containment Materials of Cultures

The culture vessels, both dishes and tubes, were of quartz glass. Under certain conditions (heated steam), corrosion of the surface of the quartz glass took place [see sections 6.3.2 and 9.1.4]. The resulting silicate suspension may have adsorbed potassium from solution causing a decrease in the measured amount of this element. An experiment to investigate such possible adsorption was never completed.

10.1.2.3 Other Glassware

All measuring glassware was tested and calibrated for accuracy and temperature variations.

10.1.2.4 Chemicals

All chemicals were of "analysis quality". A published list of trace elements present in the "suprapure" nitric acid was quoted by Holleman. This showed copper concentrations to be at potentially toxic concentrations (over the complete course of an experiment). No steps appear to have been taken to either deal with or investigate this further.

10.1.2.5 Gas Supply and Agitation

The supply of carbon dioxide gas to the cultures was highly variable. The effects of this on growth and health of the *Chlorella* cultures was not examined. In the latter experiments where the gas stream was used for agitation no comparisons were made between the efficiency of this method and of the shaking bath. Sinking and clumping was a continual problem preventing the formation of a completely homogeneous culture. Foaming proved to be another problem with losses of algae due to their deposition on the sides of the tubes. The use of Vaseline smeared thinly on the gas inlet tubes largely solved this problem. [See sections 6.3.2 and 8.1.2].

10.1.2.6 Lighting

From the literature obtained by Holleman on this subject it is clear that not only the light intensity but also the type of light source has a profound effect, not only on the growth, but also on the physiology of a *Chlorella* culture (e.g. Tipnis and Pratt, 1960). It is not clear whether the motive for the switch from a tungsten filament bulb to fluorescent tubes was purely one of convenience. The light intensity, for experiments V and VI, was however considered in some detail on the basis of the literature on the subject. The light intensity received by each culture tube was found to be equal. See also section 9.1.2.

10.1.2.7 Sterilisation

A number of measures were implemented, including a comparison of the effects of autoclaving as opposed to ultra-filtration of the nutrient solution. No significant difference between the two methods was found, based on algal growth. See also section 8.1.2.

10.1.3 Nutrient Solution

The nutrient solution used throughout was that of a slightly modified solution of Kuhl [section 7.1.3]. The main modification was that the magnesium content was reduced by 90%. This was done against the initial recommendations of section 3.1.5. Nevertheless the idea that a transmutation is most likely to occur in the presence of a deficit of an essential element was for Holleman a strong one. The element magnesium is essential for photosynthesis and thus for the whole "organic process" within the plant.

10.1.4 Algal Cultures

10.1.4.1 Production of Cultures

The production of *Chlorella* is detailed in sections 7.1.4 and 8.1.4. The cultures were, whilst often variable in results, highly resilient to a wide range of storage conditions.

10.1.4.2 Meassurement of Cell Numbers

Strictly speaking, the methods given in sections 7.1.4 and 8.1.4 were indirect and direct methods of measuring chlorophyll content. Only with *Experiment VI* was cell number directly counted.

The use of these and dry weight production may all be used to assess the general health and vitality of the algal cultures (see section 10.2.2 for details). Cell size and the number of autospores produced per cell division were not however measured. In one or two experiments associated with Holleman's synchronisation studies, a growth constant was calculated. This was not described in 9.1.3 as insufficient details were given in his notes.

Holleman did not explicitly make comparisons in his notes between direct and indirect measures of chlorophyll production and/or cell number (dry weight was also measured in one or two cases), though the data were recorded every few days for many experiments.

10.1.5 Procedure

10.1.5.1 Growth of Cultures

It is interesting to note that for experiment V the culture volume was reduced to 10% of its original value, as used in experiment II, presumably making accurate measurements more difficult [this potential difficulty was, however, never mentioned]. Nevertheless, it did enable not only duplicate cultures to develop in parallel, but also their equivalent controls to be duplicated as well, in the same culture chamber since more tubes could fit in.

Section 9.1.4 gives details of the use of a regular light/dark cycle which on its own was able to produce synchronised *Chlorella* cultures. The experimental procedure described in the literature also included the synchronised dilution of the cultures in order to attain a constant, low concentration of cells. Such dilutions increase the homogeneity of the immediate environment of every individual cell. However, it appears that regular dilutions were not conducted by Holleman. Any culture dilutions (i.e. by the addition of a measured sample of the experimental culture solution to a calculated volume of new nutrient solution) would also dilute the culture medium in which they were growing. As a consequence any accumulated transmutation effects would also be diluted. See 6.3.3 for details.

10.1.5.2 Ashing and Provision of New Nutrient Solutions

The ashing procedures described in sections 7.1.5, 8.1.5 and 9.1.4 were increasingly simplified and improved after much experimentation. The alkalisation of the ash was suspected to be due to a reaction between the acid phosphates and the nitrates, but probably involved the polyphosphates which had been formed by the algae and by the heating as well. This resulted in the removal of the acid forming ions resulting in an alkaline solution, which Holleman neutralised by the addition of extra nitrate ions in the form of nitric acid. The formation of polyphosphates by the algae was dependant on the culture conditions which, for instance, was dependant on the amount of nitric acid present, which in turn depended on the amount of polyphosphates present. Thus it was found that the pH results were highly

variable. Whilst the subject of many experiments, unfortunately pH measurements were not made during the running of the main experiments to check up on this. See section 10.2.2 for further details.

The need to add extra nitrate ions, in the form of nitric acid, to the redissolved ashes to produce usable nutrient solution meant that there was a significant difference in the chemical make-up of the reconstituted nutrient solutions from the original Kuhl solution. Apparently the phosphates that were originally present in the Kuhl solution acted as a pH buffer solution [i.e. they were able to maintain the pH of the nutrient solutions despite variations in the chemical environment that would otherwise increase or decrease the acidity of the solution]. Kuhl and Lorentzen (1964) further state that *Chlorella* is unaffected by high phosphate concentrations. Therefore a constant pH environment may be better maintained by increasing the phosphate concentration of the original Kuhl solution, and could be used instead of the nitric acid additions (this was proposed in section 9.1.4). It was also recorded, however, that under certain conditions a great part of the ashed *Chlorella* may consist of phosphate originating from inorganic, condensed phosphates (polyphosphates) which can be accumulated by *Chlorella* to a great extent (see Kuhl, 1960 and 1962b for further details). Such polyphosphates were implicated by Holleman as causing great difficulties in chemical analysis (see especially section 10.1.5); thus it may be for this reason that Holleman used nitric acid. It was also quoted from the literature that increasing the nitrate concentration increases the culture density. See however, section 9.2.2.

It is worth noting that all changes in the reconstituted nutrient solution would accumulate; thus the volatilisation of any chemicals during ashing could also have an effect on algal health. See section 10.2.2.

10.1.5.3 Controls

A number of different controls are described in sections 7.1.5 and 8.1.5. The conclusion that I have come to was that Holleman considered it important that the control treatment should be as near to identical as possible as those of the parallel growth cultures, except that the control algae should be dead. Exclusion of light and heat treatment were two successful methods; the use of selective poisons were also considered but not tried. However, in experiment V, whilst the experimental cultures and the controls ran (in general) simultaneously, the particular control and growth pairs that were to run for the same number of cycles did not run together. There was not enough room in the culture chamber for them to run at the same time. This decision lends further weight to the idea that Holleman did not consider external (cyclic) influences such as the phases of the moon [or other so called cosmic influences] to be relevant (see Hauschka 1983). The 6 cycles of experiment V for example, despite the fact that each growth cycle was only 14 days long, lasted a total of 5 months (not including a planned 1 month break and a further 3 months hospitalisation). Furthermore, it took another 4 months for the ash solutions to be analysed; a grand total of 13 months from start to finish. See section 10 for a further consideration of Holleman's outlook on the subject.

10.1.5.4 Chemical Analysis

Details are given in sections 6.2, 7.1.5, 8.1.5, 9.1.4 and 10.2.3. The main elements that were analysed - with varying degrees of success - were potassium, sodium, calcium, magnesium, nitrogen (or nitrate), and phosphorous (or phosphate).

The reason why potassium was chosen as the main element to be investigated was never stated; there are, however, very strong practical and theoretical grounds for its having been chosen. Historically it has often been implicated as taking part in transmutation phenomena. It is also easily analysed to a high degree of precision by flame photometry and capable of independent analysis by the Kalignost method. The use of sodium as a neutral reference was considered to be a an extremely clever one by Michel Haring (personal communication, November 1995). The agreements between the controls of experiment II (see section 7.2.1, figure 3 and figure 4) of both Kalignost and flame photometry for potassium and also with the sodium gave strong support for the methods chosen by Holleman. It also prevented the easy possibility of analytical error for the experimental results. Nevertheless, I have considered it my duty to attempt to find such an error (see my foreword to this work, as well as sections 10.2.3 and 11). The potential error in potassium content measurements, that may have been the result of the necessary filtration of any precipitate found in the ash solution before analysis, remains unproven. The improved hydrolysis of the ash in subsequent experiments meant that such precipitates were no longer a problem.

The analysis of calcium and magnesium proved extremely difficult. The presence of polyphosphates apparently hindered their being analysed. Nitrates and phosphates were analysed with varying degrees of success in an attempt to understand the chemistry of the ashing process. This [presumably] led to an improvement in the hydrolysis of the polyphosphates. I found it difficult to follow the procedures adopted by Holleman and to assess the success of his analyses.

Holleman quotes Steiner (1924) as stating that it is possible for potassium, via an unknown intermediate step, to transmute into nitrogen. Holleman put foreword the suggestion that this intermediate step could be the noble gas, argon. Thus Holleman refers elsewhere to Picket's analysis of argon given off from yeast. The presence of argon in biological material would conventionally be considered as coming from the radioactive decay of the potassium isotope ^{40}K.

Iron and sulphur analyses were considered but were never done [sulphate was attempted but unsuccessfully]. Carbohydrate and protein were also considered for analysis, but no records have been found of this being done.

10.2 Results

10.2.1 Analytical Results

These are given in sections 7.2.1, 8.2.1 and 9.2. The results of experiment II are further considered in sections 10.1.5, 10.2.3 and 11.

The anomalous results of the sixth cycle of *Experiment V* (section 8.2.1) are noted here

as being associated with two different calibration solution values and were discounted by Holleman.

The associated measurements, also given in 8.2.1, of the potassium contents of the 5 remaining (at the time of the analysis) inoculation cultures and of the 2 Kuhl solutions, with and without hydrolysis are also worth noting. The higher than normal values of the inoculation cultures may most simply be explained by the fact that they came directly from the Biophysics department. Thus the potassium content of their nutrient solution was presumably slightly higher than that prepared by Holleman. The lower values of the Kuhl solutions were, as expected, approximately 4% lower than that of the potassium value recorded for the cycle one cultures (see section 7.2.1 for further explanation). These results also clearly demonstrate that the hydrolysis procedure, of itself, has no effect on the measured potassium concentration.

10.2.2 Health of Cultures

The clumping and associated sinking of the algae could only partly be due to insufficient agitation. It may be, apparently, a sign of ill health. The importance of its occurrence with regards to the maintenance of a homogeneous cell population is obvious. Those cells on the inside of a clump do not have as good an access to light and nutrients as those on the outside. It is interesting that the low starting densities of the cultures associated with experiment VI were associated with low levels of clumping. Clumping and sinking also made it at times impossible to obtain a valid optical density reading.

It is perhaps here a good point to consider the general population dynamics of a *Chlorella* culture. The growth curve, of cell density against time, is a classic "S" shaped curve. The initial portion of the curve shows exponential growth; the middle section, as the individual cells start shading each other, is approximately linear; as growth continues they proceed to use up the available nutrients and so growth slows, eventually, to zero. Holleman found that this whole process took approximately 14 days. Growth would thus be limited, firstly by shading and then by the first (essential) element to be used up. This was intended to be magnesium, in the hopes of forcing the *Chlorella* to replace it by means of a transmutation. The plotting of such growth curves for cultures grown under different conditions could enable a number of different factors to be quantitatively considered and compared, i.e. growth rate, effects of growth limiting factors, etc.

The dilution procedure that may have been used in experiment VI was a compromise to enable maximum growth and to accumulate any evidence of transmutations having occurred. These were consistently the two objectives that were to be striven for in the experimental design. Measures of "growth" are varied and were only considered by Holleman in very general terms. Holleman normally refers to "growth" when referring to cell population growth. Only with experiment VI is growth also used to refer to the increase in size of an individual algal cell, rather than the growth in size of the total number of cells in the culture. Holleman referred more than once to Steiner's reference to the "organic process" upon which transmutations were supposed to depend. Steiner (1924) refers to the "organic process" in

very general terms. The two types of growth mentioned above are not necessarily equally affected by the same culture conditions. In fact, a casual glance at the literature demonstrates that *Chlorella* presents a wide range of physiological responses to different culture conditions. On the basis of Holleman's early *Chlorella* results, he considered that transmutations may very likely be associated with particular physiological, or "organic" processes. Thus the synchronisation experiments associated with experiment VI were initiated.

Holleman, a short while earlier, had observed that a heat treated control of experiment V had started to grow and in fact showed remarkably strong growth. [This control culture was started before Holleman's extended period in hospital, during which time the cultures and controls were stored. It was on his return that the beginnings of cell growth were noted. My first reaction was the possibility of contamination which Holleman did not, on paper, consider.] A small experiment (section 6.3.2) proved that insufficient heating would fail to kill all of the algae. After further consideration (taking into account that this aberrant control culture grew exceptionally strongly) Holleman came to the conclusion that perhaps the assimilative growth process was not responsible for a change in the *total* [Holleman's emphasis] mineral composition. Possibly it was a "dissimulation process", i.e. a breakdown or decay process [?] that was responsible for the occurrence of biological transmutations. As support for this idea he referred to Steiner(1924); there he describes compost [Holleman repeatedly uses the inappropriate term "manure"] formation with the aid of the [biodynamic] preparations 1-7. The problem for Holleman was how to follow this compost/decay ["manure"] process *in vitro* , in an aqueous solution. He considered the *warming* or poisoning (by chlorine, etc.) [presumably] of a *Chlorella* culture. By means of this hypothesis then, the exceptionally strong growth of the heat treated control may be explained as follows: the heat treatment, which failed to kill all the algae, caused the majority of the algae to decay. This decay process may have been accompanied by a transmutation phenomenon which could have been responsible for increasing the amount of the growth rate limiting element of the nutrient solution; this would enable an increase in *Chlorella* growth to take place. [It is worth noting, however, that later, "normal" cultures also attained such growth densities].

This consideration that the assimilation process, which only occurs in the light, may not be responsible for transmutation phenomena helps explain why Holleman considered the dark cycle of a synchronised culture, i.e. the reproductive phase worthy of particular attention. It is to be noted that the consideration that photosynthesis [assimilation] and growth occurs only in the light and that reproduction and respiration [dissimulation?] occurs in the dark is a gross simplification of the enormous variety of biochemical or physiological processes [i.e. the overall "organic process"] that takes place in such a small (4-10 thousandths of a millimetre) single celled organism. I often had the feeling that Holleman's awareness was directed mostly to the chemical phenomena with which he was familiar. Of the almost infinite complexity of the workings within such a tiny cell, Holleman made little comment.

He wrote in a letter, in 1991, that the aim of his research was to enable "a known quantity of mineral substance to be exposed to the influence of an organism, not once but six times". From this I understand the object of his study to have been the "known quantity of mineral

substance". I have the impression that most of the large quantity of *Chlorella* literature was only reviewed by Holleman during his later synchronisation research.

10.2.3 Sources of Error

The potential error that may have caused the initial disappearance and subsequent return of potassium (section 7.2.1) was not included in Holleman's privately circulated German language report. Over the subsequent 7 years of experiments, a few addressed the subject of what Holleman referred to in his laboratory notebooks as "trivial reasons" for such a result (see section 6.3.2). None of them were ever completed: other experiments were considered more important; objections were made to other such experiments testing for "trivial reasons" on a variety of technical grounds [some entirely valid, others less so]; and arguments were also found so as to make the remainder appear unlikely to be relevant [again some of this reasoning was not always clear when referred back to the original aims of these tests]. In such cases I like to remember the sentiment expressed by the great fictional detective Sherlock Holmes, that *everything* must be investigated, no matter how improbable. These tests were not (unfortunately) carried through to completion. He allowed his own personal judgement (conscious or otherwise) to get in the way of a thorough investigation.

The potential error mentioned earlier was that the ash solutions to be used for chemical analysis were *sometimes* filtered due to the appearance of an insoluble precipitate. It is possible that this filtrate contained a small but significant amount of the potassium. The formation of this precipitate, which was not dissolved by treatment with $HClO_4$ for ash analysis, was infrequent and unpredictable. However, the improvements in culture conditions, ashing and ash hydrolysis procedure successfully reduced the occurrence of such a precipitate. Thus such an [hypothesised] reduction in the amount of potassium available for analysis was very much less likely to occur. So it may have been that the subsequent attempts to repeat these results have proved unsuccessful. [The experiment that unsuccessfully attempted to test for the most probable "trivial" explanation (as described in 6.3.2) was never repeated; in contrast, the large experiments to replicate the suspected transmutation which was recorded in experiment II were, however, repeated a total of four times]. Therefore the possibility of a "trivial reason" as opposed to a transmutation having occurred must still remain a possibility. [At the time of writing the relevant laboratory notebooks are unavailable for a further investigation of this subject].

The letter quoted from at the end of the last section (10.2.2), dated 2 years after his last recorded *Chlorella* notes, states the doubts which he had after the unexplained potassium disappearance and subsequent reappearance, of the possibility that a brutal error had been made. This echoes a letter written some 40 years previously expressing the grave doubts that he had had when he, for the first time, failed to replicate the results of Herzeele's transmutation work on a wide variety of plant material. Again, he [eventually] does not allow this to get in the way of his faith in the *possibility* [my emphasis] of the existence of biological transmutations. The absence of a proof, proved [or disproved] nothing. At the age of 86 he expressed the desire, despite his no longer having access to a laboratory

and his increasing infirmity, to finish the work that he had started, including writing up his earlier replications of Herzeele's work. This was, unfortunately, work that now is for others to continue.

11 Conclusions and Recommendations

The materialistic approach to chemistry began in 1789 with Lavoisier stating the Law of the Conservation of Matter as being axiomatic. Exactly 200 years later Professor Dr. L.W.J. Holleman stopped work on his own attempt to prove that these chemical laws do not [always] apply to living organisms. This provisional review of Holleman's *Chlorella* work is [and was] written with the intention that others may be able to continue this work.

The reasons for and against such further work were given by Holleman in section 5. Apart from a large body of theoretical and experimental research on sub-atomic physics, the results of which strongly preclude the possibility of biological transmutations, no direct experimental proof for the conservation of matter and the indestructibility of elements has been conducted since the 19th century; no complete elemental analysis of the development of a biological organism in a closed system has been done using modern analytical methods. Further research work is therefore strongly recommended. The basis of such research [by definition] should be essentially empirical rather than based on theory.

I recommend that the exact details of Holleman's *Chlorella* experiment II (sections 6.3, 6.3.1, 7 and 10) be replicated as closely as possible; the "improved" repeat experiments failed to produce any measurable effects. It is also recommended that the insoluble precipitate occasionally observed after ash hydrolysis should be investigated as a possible cause of "trivial" error. In fact, I consider that his chemical analysis difficulties are very much worthy of further research, independent of the rest of this work. If the observed potassium results remain unexplained then all subsequent improvements adopted by Holleman (covered in the previous section and elsewhere) should be considered. The method described for experiment VI in section 6.3.3 showed the greatest potential for studies on the possible role of the different developmental stages of *Chlorella* (see also sections 9 and 10).

The philosophical background of Holleman and his work, as detailed in 10, should not be considered as compulsory reading. The Goethean phenomenologist and Anthroposophist Dick van Romunde (Beekman1992) stated that "there is no compulsory connection between anthroposophy and phenomenology." I have included it, however, for I felt that it was difficult to separate Holleman's beliefs from a complete understanding of his research. Nevertheless, I feel that the quality of such work can only be improved should the approach described in 10 be adopted. For a further understanding of Wim Holleman and his motivations I have taken the liberty to include a translation of his obituary, as co-written by his close friend Dick van Romunde and his daughter Sophia Holleman (Appendix I).

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[I have only seen those marked with an asterisk].

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Appendix I: An Obituary of Professor L.W.J. Holleman

Leendert Willem Jacob Holleman.

23rd September 1906 - 13th November 1994.

Our noble friend Wim Holleman has closed his earthly life and began, on the 13th November, the course that shall lead him through pure spiritual regions.

We would like to give a short sketch of a few phases of the course of his life, which is now closed. We have chosen three images which relate to his youth, middle age and to the phase in which he was older and more mature.

When he was two years old his father died. Between his second and twelfth years he lived with his mother, brother and two sisters in Baarn. His mother was a gifted pianist who often accompanied singers; the house was pervaded with a beautiful wealth of sound which the little Wim absorbed with great intensity. His being obtained the nourishment which he eagerly longed for. Even at an early age he obtained piano lessons from his mother and he developed fast and easily. We should always imagine the little Wim as a specially beautiful, finely built child. He was steeped in song and music. Poetically expressed, it was as if he had fallen from out of the music of the spheres.

After struggling through secondary school in the Hague, which did not suit him, and having deepened his love of music under the inspiring musicologist Van de Waal, he went at the age of seventeen to study chemistry in Leiden.

Just before, on the basis of their fruitful conversations, his cousin Riet had handed him a copy of *The Philosophy of Freedom* [Steiner], which he studied with great intensity. His choice for the study of chemistry can be associated with the fact that the fascinating play of the visible chemical processes is connected with the same world of numbers which manifests

itself in music. He was, certainly later, strongly under the influence of this relationship. And this leads to the second image.

After his PhD, he worked for many years as assistant in Leiden, before going to Indonesia in 1936, where he obtained a leading position in the Buitenzorg College of the National University. There he married Ibolya Mancza in 1938, whom he knew from his time in Leiden. In 1951 he was appointed professor. His inaugural address gives a clear image of the state of Wim Holleman's soul and spirit at that time. In it he sketches the development of chemistry since the Middle Ages. The ancient alchemy connected a very limited number of chemical processes to a large wealth of inner experiences. The making of gold still meant at that time the purification of the soul life, so that it attained a quality of gold. The purification was felt as a musical experience, connected with the endurance of pain. The further [historical] development of alchemy is followed, step by step, in the speech; it eventually leads to its [materialistic] opposite. Present-day chemistry (1951) connects an overwhelmingly large number of processes with a poverty of inner experience. The concepts remain almost totally limited to mechanistic phenomena in a world of unimaginably small particles. Wim Holleman ended his speech with the wish that we may have a prospect of a future rebirth of the inner wealth that could liberate chemistry from out of her [present-day] prison. He uses a quotation from the independent chemist Liesegang: I enter into no prison.

On his return to the Netherlands, Wim settled in Baarn after a while and joined the staff of the Pathology Department of the University of Utrecht. He was allowed, alongside his routine work, to devote a modest part of his time to some research of his own. His strong, inner, musically disposed susceptibility and sympathy protested against the accepted fact that the underlying principles, even for living processes, must be sought for in bone-hard immutability. That the chemical elements in organisms - for example potassium or calcium - cannot change themselves into other elements, appeared to him to be an unmusical rigidity. He found support for his ideas from Rudolf Steiner, who describes the reality of such a transposition. Towards this reality Wim directed his research. He held as an inner truth that the chemical life processes are in such a way under the continual influence of that which the ancients called the harmony of the spheres, that all that becomes fixed must be able to be broken down. It was as if he, in his own being, experienced this music so strongly that he experienced its workings in such a way that it affected his whole body. He succeeded in showing that potassium in algae must indeed be subject to transmutation into other elements.

In these three images, taken from three life phases, the figure of Wim Holleman speaks the same language: of a being, filled with nobility and amiability, penetrated by heavenly music, a noble Christian.

Sophia Holleman and Dick van Romunde:

Mededelingen, June 1995.

Appendix II: About the Holleman Stichting

The Professor Dr. L.W.J. Holleman Stichting (Trust) was set up in 1995 by Ms Sophia Holleman, (secretary and treasurer) and Ms Jannie Moeller, (chairperson). Its aim is to enable and financially support biological transmutation research, such as conducted by Professor L.W.J. Holleman; related activities are also included.

By means of lectures and articles, the Stichting wishes to make people aware of the results of this work and of its ecological importance in relation to agriculture.

The Stichting is supported by grants and donations; gifts and legacies; etc.

A copy of the constitution is available on request. Any general enquiries, donations, etc. are to be addressed to:

Sophia Holleman
Burgstrasse 8
Dornach
CH - 4143
Switzerland.

The Stichting would especially be keen to hear from anyone interested in furthering this research.

Enquiries of a technical nature should be addressed to:

David Cuthbertson
50 Balsdean Road
Brighton
Sussex
BN2 6PF
United Kingdom.

Acknowledgements

In the foreword at the start of Holleman's draft review he gives acknowledgement for the help [and/or tolerance!] of a number of colleagues. It had later been deleted, presumably because it became out of date. As his work progressed, others also became worthy of his thanks. These I wish to recognise here, posthumously, on his behalf; only the names of those recorded in his laboratory notebooks and other notes though, may now be recorded here. Speaking to a few of his close friends and colleagues, all of them were aware of his work but none felt able to say that they were in any way particularly connected with his work.

Mrs W. Terpstra of the Biophysics department has already been acknowledged. She appears to have been replaced by 1985 by a Mrs Verheyden. JW de Groot deserves considerable recognition for being the only person recorded as directly participating in the design and implementation of some of his experiments. Mrs Visser and Mr Bokhorst of Kraaybeekerhof [the Netherlands Biodynamic agricultural research institute] for helping him with the flame photometric analysis of his ash solution samples of experiment V. During his synchronisation culture work he recorded an appointment with a Dr. Gielis about *Chlorella* and literature.

I would also like to extend my apologies to Ir. Kelderman from whom Holleman borrowed a book by Louis Kervran which was not returned!

My own acknowledgements firstly are extended to Sophia Holleman for her struggles to keep her father's research alive, and also to Jannie Moeller for helping in the creation of the Professor L.W.J. Holleman Stichting. Both have been of tremendous help and support during my time with this project.

I have found help and advice from a surprising number of people from a wide range of backgrounds; sadly they are too numerous to record here. In no order of merit, and for a number of different reasons, a few noteworthy individuals are mentioned here. Reinout Amons; Joke Bloksma [on behalf of the Bolk Institute as a whole]; Jan van Gils; Michel Haring; Ir. Kelderman; Jan-Diek van Mansveld; Peter Pandelaars; Dick van Romunde; extra special thanks are reserved for my very close friend Judith Lorand for her support and understanding, especially when things became difficult.

This project has, for me, been a journey of discovery. As if the Dutch language wasn't enough of a challenge, my lack of relevant specialist knowledge made the understanding of Holleman's notes more challenging than any work of detective fiction. On top of that were my own personal explorations of - to quote from the sub-title of a book - "a spiritual understanding of nature on the basis of Goethe's method of training observation and thought". I would like to dedicate this work to all those whom I met during my very special three years in Holland.

David Cuthbertson, February 1999.