NARRATIVE OF AN INVESTIGATION CONCERNING AN ANCIENT MEDICINAL REMEDY AND ITS MODERN UTILITIES

The *Symphytum officinale* and its Contained Allantoin

BY

CHARLES J. MACALISTER, M.D., F.R.C.P

Hon. Consulting Physician to the Liverpool Royal Southern Hospital, to the Royal Liverpool Children's Hospital and to the Moore Cottage Hospital, Bourton-on-the-Water

Together with an Account of the Chemical Constitution of Allantoin

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A. W. TITHERLEY, D.Sc., Ph.D.

Formerly Lecturer on Organic Chemistry in the University of Liverpool

LONDON

JOHN BALE, SONS & DANIELSSON, LTD.

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LEE FOUNDATION FOR NUTRITIONAL RESEARCH

MILWAUKEE 3, WISCONSIN

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FOREWORD

THIS little book contains the narrative of an investigation of the common Comfrey, which led to the discovery that its roots contain relatively large amounts of allantoin.

Following this came an inquiry as to the therapeutic uses of allantoin, which appears to be the active principle to which the " virtues " attributed to the Comfrey in bygone days are ascribable.

Allantoin induces cell proliferation in a physiological way and it is this quality that renders it useful not only as a vulnerary but also because, as a promoter of leucocytosis, it helps to establish immunities in some infective conditions.

Much of the earlier work was carried out many years ago and some of it was published in the *British Medical Journal*. New matter is included in this record which has been written by way of opening the door for further investigations by those who may feel interested enough to avail themselves of clinical opportunities which, in my retirement, I do not possess.

I am indebted for much help to my colleagues and to my professional friends, to all of whom my grateful thanks are accorded.

Bourton-on-the-Water,
1936.

SUMMARY OF CONTENTS

AN ANCIENT REMEDY AND ITS MODERN UTILITIES

The *Symphytum officinale* or Common Comfrey and its contained Allantoin

ABOUT twenty-five years ago (1910-1911), when endeavouring to make some investigations concerning cell proliferants, I happened to remember that in 1896 I had published a paper in the *Lancet* on "Blood as a Therapeutic Agent," in which were incorporated some impressions which I had formed concerning bodies contained in blood which I thought might inhibit irregular cell growth. On looking up this paper I found in the same number of the *Lancet* an exceedingly interesting address entitled "Some Surprises and Mistakes," by Professor William Thompson, President of the Royal College of Surgeons in Ireland. He recorded the case of a man who was suffering from a tumour involving the nose and antrum which, on being removed, was declared to be a round-celled sarcoma by Dr. O'Sullivan, Professor of Pathology in Trinity College, Dublin. The growth returned and the patient consulted Sir Felix Semon, on whose advice the jaw was removed and at the operation the tumour was found to occupy the whole of the antrum. The base of the skull was everywhere infiltrated by it. It had perforated the septum of the nose to which it was adherent, and had entered into the opposite

(left) nostril. A month later the growth had again returned, it bulged through the incision and almost closed the right eye. It was blue, tense, firm and lobulated, but it did not break. Further operation being out of the question the man was sent to his home. About three months afterwards the patient walked into Professor Thompson's study looking in better health than he had ever seen him. The tumour had completely disappeared from the face and there was no trace of it in the mouth. He had no pain, and after having an obturator plate made to fill the opening which was left by removal of the hard palate he went home apparently well. He told Professor Thompson that he had treated it by applying poultices of Comfrey and the swelling had gradually disappeared. Professor Thompson said in his paper: "I am as satisfied as can be that the growth was malignant and of bad type . . . I know nothing of the effects of Comfrey root but I do not believe that it could remove a sarcomatous tumour."

There have been one or two cases in my own experience where undoubtedly malignant growths spontaneously disappeared, and the one which I have quoted may have been another example, but it set me thinking about the possibility that Comfrey might contain some substance capable of controlling or stabilizing cell-growth, and it naturally led to an investigation of the literature of Comfrey and to experiments being made with it clinically. At the time I had never heard of Comfrey being used medicinally. It was certainly not included among the botanical agencies dealt with in classes of materia medica when I was a student, but it became evident from a reading of the old Herbals that it was regarded as an important and valuable remedy in bygone days.

HISTORICAL

COMFREY belongs to the Boraginaceæ or Borage-worts. It is referred to very casually in the books on Materia Medica, written after the middle of the nineteenth century. Pereira[1] (1854) says that formerly several Borage-worts were used in medicine and he mentions among them the *Symphytum officinale*, but he says "they possess little medicinal value (though formerly many virtues were ascribed to them), and are now obsolete."

According to the Oxford English Dictionary the name Comfrey was of middle English origin and is attributed to the old French confirie or confiére, no doubt the equivalent of the modern confire—to preserve. It was also called Consolida or, in some of the English Herbals, Consound and was a member of a class of remedies referred to for instance in the Pharmacopœia Londinensis Collegarum (1668) among the Radices or Roots as the Consolida or *Symphytum major*, and among the Herbs, Leaves and Seeds the Consolidæ include, besides the Comfrey, the *Consolida media* (the Bugula) and the *Consolida mimina* or Daisy, also *Consolida Saracenia* or Solidago, a variety of Comfrey having knobbed roots. This was called the true Saracens' Consound or Wound-wort because it was used by the Turks and Saracens for healing wounds. William Salmon in his English Herbal (1710, page 213) classifies it among the agglutinatives or symphitica, "which is the reason that 'Comfrey' is called Symphytum because of its glewing quality." In several Herbals it is spoken

[1] Pereira, "Elements of Materia Medica, 1854."

of as the chief vulnerary for the same reason. Comfrey is known to English botanists as the *Symphytum officinale*, and be it noted that the word Symphytum is derived from the Greek Σύμφυτον, "a facultate glutandi," i.e. from its glueing properties. The Latins used the word Consolida for the same reason from Consolidare "to soder, close or glew up" (Salmon). Hence the English equivalent Consound often used in the Herbals.

When the Comfrey became *Symphytum officinale* in England I am not sure. The term Officinal first came into use in its application to medicines, according to the Oxford Dictionary, in 1693; the Latin *Officina* being applied to the storeroom of a Monastery in which Medicines, etc., were kept, and so herbs, plants, drugs, etc., kept in stock in an Apothecary's shop became "Officinal" or as being of recognized utility. Comfrey was never introduced into the British Pharmacopœia but it is described in Squire's Companion to the British Pharmacopœia (17th edition, page 619) as having astringent, mucilaginous and glutinous properties. Its author knew a bone-setter who had rendered himself famous by treating fractures with a pulp made of the scraped root spread to the thickness of a crown piece upon cambric or old muslin which was wrapped round the limb and bandaged over. It soon stiffened, giving great strength and support to the part, and the bandage was not removed until the limb was well.

Squire mentions that Comfrey was officially recognized in the following Pharmacopœias: In Belgium as *Radix symphyti*; France *Consonde*; Mexico *Sinfito*; Portugal *Consolida major*; Spain *Sinfito major*; and in no others.

There are three varieties of Comfrey referred to in the Herbals: (1) The *Symphitum majus vulgare* or Common Great Comfrey; (2) the *Symphitum majus flore purpureo*—the Great Comfrey with purple flowers; (3) the *Symphitum tuberosum* or Comfrey with knobbed roots, which is the Saracen Consound formerly mentioned. This latter has several species and was probably selected on account of the largeness of its roots. All the varieties were used, however, the roots being gathered during the winter months (October to March). The leaves, also employed, were gathered in June and July during and after inflorescence.

The medical chronology of the plant is interesting on account of its antiquity. In the new English Dictionary, Saxon Leechdom is quoted (*c.* 1000) "This Wort strengthens the man," and it states, "Ad fluxum Sanguinis accipe de Confirma, hoc est consolida." In the Saxon Herbarium it was thus recommended for one "Bursten Within"; the leaves were roasted in hot ashes and mixed with honey and then taken fasting. There are many references to Comfrey, and I cannot do better than quote some details of its history from a paper by the late Professor R. J. Harvey Gibson[1] which gives a very good historical summary. He says: "In Sarracenius's version of Dioscorides, published in 1596, descriptions are given of two species of *Symphytum*; Chap. IX (Liber II) is headed 'De symphyto petraeo,' and Chap. X 'De Symphyto Altero.' The 'roots' and their 'vertues' are described in the following terms: 'Radices demittuntur foris nigræ, intus candidæ, glutinosæ, quarum etiam est usus. Tritæ et potæ sanguinem excreantibus ruptisque proficiunt et recentia vulnera impositæ glutinant; carnum quoque frusta quibuscum conjunguntur cogunt

[1] Note on the Anatomy and Herbal History of *Symphytum officinale* by R. J. Harvey Gibson, M.A., F.Z.S., Prof. of Botany, University of Liverpool, *Pharmaceutical Journ. and Pharmacist*, Jan. 27, 1912.

ita ut coalescant. Cæterum inflammationibus, præsertimque sedis, cum Senecionis foliis utiliter illinuntur.'"

Bock, in his work, "De stirpium" (Kyber's edition, 1552), quotes and expands this sentence from Dioscorides, and describes the then recognized methods of application of the plant, internally and externally. Fuchs also (1542) quotes the same extract, and also the views of Galen and Pliny, which are practically in the phraseology of Dioscorides.

In W. Turner's "Herball" (1568) the following occurs: "Of Comfrey Symphytum. The rootes are good if they be broken and dronken for them that spitte blood, and are bursten. The same, layd to, are good to glewe together freshe woundes. They are also good to be layd to inflammation, and specially of the fundament, with the leaves of groundsell." This account is also merely a condensed translation of Dioscorides.

Dodoens, in his "Cruydtboeck," translated by Lyte (1578), expands Turner's statement, adding that when "mengled with sugar, syropes, or honny . . . are good to be layde upon all hoate tumours." Quite similar statements as to the value of Comfrey rhizome are made in Bulleyn's "Herbal" (1562) and in the "Adversaria" of Pena and Lobelius (1570), and the "Stirpium Historia" of the latter author (1576), also in the epitome of Camerarius (1586). J. Bauhin ("Historia," 1651) expresses his concurrence in the views of the sixteenth century herbalists as to the curative value of decoctions of Comfrey in all cases of wounds, blood-spitting, or even broken limbs.

Gerard's "Herball" (1597) repeats, but at somewhat greater length, the same account. He asserts the efficacy of Comfrey also in healing up "ulcers

of the lunges" and "ulcers of the kidneies, though they have been of long continuance." Parkinson ("Theatrum Botanicum," 1640) gives the same general description of the "vertues" of Comfrey, but adds: "The rootes of Comfrey, taken fresh, beaten small, spread upon leather, and laid upon any place troubled with the gout, doe presently give ease of the paines; and applyed in the same manner, giveth ease to pained joynts, and profiteth very much for running and moist ulcers, gangrenes, mortifications, and the like." There is a marble statue of John Parkinson (born 1567), Apothecary to King James I, outside the Palm House, Sefton Park, Liverpool.

In the "Compleat Herbal" of Tournefort (1719) a long account is given of the "vertues" of Comfrey and of its general characters. It is interesting that this author states that "upon a chymical analysis the Comfrey yields many acid liquids, much earth, very little sulphur and no concreted volatile salt but a small quantity of a urinous spirit and a very moderate quantity of fixed salts." Tournefort closes his account with the observations of Hieronymus Rensuerus "that a charlatan cured a certain person of a malignant ulcer, pronounced to be a cancer by the surgeons, and left by them as incurable, by applying twice a day the root of Comfrey bruised, having first peeled off the external blackish bark or rind; but the cancer was not of above eight or ten weeks standing."

By the end of the eighteenth century Comfrey seems to have declined in popularity among physicians; thus Woodville ("Medical Botany," 1794) writes: "A supposed vulnerary efficacy, for which this plant was formerly in great repute, and to which it seems to owe its name" (Comfrey = confirma), "will now be considered as nothing in

its recommendation." He adds: "The mucilaginous matter is the only medicinal principle, and may be used as an emollient and demulcent."

There are many other references to Comfrey which all tell the same story, and it is unnecessary to elaborate the evidence that it was at one time held in high repute by the medical profession. An interesting point is the fact that in country districts it is still valued by agricultural and other workers on account of its curative properties. When visiting a farm at Tarvin in Cheshire many years ago, I was interested to find that its owner always kept a bed of Comfrey in order that he might provide villagers with it when occasions arose. Dr. Walter Moore, of Bourton-on-the-Water, has recently informed me that, within his recollection, plots of it were grown as a food for cattle, on account of its reputation for producing milk, rich both in quality and quantity. Another interesting Gloucestershire reference is contained in a letter published by Mr. Edwin Green of Cheltenham[1] in 1912 in which he wrote that in Gloucestershire this plant when cooked in the same way as spinach is used very largely by many people during the spring. He stated that it is well known there as a blood purifier not only for human beings but also for cattle and horses, the effect on the latter being to produce a wonderful glossy coat. He knew a gentleman near Cheltenham who had a field of 4 acres in which nothing but this plant was grown. He gave it not only to his horses but was never without a dish of it, when in season, instead of spinach.

One more point before leaving the history of this interesting plant. Dr. Edward Nicholson, of Neuilly (Seine), who furnished numerous references when I was first working on this subject, stated in one of

[1] *Daily Mail*, March 27, 1912.

his letters (Nov. 15, 1911): "I am quite at one with you in the examination of forgotten 'Worts.' Certainly until the manufacture of the new class of synthetic medicines prevailed, one could count on one's fingers medicines that had not been discovered by old women or savages, and now one finds that some of these synthetic remedies are scientific imitations (however unconsciously) of the 'principles' contained of the old Herbals."

No doubt in the past, as in the present, the introduction of new methods of treatment led to the disuse of old ones. Fashions in medicines changed then as now, and many really valuable natural remedies belonging to the periods of rational empiricism were left behind and forgotten or relegated to the limbo of lost reputations.

I myself have heard Comfrey spoken of as an "old woman's remedy," and I admit it in the sense that it probably dates from the time when woman was the Priestess of Medicine. I referred to this in a paper on "The Psychology of Nursing," written for the *Cripples Journal* in 1928, in which I stated that "Nursing is a profession which pre-eminently belongs to woman, and the psychology of the nurse is essentially that of woman, who throughout the ages has been regarded as the possessor of inborn gifts and attributes which render her the natural tender of the weak and sick and of the maimed and hurt . . . this is related to the maternal instincts which are present, although they may be dormant in a greater or lesser degree, in every woman; instincts concerned not only with the propagation of the race but with its preservation. The experiences which woman acquired in prehistoric times regarding the care of her offspring during childhood and beyond was probably associated with some knowledge which would be handed on from mother

to daughter. So it would happen that adults appealed to woman as to a mother when smitten with illness." She was the Witch or Wise Woman of those days—and it is within the realms of possibility that Comfrey was among the "simples" employed by her, and may truly be called an old or ancient "woman's remedy."

Some idea of the traditional therapeutic virtues of Comfrey may be gathered from the names by which it was popularly known. For instance Knitbacke (Gerrard 1597), Comfort Knitbene (Scotland). In Aberdeen it was called Comfer Knitbeen, and a preparation made by boiling the root in oil or lard was extolled by old women for hardening and strengthening fractures. This property also accounted for its being called Bone-set or Knit Bone in Lancashire. It appears to have been used both internally and externally in fractures in all districts.

A rather amusing letter received from a doctor in Lancashire in 1912 illustrates the faith of the people in Comfrey as a healer. He wrote: "Three years ago I was called to see a girl with gastric ulcer, hæmatemesis and severe vomiting and treated the case in the usual orthodox manner. In three weeks the patient was able to return to the mill. When congratulating the mother on her daughter's speedy recovery the old woman said to me:

"'Do you mind my telling you something Doctor?' On my replying in the negative—

"'Well,' she said, 'my girl has never had a drop of your medicine and all she has supped is pints of strong Comfrey tea.'

"Since this occasion I have found it an excellent sedative for the gastric mucous membrane."

CLINICAL OBSERVATIONS WITH COMFREY

HAVING become acquainted with the history of the plant and the kinds of maladies for the treatment of which preparations made from it had been employed, the next procedure was to try it out and discover whether it possessed any of the therapeutic properties ascribed to it. Professor Harvey Gibson had obtained a large quantity of the ground and unground rhizome, some of which was handed to Dr. Titherley, then head of the Organic Chemistry Department, University of Liverpool, and Mr. Norman Coppin, who was working in the laboratory of Professor Benjamin Moore (Biochemistry), and while they were making a careful chemical investigation of it, which took a considerable time, I proceeded to dress the only ulcer which was then available with a strong infusion made from the powdered root.

This case was an exceedingly unpromising one because it was a "rodent" of about two years' duration, not a simple ulcer.

The patient was a woman aged 87, and it seemed a suitable case for observation following the history of the malignant growth recorded by Professor Thompson which originated this research.

It was a stroke of fortune that this case was the first one to be experimented upon because, having resisted all kinds of previous treatment, the marked epithelial growth which took place upon it seemed more likely to be the result of a specific action of the application than would have been the case in an ordinary varicose ulcer for instance.

It was a very large ulcer (measuring 4 by 3 inches) involving the skin and deeper structures over the upper thorax and it was slowly spreading, more especially by its upper margin which was high and undermined; the other margins were less raised. The base was irregular and there was some sero-purulent discharge. After being dressed with the mucilaginous infusion for about a week the surface cleaned and a distinct ingrowth of epithelium could be seen taking place from some of the marginal points. Later on the upper margin flattened somewhat on its inner aspect, the undermining vanished, and after growing here and breaking down there for a time the epithelium became stronger and closed in to a considerable extent.

By this time Dr. Titherley reported to me that he had obtained a definite, so-far unidentified, crystalline body from the root and he was able to give me sufficient of this to experiment with. Since it was very sparingly soluble in water, quite a large amount of solution was made which was now used as a dressing for the ulcer instead of the infusion. With this application the skinning over process took place more rapidly and in the course of a month was all but completed. Unfortunately the taking of a photograph was put off in the hope of getting a picture of the completely covered ulcer.

It was never obtained however because through the perverseness of fortune the aged patient contracted influenza, which was epidemic in the Institution at the time and she died of bronchitis. As a matter of fact this was the only ulcer initially treated with the infusion of Comfrey root alone, and the fact that epithelial growth was further hastened after dressing it with a solution of the crystalline body suggested that this might be the active principle which promoted healing.

That infusions of the root are very active, however, was indicated by descriptions of cases treated with it which came from a variety of sources. One of the most striking was published in the *British Medical Journal* of June 8, 1912, by Dr. Charles Searle of Cambridge. The case had the following history :—

The patient was a man aged 83, first seen on October 23, 1911. He suffered from shortness of breath, and swelling of the legs on which were some ulcers due to neglect. For some months his condition was very grave; he had marked arteriosclerosis, a loud aortic systolic murmur, with a feeble pulse and low temperature. The urine contained blood, albumin, and casts, but no sugar.

During December, 1911, a fungating ulcer appeared on the dorsum of the left foot. It rapidly spread, and eventually exposed the metatarsal bones. In January, 1912, the patient's condition appeared to be hopeless, he became at times delirious, and was removed home to die. He was then treated with four-hourly fomentations made with decoction of Comfrey root. The ulcer immediately began to fill up rapidly and was practically healed by the end of April, and the patient's condition made corresponding improvement.

Several medical men were kind enough to confirm my observations from their own experiences with infusions of Comfrey for the treatment of ulcers, but further reference to this must be deferred until I come to speak of allantoin which proved to be the actual crystalline body extracted from the root.

I must state at this point that neither Comfrey nor its constituent is a specific for rodent ulcers. The case of rodent ulcer recorded which raised considerable hopes, naturally led to others being similarly dealt with, but without convincing results.

THE CHEMICAL RESEARCH

BEFORE proceeding to describe the clinical and other observations which led to the conclusion that allantoin has to do with cell growth, it may be as well to give a short account of the result of the analysis whereby this substance was isolated from Comfrey, and of some of its chemical characteristics. In his preliminary examination Dr. Titherley established the facts that the root contained :—

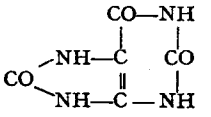
(1) Gums.
(2) Sugars, including a reducing sugar.
(3) Resins.
(4) A protocatechuic derivative or derivatives.
(5) A substance giving an intense yellow solution with sodium hydrate (not investigated further).
(6) A crystalline solid, which was isolated in a pure condition. It was very rich in nitrogen and melted at 226° C. Since from clinical observations this latter body appeared to be the physiologically active constituent of the root, Mr. Coppin was asked to devote his attention to its investigation. Ultimately Dr. Titherley and he found that the root contained about 0·8 per cent. of this crystalline substance, and by accurate determination of its carbon, hydrogen, and nitrogen contents showed that it possessed the same empirical formula as allantoin, which it greatly resembled in its chemical properties.

Allantoin

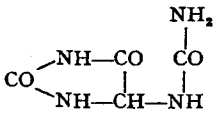
Some allantoin was now prepared from uric acid, and the product from the root was proved conclusively to be an identical substance by chemical methods. For example, allantoic acid and other derivatives were prepared both from the chemically

made allantoin and from that obtained from the root ; their melting points were the same, and so forth.

Allantoin ($C_4H_6N_4O_3$) is a compound which is obtained by the alkaline oxidation of uric acid in the cold.



Uric acid



Allantoin

It is a white crystalline substance, melting at about 226° C., with decomposition. It is only slightly soluble in cold water (0·6 per cent.), but readily in hot water. It is rather more soluble in cold alcohol, but it is quite insoluble in ether. Dry allantoin is quite stable, but if boiled with water for a considerable time it undoubtedly undergoes decomposition to some extent. It is decomposed by alkali, giving a variety of products, the nature of which entirely depend upon the conditions under which the experiment is carried out. It will be seen from Dr. Titherley's description of the chemical constitution of allantoin (p. 54) that he gives it two distinct chemical formulæ : one a single five-atom ring (mono-cyclic) with ureide side chain, the other a double (bi-cyclic) five-atom ring constitution without side chain, and he shows that one form passes into the other by the mere wandering of a hydrogen atom. Quoting his words from a letter May 3, 1933, descriptive of these peculiarities he says : " One of these forms (the double cyclic one) I call pseudo-allantoin, and I think, though it has never been proved, that it is soluble in water. At all events in one of the synthetic experiments I got a soluble form which on standing passes over into the ordinary familiar crystals. This soluble form

is too unstable to keep. I mention these facts to illustrate what I mean by saying that allantoin is a peculiar substance, and I believe that Nature somehow utilizes those peculiarities in cell metabolism."

It may be well at this point to refer to the fact that when carrying out some experiments concerning the action of allantoin on leucocytes during two periods separated by an interval of several years, I got such a diversity of results that I wrote to Dr. Titherley in 1933 suggesting a possible instability in the allantoin crystals which might be brought about by gradual decomposition. I was led to suspect this because the diversity disappeared on using freshly prepared crystals. In his reply Dr. Titherley referred to the tautomeric properties of the substance, and its peculiarity in undergoing the changes in chemical constitution, above referred to, probably due to this tautomerism. He did not think it should alter its therapeutic behaviour, however, since an equilibrium would be set up between the two forms in solution, but he considered it conceivable that this equilibrium is only slowly obtained by pure water, and perhaps more quickly if a trace of alkali or acid is present. He knew nothing on this point about allantoin, but it certainly occurs in other organic tautomeric compounds where the rate of attainment in equilibrium solution is enormously dependent on traces of other substances which accelerate the change by catalysis.

This is more or less by the way, but it has been thought well to mention it as pointing to the necessity for dissolving the crystals in distilled water. This precaution was observed in the first (original) set of experiments, but in the later one which gave such different results tap water was almost certainly used by an independent observer.

THE CHEMICAL PHYSIOLOGY OF ALLANTOIN

REGARDING the chemical physiology of allantoin, or the part which it plays in human metabolism, very little is known, but some interesting facts have been recorded which may throw light upon its action as a cell-proliferant, and may point to its having a function to perform in the bodily chemistry apart from any possibility of its being a product of purine metabolism, in which light it has generally been regarded. It is present both in animals and in plants. It was first discovered in the former, and received its name from the fact that it was found in the fœtal allantoic fluid. Later on it was identified in the urine of pregnant women. Its presence has been demonstrated in very small amounts as a normal constituent of the urines of healthy people; but that it is not an end-product of human metabolism—which means that it is probably not derived from the oxidation of uric acid, to which it is closely allied—has been shown by a good many observers, as pointed out by Dr. Ackroyd in a paper published in the *Biochemical Journal* for March, 1911. This is an important observation, and is based upon the works of Schittenhelm, Wiener, Minkowski, Poduschka, and Wiechowski, who have shown that when allantoin is given to man it can be recovered to a considerable extent in the urine; and the author himself concludes "that the whole quantity of allantoin excreted by man on a milk and vegetable diet may be derived directly from that contained in the food." You will note that I am speaking of the relation of

allantoin to human metabolism only. In the dog, cat and rabbit it appears to be a normal end-product of metabolism, and experiments made upon them seem to show that the giving of foods rich in purines, such as thymus and pancreas, gives rise in the two former animals to increased allantoin elimination; and in the latter animal the addition of sodium nucleate alone and sodium nucleate together with uric acid to the fodder, resulted in almost all the purine products being excreted in the allantoic fraction of the urine.[1] Furthermore, the same observer noted that the intravenous injection of nucleic acid caused an increase only in purine bases and allantoin, and that the ingestion of allantoin itself caused in these animals a marked increase in the total nitrogen excreted; the allantoin excreted being nearly double the amount that was ingested. The difference between the metabolism of these animals and that of man is well shown by Wiechowski, who found that, given subcutaneously, uric acid appears mostly as allantoin in the urines of the dog and rabbit, whereas in human urine about 90 per cent. is excreted unaltered.[2]

If these facts are reliable, the allantoin in human urine is mainly derived from vegetable foods and from milk, which Dr. Ackroyd found to contain about 0·019 grm. per litre, and in the healthy individual it appears to pass practically unchanged through the economy, the amount eliminated representing the amount which had been ingested.

In this relation, however, its tautomeric qualities and the influences of catalysis already referred to should be remembered.

[1] Schittenhelm and Seisser, *Zeitschr. Expt. Path. Ther.*, 1909, vii, 116-133.

[2] Wiechowski, *Biochem. Zeitschr.*, 1910, xxv, 431-459.

DISTRIBUTION OF ALLANTOIN IN THE VEGETABLE KINGDOM

OUR knowledge of this is not very extensive but it is a very suggestive fact that such analyses as have been made indicate that it is generally found in parts which are related to growth, either active or potential. We have a striking example of this in the Comfrey where it is present in the roots and terminal buds. The leaves have not yet been thoroughly investigated, but the interesting point is the large amount of allantoin in the rhizome, greater than in any plants heretofore investigated. E. Schulze and J. Barbieri[1] in 1881-82 found it in the buds of certain plants and in the bark of branches of trees and Ackroyd refers to its identification by Richardson Crampton (1886) in the embryos of wheat separated in the process of milling and in beet juice. Ackroyd[2] himself demonstrated its presence in bread, french beans and green peas, whereas it is absent in bananas and rhubarb. This regional location of allantoin in plants is interesting when regarded in conjunction with the fact that it is a characteristic component of the fœtal allantoic secretion, and related therefore to an important structure connected with the fœtal circulation, a structure along which the vessels pass which convey the fœtal blood to and from its intimate relationship with the maternal blood.

Whenever we find any substances constantly in certain parts of plants or animals, it is quite reasonable to suppose that they may be in some way related

[1] *Journ. of Prak. Chem.* [2] xxv, 147.

[2] *Biochem. Journ.*, v, 403.

to and necessary for the particular tissues which have selected them; and although the allantoin in the human embryo might be regarded as a fœtal waste product, I think we may feel justified in assuming that, in the economics of Nature, it has a function to perform, perhaps in relation to cell-multiplication, especially as it is not at once eliminated through the maternal circulation, and the same suggestion comes from the fact that it is also present in milk, the food of the rapidly growing young organism, and in those parts of some plants in which active cell-multiplication takes place.

Bearing on this comes a very interesting analogy between the presence of allantoin in the fœtal allantois and in the root of the Comfrey plant. In the earliest months of pregnancy, dating from the third week onwards, the allantois becomes relatively large, and the amount of allantoin contained in it corresponds to some extent with the size of the sack. The vessels of the chorion conveying the maternal blood to the fœtus pass through the allantois and probably derive the allantoin from it, to be utilized in the metabolism connected with growth and development. As pregnancy advances the allantois diminishes in size and at length, shortly before the child is born, it becomes vestigial and the amount of allantoin infinitesimal. Compare this with the Comfrey rhizome which in the earliest months of the year (January to March) contains from 0·6 to 0·8 per cent. of allantoin. Analysed a couple of months later it contains about 0·4 per cent. In July the amount is still further diminished and when the plant is in full growth practically none is to be found in the rhizome but it is discoverable in the terminal buds, leaves, and young shoots. This important fact may be regarded as evidence that the plant withdraws allantoin from

its storehouse in the rhizome and utilizes it for purposes of cell-proliferation. In continuation of the analogy above referred to it is interesting that the maternal milk contains about 0·006 per cent. of allantoin, a fact that may have something to do with the further growth of the child after birth.

EXPERIMENTS WITH PLANTS

THE presence of allantoin in the underground reserves and growing parts of plants, as well as its relation to the fœtus in the allantois and in milk—the food of the rapidly growing infant—added to the clinical evidence that it promotes healing in ulcerative conditions which will be referred to later, naturally led to the suggestion that experiments made upon plants might afford some information, confirmatory or otherwise, of its proliferative properties or functions. By my request Mr. Coppin planted a large number of hyacinth bulbs (the growth of which had been started in the dark) in solutions of allantoin varying from 0·1 to 0·5 per cent., and it was found that the growth of the roots was inhibited in a ratio proportionate to the amount of allantoin in the solution—that is, the stronger the solution the less was the amount of growth in the root.

Notwithstanding this diminished root growth, which was confirmed by microscopic examination, the flower stems of the plants grew in a stunted way, but none had blossomed at the end of twelve weeks, and it therefore appeared that allantoin did not promote cell-growth when added to the water in which the bulbs were growing, in fact their growth appeared to be retarded. This was disappointing, but an interesting development now ensued. It seemed certain that in plants the allantoin or other proliferative agent is stored for use in the rhizomes, buds, barks and germinal parts, and is probably elaborated there as the result of some metabolic process. There is no allantoin normally in the water or earth in which bulbs are

grown, but it or some kindred substance is evidently formed and stored in the bulb or, in the case of other plants, in the rhizomes or roots, to be drawn upon as required for advancing the processes of growth and development. This being the case, the suggestion naturally arose that one might try the effect of injecting a solution of allantoin into the bulbs, and a number of experiments were conducted on these lines, the first of which was made in a more or less casual way as follows :—

A child in the Liverpool Royal Southern Hospital had two earth-grown hyacinths in a pot. They had grown quite unequally, one having developed well with the commencement of a flower, the other being feeble, short and showing no signs of blossom.

About 15 minims of allantoin solution (0·4 per cent.) were injected on several occasions into the bulb of the latter, with the result that rapid growth ensued and it overgrew its more vigorous neighbour, and flowered before it (fig. 1).

This experiment excited the interest of the Sister of the ward (Miss Archer) and of the Matron (Miss Jolly), and they gave similar injections into the bulbs of a considerable number of plants in about equal stages of growth. Controls were used, some being injected with quantities of water equal in amount to the quantities of allantoin solution given, others being grown without any injections ; these experiments were conducted in earth-grown bulbs. In addition, some hyacinth bulbs which had just started growth, the shoots being about $\frac{1}{4}$in. high, were planted in water, and some of them were injected every third or fourth day with allantoin solution, some with water only, the remainder being controls, and the same results were obtained in every case. The allantoin evidently acted as a cell-proliferant, forcing the growth of the shoots,

and especially of the flowers (for it was noticeable that the plant often blossomed with comparatively little leaf-growth), commonly before their untreated neighbours had reached anything like the stage of flower production. The water-injected plants grew better than the uninjected ones, but nothing like so rapidly as those to which allantoin had been added.

These experiments were repeated many times with tulips (figs. 2, 3 and 4), lilies of the valley and other plants, care being taken to ensure that they were planted at the same time, in the same tubful of earth, and under the same conditions of temperature and surroundings. These results have been confirmed many times, and in the cases of non-bulbous plants the injection of allantoin solution into or below the flower-buds or into the bases of individual spikes has resulted in the production of large flowers contrasting strongly with other untreated flowers or spikes on the same plant (figs. 5 and 6).

In the water-grown plants which were injected it was noted that with the increased growth of the shoots there was sometimes a diminished growth of the roots, which were considerably longer and stronger in the controls.

This led to the impression that perhaps the roots were not so necessary to the plant if its water as well as the proliferant content were kept high, and in order to test this point a number of hyacinth bulbs were selected which had shoots $\frac{1}{4}$ in. high but no visible roots. Some of these were injected with allantoin solution, some with water, others being uninjected, and they were simply placed upon a plate without earth or water. At the end of seven or eight days the little shoots of the treated plants were opening out into leaves. The water-injected bulbs also lived, but did not grow so well, whereas the shoots of the controls browned at the tips and

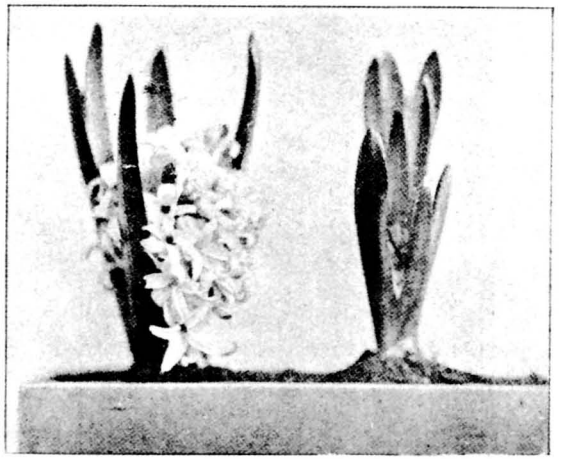


FIG. 1.

Hyacinths.—The one on the left was injected with allantoin. It was less in height and feebler than its neighbour on the right at the commencement of the experiment.

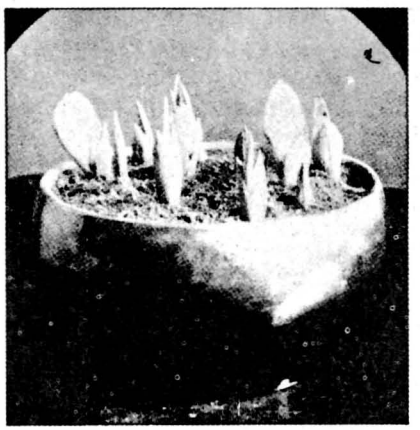


FIG. 2.

A bowl of tulips.—A central line of the bulbs were injected with allantoin. The marginal ones were not injected. Figs. 2, 3 and 4 show the stages in growth in the two sets.

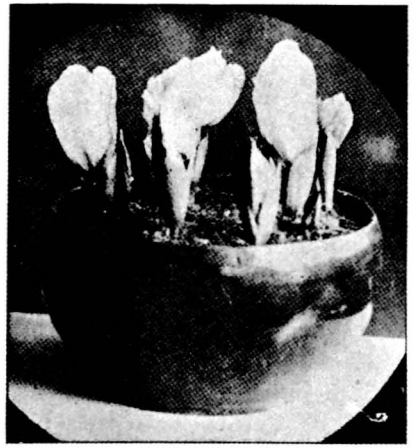


FIG. 3.

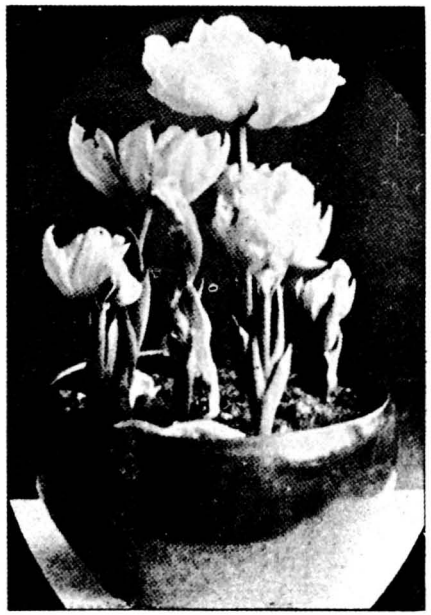


FIG. 4.

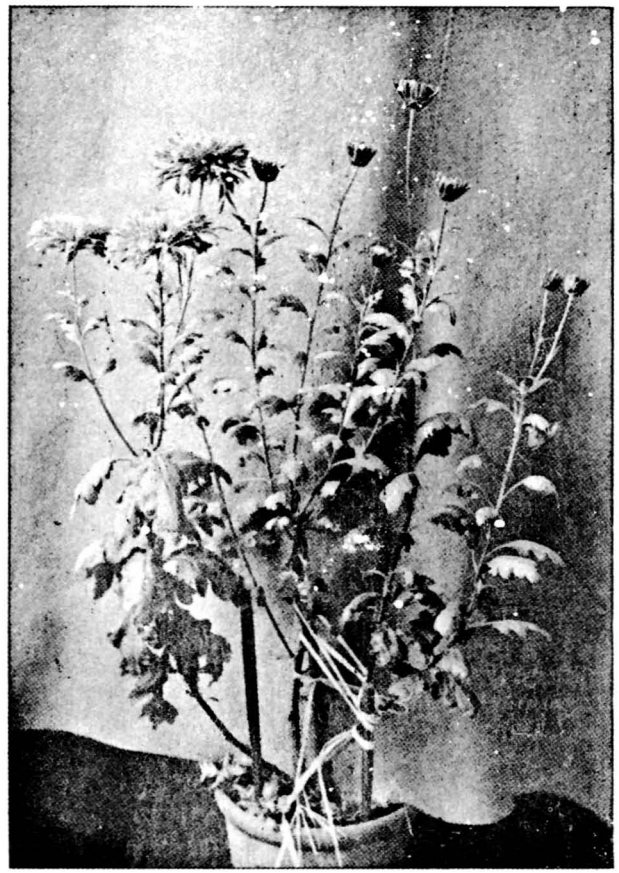


FIG. 5.

Chrysanthemum which was injected on several occasions at the base of the spike of flowers on the left. Showing the advanced inflorescence compared with the portions of the plant which are undergoing normal growth.

FIG. 6.

In this chrysanthemum the injections were given into the flower bud of the large bloom when it was at the stage of the unexpanded flower on the right. There was no nipping of the young flower buds to promote large blooms.

shrivelled up. Not a sign of roots made their appearance in any of these plants.

From these experiments one gathers the impression that allantoin is a substance which is capable of being utilized by vegetable cells in connection with their proliferative processes, just as there seems to be proof that it has proliferative properties in connection with certain animal cells, if one may judge by the way in which it promotes healing in chronic and acute ulcerative conditions which will be referred to later on. In both cases the reproduced cells are normal microscopically and resemble those from which they took their origin.

In connection with the injection of bulbs a medical man wrote to me many years ago explaining that in repeating my experiments his results were contradictory to those which I had obtained. His plants became stunted and growth was interfered with. On asking for details concerning his experiment it transpired that he had injected his bulbs with an infusion of Comfrey which he had prepared by prolonged boiling of the roots. It is quite probable that the contained allantoin would be decomposed by the boiling, but there is also a possibility that in the root there may be a controlling substance uninfluenced by heat which retards growth. A research concerning this possibility was not proceeded with.

CLINICAL OBSERVATIONS

THE original clinical observations made with allantoin were chiefly conducted in cases of superficial ulcers of various kinds, in order that the effects on the growth of epithelium might be carefully watched. In the selection of cases, great care was exercised in order to make certain that other conditions which would ordinarily promote healing were not alone operating and bringing about or starting cell-proliferation. On this account an endeavour was made to safeguard observations in the first place by dealing only with those ulcers which had refused to heal under ordinary conditions of rest and cleanliness, together with other forms of treatment, and in the second place a good many medical men were provided with fresh solutions of allantoin wherewith to treat their cases in order that our own results might be confirmed or tempered by theirs. One is rather apt, when experimenting with a substance in this way, to allow optimism to exaggerate the resultant benefits, and it is an advantage to get others to form independent judgments concerning them from their own observations.

After publishing some of these results, numerous applications for supplies of allantoin came from medical men, not only in this country but also from Canada and other Dominions, wherewith to treat cases under their immediate care, and many of them were good enough to record their results, some of which, being of great interest, will be referred to.

I was very fortunate in having surgical colleagues who were interested in this research, and numerous cases were placed at my disposal in the wards of the hospitals.

A striking case was that of a woman, aged 48, who was transferred to me by Mr. Douglas Crawford on July 20, 1911. There was a large ulcer on the dorsum of the foot and another, practically continuous with it, over the lower third of the leg. The bases were in places sloughy and even gangrenous looking, and there was a purulent discharge. She was sent to Mr. Crawford, I understood, for his opinion as to whether the leg should be amputated. The ulcer measured 5 in. by 4 in., and had been in existence for five years. Allantoin dressings were commenced on July 25. A week later the surface had cleaned and presented healthy granulations, and a rapid growth of epithelium was taking place from all the margins. On August 12 it was manifestly healing, and on August 17, i.e., in twenty-three days, this huge ulcer was reduced to the size of a pin's head. The scar was healthy and sound. The patient was kept in bed for a fortnight, and after her discharge it remained sound and well.

Another class of ulcer which quickly responded to allantoin includes those which are apt to occur in paralytic cases. We had an example of this in a girl, aged 11 years, who had a large ulcer on the dorsum of her left foot. The skin had broken down rapidly, leaving a deep circular ulcer about the size of a penny.

Since it showed no signs of healing at the end of five weeks the late Sir Robert Jones invited me to dress it with allantoin. This treatment commenced on March 10, 1911. The epithelium rapidly grew in, and in four weeks it was entirely healed.

Another case referred by Sir Robert Jones was that of a young woman who had circular paralytic ulcers on each leg. These were examples of what used to be called weak ulcers in my student days, and they had remained practically unchanged, one

of them for five months and the other for eight months, in spite of the fact that the patient had been recumbent for several weeks. On May 2 they were dressed with allantoin; on the 17th the ulcer on the right leg was skinned over and those on the left one healed soon afterwards. The epithelial coverings in this case were thin, and the cicatrices soft and flat. She was one of those patients with flail legs, the surface cold and muscles atonic, and one was not surprised to hear that one of the ulcers subsequently recurred.

I might quote many cases of various kinds which clearly confirmed the cell-proliferative qualities of allantoin. Among these were several cases of varicose ulcers, but it was found that burns and scalds of the lesser degrees were very useful fields for observation. This was because islets of epithelium, many of them at first invisible to the naked eye, formed centres from which new epithelial growths could be seen spreading from day to day with remarkable rapidity. In a letter to the *British Medical Journal* (January 13, 1912) Mr. R. W. Murray (Hon. Surgeon Liverpool Northern Hospital) confirmed the value of allantoin as a cell-proliferant as follows: "I can confirm Dr. Macalister's remarks upon the value of allantoin as a cell proliferant. Towards the end of last year there was an explosion at works in the neighbourhood of the hospital and we were called upon to treat a large number of men who were severely burnt on the hands, forearms, and face. The burns were mostly of the second or third degree, and for about a week they were dressed with gauze soaked either in a solution of picric acid or in a solution of iodine.

"Dr. Macalister asked me to try dressing them with allantoin, and kindly provided us with a

quantity of it. In the first instance it was tried on two or three cases only, but the results were so satisfactory and so convincing to house-surgeons, dressers, and nurses, that dressing with allantoin solution soon became general. It not only stimulates epithelial growth, but 'cleans up' sloughing surfaces in a most remarkable fashion. When nurses and house-surgeons are really keen about any particular line of treatment there is generally something in it. This has certainly been our experience with allantoin."

Mr. Murray's statement that allantoin cleans up sloughing surfaces applies not only to burns but to ulcers generally, and the question arose as to whether it possessed any antiseptic properties. This cleaning-up process is of course essential if healing is to occur, because healthy cell-proliferation will not take place over septic surfaces, and it was essential to determine whether the allantoin was primarily destructive to micro-organism or promoted this effect by establishing physiological conditions in the cells and their surroundings which rendered them immune to micro-organisms. Some simple experiments had previously demonstrated that, far from being a poisonous antiseptic, it possesses no toxic qualities, and rather favours than inhibits culture growths of organisms. In August, 1911, during a cruise to the Shetlands in the late Professor Sir William Herdman's yacht "The Runa," I was able to make a number of observations concerning the effects which followed the placing of samples of the plankton gatherings (which were constantly available during tow-netting operations) in sea-water containing varying amounts of allantoin. The object of this was to discover whether the allantoin was destructive to the small and simple forms of animal and vegetable life which are found in the

plankton. Owing to the active way in which they move about, the most easily observed creatures were the copepoda, but there were many other forms which could easily be watched with the naked eye. An excess of allantoin was placed in a quantity of sea-water and thoroughly shaken up, so as to make as saturated a solution as possible, and of this quantities were added to newly drawn sea-water in proportions varying from 1 : 8, 1 : 7, and so forth up to saturated strength, controls of pure sea-water being employed in every series of experiments. The net result of a considerable number of observations was that the animalculæ observed lived for nearly as long a time (about two days) in the saturated and other solutions as they did in the controls, and from this it appeared that allantoin was not appreciably inimical to the forms of life which were experimented upon.

On my return to Liverpool Dr. Alfred Adams, working in the Biochemical Laboratories of the University, made some experiments with various culture media, containing 0·2 per cent. of allantoin, and on November 1, 1911, he reported that the *Bacillus coli*, Staphylococci, Streptococci and Tubercle Bacilli were not retarded in their growth and multiplication by it.

Allantoin, then, is not an antiseptic in the usual acceptation of the term, and its action in this respect must depend upon some influence brought to bear upon the cells, whereby their resistance, stability and immunity are established and their proliferation promoted.

It has been explained that sundry independent workers had reported their experiences, and before going further one or two of these may be mentioned.

In February, 1912, Dr. Mackenzie Forbes of Montreal was supplied with allantoin wherewith

to treat a case of senile gangrene in the heel of an aged colleague who had been glycosuric for some years and had sclerosed vessels. He had been in bed for five weeks suffering much pain and the slough had not separated. The necrosed area was described as being over an inch in diameter and from half to three-quarters of an inch deep, this measurement being due to infiltration of the surrounding tissues. The necrosis extended down to the os calcis; it was not thought, however, that the periosteum had been denuded. The allantoin was at once applied on its arrival in 0·5 per cent. solution, and immediately promoted cleaning up, separation of the slough and much relief from pain. The report stated that there was a continuous improvement after the new dressing was employed, and the cavity ultimately closed with a stellate scar.

The late Dr. Arthur Wallace (Surgeon to the Liverpool Women's Hospital), reported the closure of an intractable biliary fistula following operation for gall-stones. Ten days after the first dressing with allantoin solution, applied by means of a gauze wick, bile ceased to escape and the sinus steadily healed. He reported another case of local necrosis following a pressure sore and others in which drainage tube tracks had been slow in healing which were greatly benefited by treatment on similar lines.

An ophthalmic surgeon employed allantoin dressings for a patient having a very extensive and deep burn of the eyeball and lids, due to molten copper, and expressed himself astonished at the result. He wrote: "The disappearance of the chemosis, the firm healing of the deeper layers and the formation of new tissue have been most marked and have even astonished the nurses who know nothing about the stuff."

There were many other writers on the subject but it would only labour the point to refer to their comments and expressions concerning the effects produced in the way of cell-proliferation.

Just a few words may be permitted concerning the use of allantoin in internal ulcerative conditions where no ocular demonstrations of the healing processes have been possible. Comfrey was much used by medical men in the seventeenth and eighteenth centuries for those " Bursten Within," i.e. in cases of hæmatemesis and hæmoptysis. They had much faith in it. Was this justified ? No doubt much would depend on the natures of the diseases concerned. Some would probably be ulcers in the stomach and duodenum, others were possibly malignant growths. In chest cases they evidently treated phthisical hæmorrhages with it.

In the course of the past twenty years there have been many opportunities for testing the situation, and one can only infer that whereas there are cases of internal ulcerative conditions in which improvement has been noted, there have been a good many in which the results were doubtful. One can only speak with any degree of certainty when diagnosis has been clear and where beneficial results have not followed other forms of medicinal treatment. One such experience occurred in the case of an elderly man who after suffering pain after food, vomiting of blood, melæna, and other clinical evidences of ulceration which were unrelieved by medicinal treatment, finally submitted to operation. He nearly died on the table after the stomach, having a very evident ulcer in it, had been exposed. The operation had to be abandoned and so far as local conditions were concerned he remained *in statu quo ante*. He was then treated with allantoin dissolved in a Comfrey infusion and a purely milk diet was

prescribed, with the result that he was free from signs and symptoms in a month and is still alive at 90 odd years of age.

There have been good results and uncertain ones in cases of gastric and duodenal ulcers, but my general impression is that allantoin and Comfrey are useful adjuncts to general dietetic treatment.

The following extreme case suggests that allantoin may find useful application in gastric and other internal ulcerations :—

On February 17, 1911, a woman, aged 47, was admitted into my ward in an extremely debilitated condition in consequence of a severe attack of hæmatemesis. For many weeks previously she had suffered from pain after food, and had vomited persistently for a fortnight prior to her admission. Even water caused pain and was immediately rejected. There had been melæna for some time prior to the attack of hæmatemesis for which she was immediately admitted. For twelve months she had been conscious of pain on pressure over the abdomen, and had noticed a lump in the epigastrium and extending into the right hypochondrium. This woman was so feeble that the ward sister said that she thought it was a pity that a patient so ill and advanced in disease should be sent into the hospital. I felt disposed to agree with this opinion, because on examining her abdomen I found a tumour just underneath the ribs on the right side. It was rounded, irregular, and intensely painful, and my feeling at the time was that she had a carcinoma involving the stomach, and this opinion was strengthened by the fact that she had an undoubted carcinoma of the breast. When the irritability of the stomach subsided she was given the mucilaginous infusion of Comfrey root reinforced with some of the saturated solution of allantoin. In addition

to the improvement which took place in her stomach symptoms, in the course of a month the abdominal tumour disappeared, an area remaining, however, which was extremely painful on pressure ; but in time this also disappeared, and what I had taken to be a malignant growth had vanished.

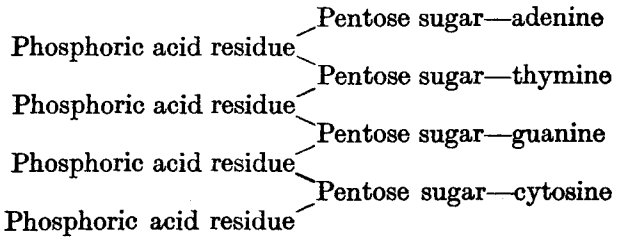
I have treated other cases of gastric and duodenal ulcers in the same way, and have reason to believe that it is a helpful measure for them.

RELATION OF ALLANTOIN AS A CELL-PROLIFERANT IN CASES OF MALIGNANCY

HAVING demonstrated that allantoin is a substance capable of being utilized both by vegetable and animal cells in connection with their proliferative processes, the next point to determine was whether it produced any influence upon malignant cell-growth. A good many cases have been recorded where cancerous, or sarcomatous growths are reputed to have been benefited by treatment with Comfrey. They are mentioned among the " vertues " in some of the old Herbals. We have a modern example in the case referred to by Professor William Thompson (p. 1), and there have been others which led to our trying the effect of Comfrey and allantoin when applied locally in cases of advanced and ulcerating cancerous growths. Except in the case of rodent ulcer referred to on p. 11, no definite signs of improvement were noted. Whether consequent mitotic changes took place was not determined, and so far as at present observed it can only be stated that Comfrey and allantoin do not appear to have any capacity for producing a somatic cell from a malignant one, or a carcinomatous or sarcomatous cell from a somatic one, and no reasonable explanation can be afforded for the occasional cures which have followed their employment.

From its influences on normal cells it can only be inferred that since allantoin leads to their proliferation it may possibly have something to do with the activities of nucleic acid. If this is the

case a study of the structure and building-up of nucleic acid may in the future prove to be of some importance. It was at one time believed that it consisted of four metaphosphoric acid residues, each linked to a pentose molecule (the sugar ribose) and to one molecule respectively of the purine bases adenine and guanine, and one each of the pyrimidine derivatives thymine and cytosine. It is to the two purine bases that allantoin may be indirectly related, since they both yield uric acid in the course of their chemical metabolism, whilst uric acid when suitably oxidized yields allantoin. Nucleic acid and sodium nucleate have for some time been known to increase the count of white blood-corpuscles in cases of pneumonia, and it has been supposed that the indirect relationship of allantoin to nucleic acid may to some extent be responsible for this. Dr. Titherley informs me that it is now generally believed that the phosphoric acid residue in nucleic acid is that of common ortho-phosphoric acid and that Levene now considers that the four nucleotides (phosphoric—pentose sugar—purine—pyrimidine) are linked through the pentose groupings thus :—



This is contrary to Kossel's formulation of the phosphoric acid residues as being linked together themselves, also each being independently linked to the pentose sugar. Another authority (W. Jones) does not link the phosphoric acid residues either in

the manner of Kossel or of Levene but links the pentose sugar residues together (not through phosphoric acid) and each independently to a phosphoric acid residue, leaving each phosphoric acid residue to contain two hydroxyl groups. Levene's formulation gives one hydroxyl group in three of the residues and two in the fourth.

It seems reasonable to suppose that cellular stability may depend upon the efficient building-up of nucleic acid, and as some of the experiments on plants have suggested, this probably results from a metabolic process in the cells. On this account there would appear to be little use in giving or applying nucleic acid (or nucleo-protein) when it is desired to build up that protein in the cells. It seems likely that they cannot make use of the fully formed material but require to assimilate the constituents required for cellular growth, structure and stability. On this account experiments might be directed towards the discovery of the effects upon cell growth of the various constituents of nucleic acid.

As previously stated, the connection between nucleic acid and allantoin is a very indirect one, and there is no real evidence that the latter builds up the adenine and guanine components of nucleic acid. At present this is in the realms of hypothesis.

The only substances which have been experimented with up to the present have been Meta- and Ortho-phosphoric acids which in dilute solutions ($\frac{1}{2}$ to 1 per cent.) have certainly been found useful in the treatment of old sinuses and abscess cavities which were showing little signs of closing. The results of washing them out with these solutions have proved very satisfactory.

Finally, a survey of the results of using decoctions of the *Symphytum officinale* and solutions of allantoin

in cases of alleged malignancy brings out the suggestive observation that nearly every case in which benefit seemed to ensue was treated either with the decoctions alone or sometimes with the addition of small amounts of allantoin. In Professor Thompson's case the patient inferred that the growth was dispersed by the local application of Comfrey poultices (p. 2). The case of rodent ulcer which improved so greatly (p. 11) was treated in the first place with Comfrey mucilage and afterwards with allantoin extracted from the rhizome. The woman suffering from an apparently malignant growth involving the stomach (p. 33) cleared up under the administration of a mucilaginous decoction of the rhizome reinforced with the synthetic allantoin.

These and other cases, not recorded in the text, lead to conjecture as to the possibility that the optically active *d*-allantoin (p. 57) in the Comfrey and other vegetable and animal structures may have biological influences on cells different from, or accessory to, those produced by the synthetic (racemic) product which, however, undoubtedly acts as a cell-proliferant and promotes healing as heretofore described. By analogy it may be surmised that natural products are superior to their synthetic isomers in their biochemical activities. Take for example the case of glucose of which the optically active *d*-form is an essential element in metabolism, whereas the synthetic *l*-glucose is not valuable as a foodstuff. The difference between the natural *d*-glucose and its image-isomer *l*-glucose is of the same type as that between the *d*- and *ι*-allantoins (i.e. in optical activity and asymmetric arrangement of atoms). In all chemical properties they are (in both cases) identical, yet biochemically the two forms of glucose are completely different and it seems possible that some difference in biochemical

activity may also exist in the case of the allantoins. Practical experience tells us that synthetic allantoin is an active agent, but the question arises as to whether the natural form found in the allantois, in milk, and in various vegetable structures may not possess virtues superior to those of its synthetic isomer.

For all we know allantoin may have some physiological association with other elements found in the animal or plant such as endocrines or vitamin B_1, which may influence its therapeutic properties. One striking point connected with its action is the relatively small amount required in solution to set going a local healing process, e.g. by application to an ulcer, or a multiplication of leucocytes in the blood which will be referred to presently, in this respect resembling a hormone.

This ends the primary investigation concerning the *Symphytum officinale* and its contained allantoin, much of which was published in the *British Medical Journal* (January 6 and September 21, 1912).

FURTHER OBSERVATIONS CONCERNING ALLANTOIN, 1914-1935

IN connection with the local application of allantoin to septic and sloughing surfaces attention has been directed to the "cleaning-up" process which takes place as a preliminary to, or concomitantly with, the ingrowth of epithelial cells from the margins of ulcers and from the islets of epithelium on the surfaces of burns. This was originally ascribed only to an increase in the vitality or resistance in the cells themselves, but the point arose as to whether this might not be brought about, in part at any rate, by some change in the cellular environment. That the cleaning-up did not result from a chemical antiseptic action was indicated by the absence of such properties in the allantoin itself, and by the circumstance that many of the ulcers had failed to clean up or heal when treated by various antiseptics before the solution of allantoin had been applied. The question thus arose as to whether this lessening or inhibition of sepsis resulted from a promotion of phagocytosis.

Some light was thrown on this by Drs. Albert Berthelot and D. M. Bertrand in a research carried out in the Pasteur Institute, the results of which were published in August, 1912.[1] This research was promoted by their notice of my publication in the *British Medical Journal*, January 6, 1912. It had occurred to them that allantoin might act by stimulation of phagocytosis, and they studied its action by experiment on guinea-pigs.

[1] *Comptes Rendus Hebdomadaires des Séances de la Société de Biologie*, lxxiii, 2nd Aug., 1912, p. 263.

Three cubic centimetres of a saturated solution of allantoin were injected into the peritoneal cavity of a guinea-pig. Eighteen hours afterwards they detected the presence of a light peritoneal exudate containing polynuclear cells. In order to obtain a more abundant leucocytosis 10 cgm. of finely pulverized allantoin were suspended in 5 c.c. of water, which after sterilization was injected into the peritoneal cavities of two animals, and twenty hours later a discharge of purulent liquid, composed entirely of absolutely aseptic polynuclear cells, was withdrawn by means of a fine pipette. They then injected similar allantoin suspensions into several other guinea-pigs, varying in weight from 350 to 400 grm., and twenty-four hours afterwards, having determined the presence of a very abundant peritoneal exudate, they injected into one of them a fatal dose of a young culture of cholera vibrio, and into the other a similar fatal dose of Eberth's bacillus. Control animals were injected at the same time with similar fatal doses.

All the control animals died in from twelve to nineteen hours, whereas the animals which had received the allantoin were unaffected and survived. The peritoneal exudate taken from these latter animals, twenty hours after the injection of the microbes, was sterile, and was composed of leucocytes, some few of which contained granules which might have been the débris of dead bacteria. From these experiments the authors concluded that allantoin was capable of strengthening the local resistance of the peritoneum against infection and of causing a considerable leucocytosis; and they suggested that the favourable action of allantoin was probably attributable, in part at all events, to this.

These experiments, and the observations made by clinicians concerning the cleaning-up of septic

surfaces, opened up a new field of inquiry, for it seemed possible that if the local application of allantoin promoted a multiplication of phagocytes, it might also, if introduced into the blood, set up a general leucocytosis, and that if this proved to be the case a controlling influence might be exerted on infective diseases, and especially perhaps in those types of cases where there is a controlled immunity. It is well known for instance that immunity from pneumonia is diminished when other diseases such as influenza (in which, by the way, there is a leucopœnia) lower the vital resistance. This resistance, strange to say, is diminished in some conditions which improve the general health. We seldom come across pneumonia among sailors or passengers when they are at sea. It is on arriving at the ports when their leucocytes are off guard that they are liable to become victims. Everybody recognizes how prone we are to get catarrhal colds after arrival in the towns from a sea voyage or from a holiday in a relatively germ-free atmosphere. This tendency to infection was recognized by the physicians of a past generation. I recollect the late Dr. William Carter about forty years ago warning me, on my suggestion of Spitzbergen for a holiday, that there was a risk of becoming infected with pneumonia on returning to my hospital work. At that time, however, I do not think that the function of the leucocytes as infection-preventers was much understood.

Pneumonia is to some extent an unsatisfactory disease on which to make unambiguous observations because severe cases often recover, whatever treatment may be adopted, and there is also the fact that the strain of pneumonia organism prevalent at one period may differ in virulence from that of another.

It so happened that the first suitable cases which came before me for observation were severe lobar pneumonias, and it will be best to record the history of this clinical investigation which was commenced in 1914, in order of sequence. One of the earliest cases treated made a somewhat unexpected recovery. The patient, a quartermaster on one of the liners, was desperately ill and his blood had a very low leucocyte count, only about 6,000 per c.mm., a point which always makes for a bad prognosis in the later stages of pneumonia. There was a possibility of there being a small amount of fluid in his chest, and thinking that its removal might help matters I needled the chest but got nothing but blood from the solidified lung. Without removing the needle I then slowly injected 2 c.c. of an 0·5 per cent. sterile solution of allantoin into the solid lung. Within two hours the leucocyte count had gone up to 15,000; later it was 20,000 and with this increment there was distinct improvement and a speedy crisis. A possible explanation, however, in a case of this kind is the fact that simple puncture of the lung will sometimes precipitate a crisis and in some chronic types will similarly promote resolution. I pointed this out long ago in a paper entitled "Puncture of the Lung in Chronic Pneumonic Conditions."[1]

In order to eliminate the possible influences of lung puncture, I proceeded to give my patients allantoin by hypodermic injection, and was encouraged to a certain extent by what appeared to be resultant benefits. For instance, in the case of a man, aged 23, whose leucocyte count in the later stage of the disease was 9,000 per c.mm., 2 c.c. of the 0·5 per cent. solution of allantoin was given hypodermically. In two hours the count had

[1] Published in the *Liverpool Med. Chir. Journ.* of March, 1901.

risen to 12,000 and after another injection it went up to about 20,000 and remained high until the crisis occurred. Such cases were encouraging but there were admittedly a considerable number of failures and one could by no means speak with certainty as to what might accrue from this method of treatment.

The point was that at that period I was only using allantoin as a kind of *dernier ressort* in the later stages of grave or apparently hopeless cases presenting indications of serious toxæmia. Many of these cases already exhibited a considerable leucocytosis which was not materially influenced by allantoin, and the unsatisfactory results were due to cardiac failure brought about by the toxæmia. In consequence of this the use of allantoin was abandoned in these grave cases; the fact remained, however, that it had conferred benefit in some cases presenting a low count, and this led to an entire change of procedure which resulted in a very different course of events. The allantoin was now given in the earliest instead of the later stages of the disease, and for the efficient clinical observations of these cases I am greatly indebted to Dr. Edward Blair Wilkinson, who was my Resident at the time, and to my student Mr. N. Green, who took a great interest in the subject and read his thesis for the Cambridge degree on it.

Allantoin and Leucocytosis.

The first experiments were carried out in normal healthy individuals, to whom a grain of allantoin in solution was given by mouth at two-hourly intervals on an empty stomach. The counts were generally taken two hours after each of three successive doses. The polynuclear cells were increased

by from 5 to 15 per cent., and it was noted by making subsequent counts that they had returned to the normal in from sixteen to forty-eight hours.

Next came the administration of allantoin to cases of pneumonia commencing at the earliest possible periods after the onset of the disease, with the result that a rapid increase in the number of leucocytes was generally observed. In many cases it was noted that the crisis was early, this being influenced by the shortness of the period, following the onset of the disease prior to the administration of allantoin. For instance, in nine cases where the crisis took place on or before the seventh day the allantoin had been given within the first seventy-two hours, whereas in six cases in which it had been commenced after the first seventy-two hours the crisis occurred on or after the eighth day.

A very striking instance of the benefit resulting from early administration was in a patient who had been operated on for gall-stones. He had a sudden rise of temperature with rigor, pain at the base of the left chest, where there were undoubted signs of early pulmonary consolidation including impairment of the percussion note, bronchial breathing and some fine crepitant sounds. No blood-count was taken but allantoin was given intensively, 2 gr. in solution at two-hourly intervals, and within forty-eight hours the temperature was normal and the condition practically cleared up. In another case which I saw at the Bourton-on-the-Water hospital[1] in 1933, where a pneumonia had commenced in a very stormy way, accompanied by pericarditis, a similar speedy resolution took place, but was followed by an empyema. (Empyema has on several occasions followed in cases of pneumonia treated with allantoin, and a conjecture has arisen

[1] With Dr. Leslie King.

as to the possibility of it resulting from what may be called a hyper-leucocytosis.)

With reference to the period of crisis Mr. Green recorded that out of 15 cases of frank lobar pneumonia treated with allantoin during a period of twelve months, the crises occurred as follows :—

One	case	on the	4th day
One	case	,, ,,	5th day
Five	cases	,, ,,	6th day
Two	cases	,, ,,	7th day
Three	cases	,, ,,	8th day
One	case	,, ,,	11th day
Two	cases	,, ,,	12th day

In nine of these cases the crisis occurred on or before the seventh day, and in six of them on or after the eighth day. In the former group the allantoin was given, with two exceptions, well within the first seventy-two hours from the onset, and in the latter group after the first seventy-two hours, with three exceptions, one of which was a very severe case with involvement of almost the whole of both lungs.

A point to note in connection with the course of pneumonias treated with allantoin (also observed by Mr. Green) is the occurrence of a pseudo-crisis generally from twenty to thirty hours after the drug was first given, the temperature dropping to normal and remaining so for a few hours, but without much relief from symptoms. Occasionally one or two such falls in temperature preceded the true crisis. Mr. Green suggested that the pseudo-crisis might be explained by an abnormally rapid increase in the leucocytes causing a temporary check to the multiplication of pneumococci and production of their toxins in the blood-stream, enabling the thermal centre to regain its balance for a while. The symptoms are not abated during the pseudo-

crisis because the lung condition remains unchanged, and the disordered circulation in the lung does not enable many of the newly formed leucocytes to penetrate and attack its contained pneumococci.

That allantoin renders a useful service in pneumonia is indicated by the relatively low mortality of patients treated with it as compared with the general run of cases treated on the usual therapeutic principles. At the time when Mr. Green worked in this investigation there were ample opportunities for making comparative observations. Over a period of two years 47 cases of lobar pneumonia had been treated in the hospital, and in that period most of the cases in my own wards had been given allantoin, but very few of those in other wards. At any period in those two years the cases would be of somewhat similar strains, or at all events they would be fairly evenly mixed. Twenty-five of the 47 cases were treated on the usual therapeutic lines, the remaining 22 cases were given allantoin by mouth, some of them with such additional medicines as might be required to meet symptoms. The mortality in the former group was 20 per cent., whereas in the latter it was under 5 per cent.

Making all allowances for conditions which might influence prognosis such as questions of age, precedent or collateral infections, etc., it seems evident that there must have been some relationship between the diminished mortality and the administration of allantoin.

During the past eight years Dr. Robert Stewart and other medical men practising in and around Bourton-on-the-Water have, without making any leucocyte counts, treated their pneumonias both in and out of hospital on the principles above referred to, and their experiences have been definitely satisfactory.[1]

[1] *vide* Addendum page 52.

During the past three years (1932-35) a series of clinical observations have been made with allantoin with a view to confirming or otherwise the presumption that it produces a leucocytosis when administered by mouth. In 1932 I sent a quantity of the crystals, which had been in my possession for a year or more, to Dr. Norman Capon of Liverpool, and his former Resident, Dr. O. Vaughan Jones, kindly carried out a series of investigations, the general results of which were distinctly confirmatory. The allantoin was given to 20 individuals whose leucocyte counts were within normal limits. To each of them he gave 2 gr. of allantoin dissolved in one ounce of water at two-hourly intervals and, in order that the leucocytosis following the ingestion of food should be eliminated as far as possible, the blood-count in each case was made from two and a half to three hours after a meal. By way of control, blood-counts were made in five cases under the same conditions, but without giving any allantoin. In every one of the cases receiving allantoin a leucocytosis ensued ranging from 50 per cent. to 83 per cent. increase in seven instances, and from 25 per cent. to 47 per cent. in the remaining 13 cases. None of the controls showed appreciable changes. The average increase was somewhat low compared with the results in some of my own original observations and those of the staff in the pathological laboratory of the Royal Southern Hospital, from ten to fifteen years ago, and one rather suspected that the crystals of allantoin might have deteriorated. It so happened that in 1934 I sent a supply of the same stock of crystals to Dr. M. Schwartzman who was interested in the matter, and he reported that in a limited number of observations his results were not convincing. This led him to obtain some freshly prepared allantoin which he administered, in accordance with the recommended instructions, to 19 people who were either in normal health or the subjects of diseases presenting no blood changes, and in course of conversation he informed me that in 17 of them positive results up to 65 per cent. increase in the number of leucocytes were obtained.

It seems just possible, therefore, that synthetic allantoin crystals, although supposed to be quite stable, may deteriorate with keeping, and if so it is advisable to use crystals which are not too old kept absolutely dry in a well-stoppered bottle. It is at present uncertain how long they will keep in an unimpaired condition, but crystals freshly prepared seven months ago, so kept, have certainly proved perfectly active in a number of recently investigated bloods.

I have already explained that solutions should be made with distilled water, and that heat short of boiling should be employed in their preparation. It is advisable to powder the crystals finely first in a perfectly clean mortar, and then use only hot distilled water, stirring well to get the solid into solution, of course using a chemically clean glass test tube or beaker and glass rod.

In conclusion it may be said that although lobar pneumonia has been the disease mainly investigated with reference to the action of allantoin in producing phagocytosis, it may prove useful in other infective conditions which might be benefited by the production of a leucocytosis. It has been found serviceable in some cases of bronchopneumonia, and in some septic infections, and even the common cold seems sometimes to have been averted when the drug has been given at the commencement. Its employment in the early stages of influenza and in other diseases associated with leucopænia might be usefully investigated.

Its utility as a promoter of healing in wounds, ulcers, and sinuses has already been appreciated in this and other countries, and it is generally recognized that the best way of applying it is on sterilized lint or gauze without waterproof coverings. Ointments having lanoline bases which are capable of holding solutions of allantoin have proved useful.

A curious suggestion has recently come from America,[1] following the discovery made by entomologists of the U.S. Department of Agriculture, that maggots of certain flies when placed on wounds promote their healing. In this connection Dr. William Robinson, of the Bureau of Entomology and Plant Quarantine, found that allantoin given off by the maggots is responsible for at least a part of the healing power they possess, and the article goes on to state that surgeons who have found it difficult in the past to obtain sterile maggots laud the discovery that allantoin, which is spoken of as a new treatment, can be made to control healing.

The impression gained as to the cell-proliferating property of allantoin is that it acts like a hormone, in the sense that such small quantities of it set going proliferations of considerable magnitude.

[1] *Drug Trade News*, April 29, 1935.

PREPARATION AND ADMINISTRATION OF ALLANTOIN SOLUTION

FOR internal administration or local application of allantoin it is essential to use freshly prepared solutions of which the most convenient strength is 0·4 per cent. This is approximately 2 grains to the ounce. Allantoin is very sensitive to the action of alkalis, and on this account distilled water should be used for making the solutions.

Although prolonged heating decomposes allantoin, it has been found that water just below the boiling point may be used for dissolving it without appreciable decomposition, provided the solution is quickly cooled.

Another point to remember is that though it is generally supposed that the crystals are quite stable and may be kept for any length of time, clinical experience has indicated that they are apt to lose their efficiency if kept too long. Care should therefore be taken to use crystals which are not too old and which have been safeguarded against damp by keeping them in well-stoppered bottles. They appear to remain stable for at least four months, and probably considerably longer if kept perfectly dry.

Solutions of allantoin should be freshly prepared in sufficient quantity for use during a period not exceeding twenty-four hours.

Experiments on plants in which older solutions have been used have shown that they are less active than freshly prepared ones.

Addendum.

Dr. Robert Stewart has treated many cases, both of lobar pneumonia and bronchopneumonia with allantoin, and has no doubt that it does good provided it is given early enough. He regards it as a defensive measure, preventive as well as curative, in that it appears to lessen the extension of the disease. His contention is that the important point is to promote an early leucocytosis, before the disease becomes systemic, and that by so doing there comes a possibility of limiting the pulmonary involvement. He has recorded notes of two cases treated in the Moore Cottage Hospital, Bourton-on-the-Water in January and February, 1936. The first was a man aged 47 suffering from a classic pneumonia for which the treatment was commenced within twenty-four hours of its onset. There was a massive involvement of his right lung. Two grains of finely pulverized allantoin crystals were given every hour for twelve doses. This was followed by a characteristic series of pseudo-crises and a final crisis on the sixth day. The second case illustrates a result following immediate treatment in a woman whose pneumonia developed while she was in hospital. She was admitted for a pleurisy of two days' duration. On the evening of her admission her temperature shot up to 103·5° and she developed signs at the right base. Two grains of pulverized allantoin were immediately given at hourly intervals and within thirty-six hours she had a typical crisis.

Dr. Stewart has found that in a country practice, where many of the patients live in distant places, time was wasted by the preparation of solutions of allantoin for administration. This led to his adopting the plan of having it dispensed in the form of powders. The insolubility of the crystals had hitherto rendered this means of administration

unlikely to be so serviceable as when they were predissolved but, judging by results, it is evidently effective.

Following this record of experiences I experimented on some bulbous plants by injecting them with finely pulverized allantoin suspended in distilled water in quantities considerably above saturation point. For instance, twenty-four hyacinths having shoots about 1 inch high were planted in a box. Twelve of them were injected with 10 minims of the suspension, the remainder being controls. The idea was to introduce a store into the bulb, which would be gradually drawn upon, thus avoiding repeated injections. In four weeks the injected bulbs were in bloom, whereas the controls had only reached the stage when the enlarging flower-buds had become visible, thus being far behind their treated neighbours. This experiment was repeated with a series of tulips and daffodils with similar results.

Having exhaustively told the story of the research which led to the discovery of allantoin in the *Symphytum officinale*, and of the evidences of its utility, it remains for others to derive conclusions from their own clinical experiences with it.

THE CHEMICAL CONSTITUTION OF ALLANTOIN

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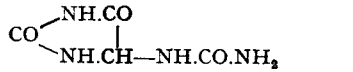
In a communication to the Chemical Society in 1913,[1] I showed that the accepted formula for allantoin originally devised by Grimaux :—



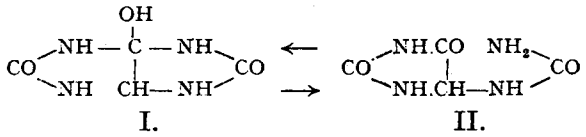
will not explain all its properties, and especially its optical inactivity. Its molecule on Grimaux' formula contains an asymmetric carbon atom, namely that contained in the CH grouping, and like other compounds containing an asymmetric carbon atom, allantoin should be optically active. By this it is meant that when a ray of polarized light is passed through its solutions the plane of polarization should not be undeflected as it would be with water, alcohol, etc., whose molecules are symmetrical, but rotated either to the right or left, as it is for example with the unsymmetrical molecule lactic acid, CH_3. CH. (OH) COOH.

An inspection of the latter formula shows that the central carbon atom is asymmetric, i.e. linked to four *different* groups, just as is the above-mentioned carbon atom in the Grimaux formula. Lactic acid appears in two forms, both optically active, in opposite senses, one, *d*-lactic acid, rotating

[1] *Journal of the Chemical Society Trans.*, ciii, 1336.

the plane of polarized light to the right, and the other, *l*-lactic acid, rotating it to the left to an exactly equal degree.

In 1913, in order to explain what appeared to be the optical abnormality of allantoin, I supposed it to be a tautomeric substance in which a double-ring formula (I) alternated reversibly with that of Grimaux (II), thus :—



I. II.

This alternation between I and II is promoted by the shift in position of one of the hydrogen atoms, believed to be mobile, a shift which by analogy with certain other types of molecule should be an easy one. Granting such a shift, involving a tautomeric change between two distinct formulæ, the optical inactivity was easily explained since Formula I is strictly symmetrical, and when it changes back to Formula II, containing an asymmetric carbon atom, it must yield an equal number of molecules which are dextro-rotatory (*d*-) to those which are lævo-rotatory (*l*-), since there is nothing to determine a preponderance of one form ; and the *d*-*l*- mixture must be optically inactive by compensation, as each form must be equal and opposite in the degree of optical rotation.

Apart from the above, there were other (chemical) reasons in favour of Formula I, being at least one of the possible tautomeric forms of allantoin, though these reasons need not be considered here.

This theory of tautomerism has quite recently (1934) received indirect confirmation by the researches in France of Fosse, Thomas and Graeve

(*Comptes rendus*, 198, 685, 1373, 1953). They have shown that it is actually possible to prepare optically active forms of allantoin if great care is exercised to prevent what is called *racemization*. Racemization is a well-known phenomenon in organic chemistry, viz. one by which an optically active compound loses its activity by conversion into an optically inactive mixture (equi-molecular of *d*- and *l*- forms). Thus, suppose a given compound is wholly the *d*-form : on certain chemical treatment, more or less drastic, this *d*-form may change into the *l*-form, and as more and more molecules of the *d*-form go over the rate slows down because the reverse thing is happening (under the given treatment) with the *l*-molecules, and finally a state of equilibrium is attained when as many *d*-molecules are being regenerated from the *l*-molecules as are being transposed into *l*-molecules. So if the process is followed by the proper optical instrument it is found that the initial dextro-rotatory compound steadily loses its activity and ends by being completely inactive, like water. It is now said to be a racemic mixture of *d*- and *l*- forms (in exactly equal amount) and the process is, as above stated, called racemization. And so it turns out that allantoin as ordinarily obtained is such a racemic mixture (or racemic compound) of the two forms, and if one of the two forms is isolated it passes with extreme ease into the racemic form, as it would be expected to do if there is an alternation between the two molecular formulæ I and II.

Now it is a commonplace of Organic Chemistry that asymmetric substances found in Nature are almost invariably optically active, while the same substances produced synthetically are inactive because racemic (there being nothing to determine a preponderance of one of the forms by synthesis).

It is also a well-known phenomenon that when a racemic mixture is fermented by any means (say by enzymes), one of the two forms is more susceptible to decomposition than the other, which sometimes is quite unaffected. To this rule allantoin is no exception. Natural allantoin when isolated from natural sources, by carefully controlled methods to prevent racemization, is optically active as demonstrated by the above authors. It is *d*-allantoin and has a specific rotation for polarized light $[\alpha]_D^{22} + 93°$ in water, and this hitherto unknown form of allantoin has been obtained from calves' urine and from certain plants, by extraction methods avoiding all rise of temperature. In all ordinary chemical properties it is identical with common allantoin, which is now recognized to be a racemic compound of *d*- and *l*- allantoin, but it might be supposed, by analogy with other optically active compounds, that there should be a biological difference between *d*- and *l*- allantoin on the one hand, as well as between them and common (racemic) allantoin on the other. *l*-Allantoin has been obtained by the above authors from the latter by by taking advantage of selective differences in the rate of destruction at 40° C. of the two forms, by an enzyme, from soya bean, called allantoinase, the *l*-form preferentially surviving, as the *d*-allantoin ferments more rapidly.

Both *d*- and *l*- allantoin are exactly alike, except in respect of what might be termed asymmetric properties such as optical activity, where they are opposite and equal. Each on warming in solution passes over into the racemic form, common allantoin.

It is well known that many of the bio-chemical manifestations of living matter are concerned with the transformation (such as oxidation, reduction, hydrolysis, etc.) of complex asymmetric compounds,

proteins, carbohydrates, phosphatides and so on, most of which display optical activity and are therefore not racemic forms. Indeed chemical asymmetry plays a fundamental role in life, as is well known from numerous cases which have been established, and probably allantoin is no exception to this. It is apparently the unstable *d*-form which, so far as is known, normally functions in the living plant and animal. When therefore ordinary racemic (*d-l*) allantoin is applied therapeutically it may be presumed provisionally that only one half of it (*d*-) is active and the other half (*l*-) is inert, but as yet nothing definite is known about this. In any case when ordinary allantoin is brought into solution by heating with water it no doubt passes partly into the symmetrical double-ring form I (p. 55) and even with cold water it is probable that a small quantity of I co-exists in solution with the asymmetric form II, and the equilibrium between these two forms, I and II, will be disturbed by numerous chemical agencies. For instance, the influence of acids and alkalis would be expected to be profound and thus the *p*H value of the solution, as representing the concentration of hydrogen (oxonium) ions will be a determining factor in this equilibrium. As is well known with *p*H 7, as it is in the purest distilled water, we have absolute neutrality, meaning a very small but definite value for hydrogen-ion concentration. With small quantities of alkali present (even as dissolved out of glass when boiled with water) the *p*H rises, and when at a value of say 8 or 9 there is a marked alkalinity. On *a priori* grounds this should favour a more rapid establishment of equilibrium between forms I and II and also a preponderance of the asymmetrical form II, because this is the more acidic of the two forms. Allantoin belongs to a group of chemical substances known as *amphoteric*, because they

function as weak acids in their behaviour toward alkalis, forming alkali salts, and as weak bases towards acids, forming salts with such acids. Allantoin, for example, yields a well-defined crystalline nitrate with nitric acid and it dissolves in caustic soda much more readily than it does in water because it forms a sodium salt (very unstable). It is also important to observe that allantoin as an acid is considerably stronger in hot aqueous solutions than it is in cold. When its aqueous solution is acid for any reason (say on heating or when acid is definitely added) the hydrogen-ion concentration increases. This, as in the case of alkali-effect, will promote a more rapid attainment of equilibrium between forms I and II, but for certain chemical reasons it is likely that it favours the predominance of the symmetrical form I.

We may sum up, then, by saying that allantoin will be very sensitive to acidity and alkalinity, as well as to high temperature, but that there is reason now (after the work of Fosse, Thomas, and Graeve) to believe that the predominating form in neutral solution (*p*H = 7) in the cold is the asymmetrical form II.

Apart from these considerations, affecting only the reversible equilibrium between form I and the two optically active forms (*d*- and *l*-) of II and readily accounting for the easy racemization of the latter, it must be noted that allantoin is a chemically unstable substance, readily converted by various chemical agencies into the other chemical compounds related to it, but in no way identical with it. The most striking of these changes is that observed with strong alkaline solutions. Here the first stage is its conversion into the salt already referred to, but on standing in the cold this salt decomposes by rupture of the ring in Formula II yielding the salt of a definite

acid, called allantoic acid, which is itself very unstable and may be converted into a number of derivatives, these being as yet but imperfectly understood. It is quite possible that some of these derivatives appear in animal and vegetable life but have not been identified owing to their very soluble, syrupy and non-crystallizable nature.