Biological relations of optically isomeric substances / by Arthur R. Cushny.

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BIOLOGICAL RELATIONS OF OPTICALLY ISOMERIC SUBSTANCES

BY

ARTHUR R. CUSHNY, M.A., M.D., LL.D., F.R.S.
Professor of Pharmacology and Materia Medica in the University of Edinburgh
(Formerly in the Universities of Michigan and London)

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THE CHARLES E. DOHME MEMORIAL LECTURESHIP

In June, 1916, Mrs. Charles E. Dohme of Baltimore generously offered to pay annually the sum of $1,000.00 to the Trustees of the Johns Hopkins University to found a lectureship in memory of her deceased husband, Charles E. Dohme, the well known pharmaceutical chemist of this city.

The purpose of the lectureship is to promote the development of a more intimate relationship between chemistry, pharmacy and medicine. The lectureship is open to scientists from any part of the world and the selection of the lecturer is made by a committee representing the departments of Pharmacology, Chemistry and Medicine.

A provision of the gift reads as follows: "My preference is to have the lectures delivered annually, but should a suitable lecturer at any time not be considered by the committee to be available my suggestion would be that the income of the fund for that year be used to defray the expenses of a research upon a subject germane to the general subject of chemistry as applied to medicine. This research is to be published in some leading chemical as well as medical journal, and whatever results

may ensue of benefit to science or industry shall be available for any and everyone."

PUBLISHERS' ANNOUNCEMENT

The publishers plan to issue the Dohme Lectures in book form. Announcement of titles will be made as each book is issued from the press.

PREFACE

It is a great pleasure to me to address once more an audience of American students, among whom I began my course of teaching and to whom I feel that I am indebted for such success as I have attained. For if the teacher may influence in some measure his pupils, it is no less true that the interest and encouragement of his students may profoundly affect the work of the teacher, especially perhaps in his earlier years. And such intelligent interest I profited by throughout the formative years which I spent in the United States.

And I feel it a special honour to have been invited to address you as lecturer on the Dohme Foundation. I never had the pleasure of Mr. Dohme's personal acquaintance, but he was known to me as one of a chemical firm of wide views and interests, who recognised the importance to industry of the advance of scientific investigation, and was very willing to help in any such advance as far as he could. The foundation suggests that the lectures should be devoted to chemistry and medicine, and I regard it as in accordance with the general character of the founder that one whose claims to chemical investigation are so slight as mine should have been invited to deliver the course this year.

A. R. C.

Edinburgh, 1925

CONTENTS

THE BIOLOGICAL RELATIONS OF OPTICALLY ISOMERIC SUBSTANCES

I have chosen as the subject of these lectures the relation to living matter of a series of compounds, which seem to me not to have received the attention they deserve from biologists. Many of the phenomena of life, such as the movement and reproduction of organisms are far removed from the processes of the chemical and physical laboratories and are hardly susceptible to such definite measurements as the presiding spirits of these departments demand. On the other hand, more definite results might be expected from the study of the formation and reproduction by living matter of chemical substances possessing the power of rotating light. The chemists have long been mildly interested in this form of vital activity, the pharmacologists have shown its importance in their sphere, but biologists in general do not seem to have appreciated it as a very definite and measureable feature of living matter. It has interested me for many years, and it seems that some account of what has been done in it might serve to direct more attention to it and perhaps lead others to take it up and advance it further.

Our knowledge of this phenomenon dates from

almost a century ago, at which time a number of French physicists and chemists were engaged in the study of the changes effected in a ray of light on passing it through various inorganic crystals and other substances. It was found that when a crystal of Iceland spar is placed in the path of a ray, the emergent light is polarised, that is, its vibrations occur in only one plane, while in an ordinary ray the ether vibrates in all planes at right angles to its axis; the crystal may be visualised as a series of parallel plates between which the light passes, the undulations only being permitted in one plane, let us say the vertical. When a second crystal is placed in the path of the polarised ray exactly parallel to the first, i.e., with its plates vertical, the light continues to pass through its interstices, but if it be rotated until they run at right angles to those of the polarising crystal, the whole of the surviving polarised rays are shut off and the light disappears. Biot showed however that if the two crystals were maintained in this position, the light reappeared when certain substances such as oil of turpentine, or a solution of sugar, camphor or tartaric acid was placed between the crystals, and inferred that these fluids rotate the plane of polarised light so that the polarised ray now meets the second crystal at a different angle; the effect is the same as if the

second crystal was not exactly at right angles to the first, and the degree to which the solution distorts the light can be measured by the amount of rotation of the second crystal that is sufficient to shut out the light after it has passed through the solution. Some solutions rotate the ray to the right (clockwise) and are termed dextrorotary, others to the left (or counter-clockwise) and are laevorotary. The same rotation is caused by these bodies in a ray of unpolarised light, but it cannot be detected in it.

A similar rotation occurred when the polarised light was passed through solid crystals and it was noted that some of these had an asymmetrical facet which inclined sometimes to the left and sometimes to the right; Herschel pointed out that the direction of rotation of light corresponded with the inclination of the asymmetrical facet of the crystal, all crystals with facets inclined in the same direction rotating polarised light in the same sense.

Such was the position about 1845 when the subject was taken up by Pasteur, who was interested in crystallography and began to study the tartrates with the view of testing whether the relations established by Herschel for inorganic crystals held also for organic substances in solution. He soon satisfied himself that the ordinary tartrates formed crystals with an asym-

metrical facet and also rotated light to the right, in agreement with the observations of Biot and Herschel. But another acid had recently been obtained from a batch of crude tartar, which was said to resemble the ordinary tartaric acid in every respect except that it did not rotate the plane of polarised light. There is an amusing account in the "Life of Pasteur" of his exploring expedition over half of Europe in search of this acid which

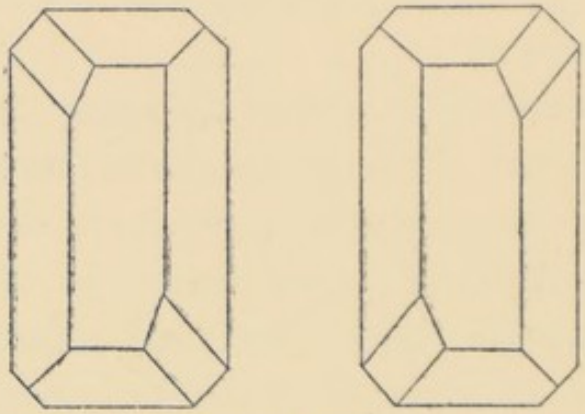


FIG. 1. HEMIHEDRAL CRYSTALS, MIRROR IMAGES OF EACH OTHER, ROTATING POLARISED LIGHT TO RIGHT AND LEFT RESPECTIVELY

had been named paratartaric acid, and how he finally obtained a supply. You may wonder at the interest excited by this question, but several distinguished chemists had found the two acids identical in all respects except in regard to their effect on polarised light, and this seemed to shake the foundations of chemical science, which held that differences in behaviour indicated differences in composition, and was still innocent of the knowl-

edge of structural formulae. Pasteur anticipated that further differences would be found between the two acids and in particular in their crystalline form, that while tartaric acid had asymetrical (hemihedral) crystals, the "paratartaric" form would prove to have symmetrical ones. On examining the tartaric crystals he found them hemihedral as he had expected, and on examining the paratartaric crystals, he found them asymmetrical also. Few of us go far in investigation without meeting with similar tragedies in which a beautiful theory is overthrown by a brutal fact, and it may be some consolation to remember that even the greatest are not immune from such disheartening experiences. In this case disappointment was shortlived, for a new interest was aroused by the observation that while the paratartaric crystals were all hemihedral, they were not identical; half of them had an asymmetric facet directed to the left, the other half to the right. And on collecting a few of each of these groups and dissolving them, each was found to be optically active, the one group rotating light to the right in the same way as ordinary tartaric acid, while the other rotated it to the left to exactly the same extent; the latter thus proved to be a new hitherto unknown acid, which is now known as *l*-tartaric acid. There were then three forms of tartaric acid, the ordinary or

d-tartaric, Pasteur's new acid or *l*-tartaric, and the mixture of the two in equal parts which had no rotatory power, the old paratartaric or racemic acid, which is now designated *dl*-tartaric acid.

Subsequent investigations showed that there is a fourth acid also inactive towards light but not resolvable into active acids, owing to internal compensation. Fortunately Pasteur did not come across this at that time, for it might have delayed his further development of this problem by confirming him in his preconceived theory, that the inactive acid would have symmetrical crystals.

A touching scene is described by Pasteur when he demonstrated his result to Biot, who had first shown that tartaric acid is optically active and who was now an old man and a little sceptical of revolutionary advances. "My dear boy," said the old scientist, "I have loved science so much all through my life that this discovery of yours makes my heart throb."

Pasteur had thus solved the problem of the tartaric acids and introduced the first pair of optical isomers, bodies which are identical in their physical characters and rotate polarised light to the same extent but in opposite directions and often form crystals in which the facets are directed in the one case to the right, in the other to the left. The crystals are thus mirror images of

each other and they cannot be superposed on each other so as to coincide. Pasteur described the crystals as resembling the two hands in their resemblance, and in the impossibility of superposing them, while one hand can be superposed on the mirror image of the other.

He examined many other substances presenting this property of rotating the plane of light, and points out that this is found in most natural organic products or at any rate in those "which play an essential part in the phenomena of vegetable and animal life," while no synthetic substance exhibits this power unless it derives from one that already possesses it. Among the most important of these bodies are the proteins, and he speculates upon whether life would be possible if the rotation of these were to the right instead of to the left.

Pasteur insists so much upon the asymmetry of the molecule of these optically active bodies, that it might almost be supposed that he had anticipated a discovery made simultaneously by Le Bel and Van't Hoff some twenty-five years later (1874). But while Pasteur's asymmetry refers to the crystalline form, these later observers made the important discovery that optical activity is always associated with an asymmetrical carbon atom in the molecule. Asymmetrical nitrogen

and other elements also give rise to optically active substances, but I shall not touch on these at present but shall confine my attention to the asymmetric carbon atom, that is, to substances in which each of the valencies of C is satisfied by a different radicle. The interest of this discovery is mainly chemical rather than biological, though it has been of value in interpreting some biological phenomena.

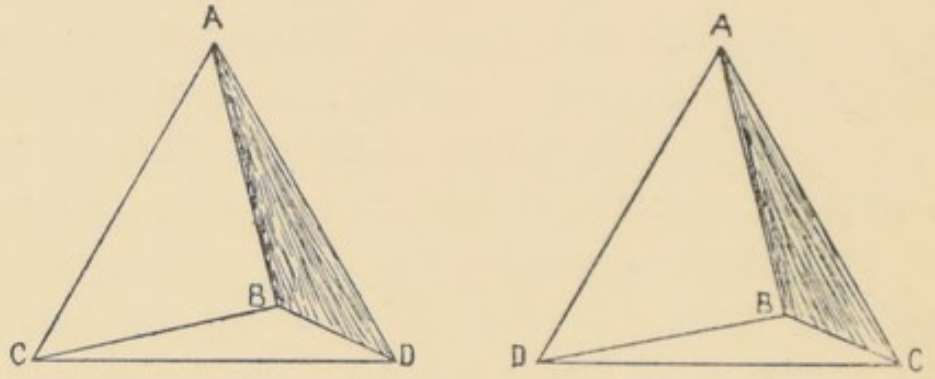


FIG. 2. DIAGRAMS OF AN ASYMMETRICAL CARBON ATOM, i.e., ONE WHICH IS ATTACHED TO FOUR DIFFERENT RADICLES A, B, C, D IN SUCH A WAY THAT THE ONE FIGURE IS THE MIRROR IMAGE OF THE OTHER

This doctrine as originally stated was universally heralded as marking a great advance in structural chemistry. But in some instances active compounds have been described which do not exhibit an asymmetric carbon in the simple definition of the term; further qualifications and extensions have therefore been introduced and these seem to reduce the value of the rule at any rate to

the inexpert in the spatial expression of chemical structure. The relations may be shown in the simplest form in the diagrams of figure 2, in which the carbon of the tetrahedron is attached to four different radicles A, B, C, D. The faces cannot be brought to coincide, but are mirror images of each other. The properties are identical except in the direction in which they act on polarised light as long as they remain mirror images of each other. It follows that they cannot be separated from each other by any ordinary chemical reagents, and a mixture of equal quantities of two optical isomers, such as of the *d*- and *l*-tartaric acids, behaves as an individual substance, is not optically active, and in fact may give no indication under ordinary manipulation that it is a mixture. We have seen the difficulty of analysing racemic acid into its two components and how Pasteur only succeeded by mechanically selecting the crystals, and this method could not have been applied to most racemic bodies.

An optically active substance often tends to become inactive either spontaneously or on comparatively simple manipulation. For example Pasteur found that on heating *d*-tartaric acid with cinchonidine it lost its effect on polarised light through being altered to the racemic (*dl*) form. Half of the original *d*-substance was thus changed

by the manipulation to its isomer of the opposite rotation. This would suggest that the natural form, for example the dextrorotary, is less stable than the laevo, but this is not so, because they come to equilibrium at *dl*, i.e., $d = l$ in amount, whereas if one active form were more stable than the other, equilibrium would encroach more or less upon the territory of the less stable. When an asymmetric carbon compound is built up by synthesis, it is never optically active but always racemic, since the two isomers are formed in equal amounts, there being no reason why one should be in excess.

Quite a different result follows however when instead of using the ordinary chemical reagents, substances which themselves are optically active, are employed. This was discovered by Pasteur in his second method of separating the tartrates, by means of cinchonidine; when *dl*-tartaric acid is treated with this alkaloid and the solution is allowed to evaporate, the first crystals consist of cinchonidine *l*-tartrate, while the corresponding *d*-tartrate is more soluble and remains in solution. Thus while the two isomeric tartrates of the alkalies cannot be separated, since their properties are identical except in the direction of rotation, the isomeric tartrates of an alkaloid which is itself optically active, differ in their solubility and in many other qualities; in fact they may resemble

each other no more closely than other organic salts. Thus the two isomeric tartrates of cinchonidine differ in their water of crystallisation, their specific gravity, and in their tendency to undergo oxidation on heating. This has been elaborated by Fajans[1] in the case of the optically active camphorcarboxylic acids, which he showed to differ in the readiness with which they release CO_2 in the presence of various alkaloids; for example *dl*-camphorcarboxylic acid treated with quinidine becomes laevorotary from the catalytic decomposition affecting the *d*-component especially, while with quinine the *l*-camphorcarboxylic acid is more rapidly decomposed and the solution becomes dextrorotary.

The combinations of two optical isomers with another active body thus differ in their qualities in many ways. This is readily intelligible for we are no longer dealing with isomers of the same kind; these combinations are no longer mirror images. For if two optical isomers *d*-A and *l*-A are treated with another optically active base *d*-B, the resulting salts will be *d*-A *d*-B and *l*-A *d*-B, which no longer possess equal rotatory power and are no longer complementary images, and have no such similarity in their characters as is characteristic of the optical isomers. Such imper-

[1] Zeitschr. f. physik. Chem., **73**, p. 25, 1910.

fectly isomeric bodies occur widely in nature; for example, quinine and quinidine are isomeric in structure and in part of their molecules differ only in the direction in which they rotate polarised light, but attached to this is a part which is identical in each and these alkaloids are therefore not true optical isomers and can be separated by ordinary reagents.

There is no definite rule for this method of separating optical isomers. For example if we desire to precipitate *d*-tartaric acid from the racemic mixture by means of an optically active alkaloid, there is no reason to select a dextrorotary one in preference to one of the opposite sign. The characters of the combination of acid and alkaloid are not determined by their resembling or differing in the direction in which they rotate the plane of polarised light, but by the structure of the molecule in general; this is of course true for all similar operations, for example, the best precipitant for sulphuric acid or lead can only be found by actual experiment.[2]

[2] A good example of this is given by Fajans (Zeitschr. f. physik., Chem., **73**, p. 25, 1910) who investigated the liberation of CO_2 from camphorcarboxylic acid by the catalytic action of a number of alkaloids and found that the laevorotary quinine acts especially on the *l*-camphorcarboxylic acid, yet cinchonine (dextrorotary) can be substituted for quinine; on the other hand, nicotine (laevorotary) and quinidine (dextrorotary) both act specifically in breaking up the *d*-camphorcarboxylic acid.

An active acid combines with an active base in the ordinary way; it is true that Erlenmeyer[3] believes that an active base such as cinchonine has a somewhat greater avidity for one isomer than for its counterpart, but it is not yet established that this is not the result of the difference in solubility of the product arising from the conjunction rather than any more mystical affinity.

The most that we can say at present is that when two optical isomers combine with another active substance, the two products behave in different ways, but there is little evidence that there is any special affinity determined by the direction of the rotation.

Pasteur had been impressed from the first with the abundance of optically active bodies in nature and the impossiblity of forming them by ordinary synthesis. For as he pointed out, if synthesis is carried as far as possible, it can only result in equally balanced (racemic) bodies, since the two components are chemically identical with each other and each would be formed in equal amount. Only in the presence of an actively rotating substance can such a synthetic racemic body be separated into its components and thus acquire the property of active rotation through one isomer

[3] Biochem. Zeitschr., **97**, p. 261, 1919.

being destroyed or eliminated. The presence of active bodies in plants and animals thus tends to the separation of other active substances and this explains the propagation of optical activity in nature. Given an optically active substance, the chemist can form further rotating substances in the same way as plants or animals. And Fischer and Slimmer[4] have succeeded in synthesising more complicated substances on a groundwork of sugars and then dividing between the new and the old, so as to obtain two optically active bodies from the original one.

The propagation of these active bodies was thus accounted for by the previous existence of similar substances in living tissues. But a further question followed immediately, namely, how the first active substance was developed. This is a parallel to the difficulty which arose in the case of the old rule *Omne vivum ex ovo* when it was asked where the first life was found. In fact our enquiry really is a particular case of the earlier one, but is put more definitely, for while the qualities of an egg are indefinite and various, there can be no haggling about the chemical question "Where and how did a substance first acquire optical activity?" When a carbon is supplied with four different

[4] Sitzungsber. k. preuss. Akad. der Wiss., 1902, I, p. 597.

radicles by ordinary synthesis, it is of course asymmetric, but the compound is racemic. It has been suggested that if the synthesis were performed under asymmetric forces, for example in the magnetic field, or under polarised light, an optically active substance might result; and Byk has pointed out that light is polarised by reflection from the surface of the sea and thus an asymmetrical force is provided in abundance if it were sufficient to form optically active substances. But experiments to prove this have never been successful, and the origin of optically active substances has thus assumed an interest in the evolution of life; chemists now see little difficulty in the synthesis of organic substances and even of proteins under ordinary forces, but this synthesis is not sufficient unless it is followed by the development of optical activity. Unless these efforts are more successful than hitherto, "we are apparently compelled to assume the intervention of some unknown supernatural forces in the origin of life" (Bayliss).

From Pasteur's original enquiry on the form of a crystal we have now reached the higher theology and enter upon fundamental questions of interest to wide circles; I will not say that these are challenging the evolution of optical activity, for the difficulty is not recognised, and it may be that its

solution will be reached before it is widely appreciated. It is necessary in order to differentiate between two optically isomeric substances that they should no longer be mirror images, and one way in which this is done is by the addition of another active body by the second method of Pasteur. It is possible, however, to imagine molecules which in ordinary conditions are mirror images, but in which a swing may be set up so that they temporarily fail to maintain their likeness, though they soon return to it. This "relative isomerism," suggested by Van't Hoff, has been studied by Erlenmeyer[5] in the asparagins

$$\begin{array}{l} CH_2 - CONH_2 \\ | \\ CH(NH_2)\cdot COOH. \end{array}$$

At ordinary temperatures these are *l*- and *d*-isomers identical in all features except in their influence on light, and a mixture cannot be separated by ordinary symmetrical reagents. When however a mixture is heated for some time and is then allowed to cool, the laevorotary form separates out first, and a difference is found in other respects, such as in the colour of the copper salts; this indicates that they are no longer optical isomers; they tend to resume their original rela-

[5] Biochem. Zeitschr., **52**, p. 439. 1913,

tionship on keeping. Erlenmeyer suggests that the action of heat is to exaggerate the rotation so that a twist is caused in the link between the two halves of each molecule and they are thus no longer mirror images and therefore differ in their physical qualities. In fact we have a parallel condition within a molecule to that utilised in the second method of Pasteur.

If the two isomers can be induced to react differently by warmth or other forces, the difficulty of the formation of the first optically active substances is removed, and when the first optically active body has been evolved, its propagation may be explained readily by Pasteur's method, though it is possible that this may continue to be accompanied by that described by Erlenmeyer. The latter suggests that asparagin, which is so widely distributed in living matter may have been the first substance possessing optical activity which was evolved. In any case these experiments suggest that it is unnecessary to postulate a creative act to account for the optical activity of living matter, for given an asymmetric carbon atom in a molecule, one active form may be evolved from the synthesised racemic form if it can be changed from being the exact mirror image of its opposite, be it by heat or by some other external force. It is no longer necessary to have

a pre-existing optically active form. And there seems hope that optical activity may not become a theological bone of contention. This method of separation by Erlenmeyer may be regarded as the third method in which two optical isomers may be separated from each other.

RELATION OF ENZYMES AND OPTICALLY ACTIVE BODIES

After his success in separating the active isomers from racemic tartrates by mechanical and chemical methods, Pasteur showed that they could be differentiated by moulds and yeasts, for when a solution of ammonium racemate was allowed to putrefy, it soon began to become laevorotary and this power, slight at first, increased to a maximum at which there was no *d*-tartrate present in the liquid, but only *l*-tartrate; later the laevorotation lessens showing that the *l*-tartrate is also attacked though it is less susceptible than the dextro variety. He points out that the same phenomenon is present here as in the reactions with alkaloids, the differentiation between the two isomeric tartrates occurring in both cases through the presence of some optically active substance; this view of course he could not actually demonstrate, and we are still unable to do so since we have no chemically pure ferment. But every enzyme that is effective

rotates polarised light, and the origin of these bodies and their close association with proteins is regarded as sufficient basis for Pasteur's view. Even if the enzymes should prove to have no effect on light themselves, they always act in association with bodies which rotate it, and this is all that is necessary to explain their power of differentiating the isomers.

At this point we take leave of Pasteur, who is not further concerned in the development of the isomers, his energy being henceforth devoted to the elucidation of infections of plants and animals with the results that all know. You will perhaps allow me to point out again the extraordinary course of his studies. His investigations began in the recondite subject of crystallography, a subject which appears to be as far removed from biology and medicine as any; within a few years he had become the greatest genius in medicine of his time. Perhaps he would have arrived there in course of time wherever in chemistry he had begun, but one cannot help feeling that the special relations of these isomers to living tissues proved a very direct path to a Pasteur, whose nature required him to follow the gleam, wheresoever it led.

For example, there seems to be a close connection between his view that the isomers only

arise in living matter and his strenuous denial of the possibility of the spontaneous generation of life. One cannot help wondering at his mobility, at his daring in leaving a subject in which he had already made his mark, to follow perhaps a will-of-the-wisp across the whole domain of biology. The question presents itself whether such a career is possible at the present time of subdivisions of divisions of science, where each one works his little garden plot and fears to even look over his neighbour's boundary fence. Would any crystallographer of to-day venture into even the frontiers of biology, not to speak of forcing his way into the secret places of pathology and medicine, and what would the pathologist say to such an intruder? Would he not rather hand over a question which was getting beyond his narrow experience to some specialist on the other side of the wall, who, uninterested in it, would perhaps lay it aside. It is beyond question that the advance of knowledge necessitates a subdivision of subjects such as was unknown until now and undreamed of a few years ago. But how does this bear upon future Pasteurs? Will they dare to burst through the barriers and wander as they will through other men's demesnes? Is our extreme specialisation in early years adapted to train Pasteurs?

The fermentation which Pasteur employed is now known to be due not to the living cells of the yeasts and moulds, but to enzymes which they contain, and since his time these have become a method of investigation in common use in chemistry; in fact the chemists are inclined to claim them and the changes they induce for chemistry as against biology proper. The reactions of the enzymes to optically active isomers have been the subject of a large number of researches of which I can mention only a few as examples.[6]

Many of these researches have been made with intact organisms such as moulds, yeasts or bacteria, others with enzymes derived from them, or from the digestive secretions or the fluids expressed from the organs of the higher animals. In all of these the results of the activity show close resemblances, and it is unnecessary to differentiate between them in principle, though they present every variety in detail. Pasteur's observation of the choice by the moulds of one tartrate in preference to the other still remains the type. Another well-known set of observations are those of Fischer,[7] who found that a number of sugars, such

[6] A full account of the subject up to 1910 is given by Fajans, and much of its later developments by Hirsch (Einwirkung d. Microorganismen auf Eiweisskörpern, Berlin, 1918).

[7] Zeitschr. f. physiol. Chem., **26**, p. 60, 1898.

as *d*-glucose, are readily fermented by yeast, while their mirror images are untouched by it at any rate for many hours.

Again, Dakin[8] showed that the lipase of pig's liver splits up the esters of *dl*-mandelic acid, $C_6H_5-CHOH\,COOH$, but acts more quickly on the dextrorotary ones. Fischer's results with the methyl-glucosides are especially striking; α-methyl-*d*-glucoside is hydrolysed by yeast into methyl alcohol and glucose, while its mirror image, α-methyl-*l*-glucoside, is unchanged. Similarly with a parallel series, β-methyl-*d*-glucoside is broken up by emulsin, while β-methyl-*l*-glucoside resists it. The synthetic racemic polypeptides under digestion with pancreatic juice free the natural laevorotary amino-acids, while the dextrorotary complements remain unchanged. Racemic arginin under the action of arginase forms *d*-ornithin and *l*-arginin,[9] so that the dextrorotary component is digested by preference and the laevorotary remains unchanged. Racemic *dl*-leucine and *dl*-glutamic acid exposed to penicillium react in the same way as tartrates,[10] becoming laevorotary from the *d*-body being destroyed in larger proportion; similar changes are seen in many other amino-acids exposed to moulds or yeasts or bacterial enzymes.[11] The tyrosinase of Russula delica[12] added to the isomeric forms of tyrosin colours the natural *l*-tyrosine first, then the *dl*-form and last of all the artificial *d*-variety.

[8] Journ. Phys., **30**, p. 253, 1904; **32**, p. 199, 1905.

[9] Riesser. Zeit. f. physiol. Chem., **49**, p. 210, 1906.

[10] Schulze u. Bosshard. Zeit. f. physiol. Chem., **10**, p. 134, 1886.

[11] Herzog. u. Meier. Zeit. f. physiol. Chem., **59**, p. 57, 1909.
F. Ehrlich. Biochem. Zeitschr., **1**, p. 8, 1906; **8**, p. 438, 1908.

[12] Abderhalden u. Guggenheim. Zeit. f. physiol. Chem., **54**, p. 331, 1907.

The reaction between an individual enzyme and a pair of optical isomers is always of the same character, just as that between them and an alkaloid is constant, but there is no uniformity in the effects of any individual enzyme on different sets of isomers. This was early shown by Lewkowitsch,[13] who pointed out that penicillium glaucum leaves the *d*-lactic, *d*-mandelic and *l*-glyceric acids relatively unchanged, while oxidising their mirror images from a racemic mixture; on the other hand it rejects *l*-asparagin, *l*-glutamic acid and *d*-leucin, and oxidises their mirror images. This fickleness has been observed in many other instances, and it is obvious that an enzyme may select the dextrorotary component of one pair of isomers, the laevorotary of another. The enzymes cannot be classified into those destructive to dextrorotary and those destructive to laevorotary bodies, for they differ in the isomer selected with each pair they meet.

In the same way there is no uniformity in the behaviour of different enzymes to a pair of isomers. For example while penicillium destroys *d*-tartrates, some other organisms attack the *l*-form by preference; similarly *dl*-mandelic acid exposed to penicillium becomes dextrorotary,

[13] Ber. d. d. chem. Gesell., **16**, p. 1658, 1883.

while saccharomyces ellipsoideus renders it laevorotary, the two enzymes differing in the relative amount of the isomers that each destroys readily. *L*-tyrosin or *dl*-tyrosin gives rise to *d*-p-hydroxyphenyl-lactic acid when exposed to Bacillus proteus while the laevorotary isomer is formed by Bacillus subtilis.[14]

From such observations it appears that neither the enzyme nor the substrate alone determines the resultant of their reaction; an enzyme may select one isomer of one pair, the other of another, while on the other hand one enzyme may destroy the dextrorotary component, another the laevorotary of a racemic mixture.

While there are thus many instances in which the enzymes act preferentially on one or other of two optical isomers, this is not a universal rule, and it would be erroneous to state that the ferments differentiate between these mirror images as a whole. In some cases each isomer is destroyed at the same rate; for example, *dl*-lactic acid subjected to certain ferments is slowly destroyed without developing any optical activity whatever; that is the dextrorotary and laevorotary isomers are acted on equally.[15]

[14] Sasaki, Acta Schol. Med. Univ. Imp. Kyoto., **1**, p. 103, 1916–17. Tsudji. Ibid., p. 439.

[15] F. Ehrlich. Biochem. Zeitschr., **63**, p. 379, 1914.

When enzymes do differentiate, their choice is not absolute; they prefer one isomer, but they generally act on each of them though in different degree. For example, Pasteur noted that his solution of tartrates originally inactive, when exposed to the moulds became laevorotary and the extent of rotation increased till it reached a maximum and then declined; he interpreted this as indicating that the *d*-tartrate was first destroyed and later the laevorotary. And the same choice has been observed in many other instances. The degree of specificity for one component of a racemic mixture varies in different experiments; for example, Fischer found that zymase destroyed *d*-glucose rapidly while *l*-glucose remained unchanged for many hours, and he concluded that the reaction only occurred with one isomer while the other was incapable of combining with the ferment. But it is now held that both isomers are susceptible of change by an enzyme which is capable of acting on either, though the velocity may differ greatly.

We may take as types the lactic acids, which I have mentioned as being equally readily destroyed by certain ferments, that is, the reaction of the two isomers is 1:1; the glucoses subjected to yeast whose relation seems nearly $1:\infty$, and the tartrates fermented with penicillium in which each isomer is attacked though unequally. In the long series

examined, every variety between the extremes may be met with.

As a general rule the enzymes act by destroying one of the isomers while leaving the other relatively intact so that a previously inactive mixture of the two begins to rotate the plane of light from one of the isomers no longer being balanced by the other. Sometimes more complicated processes may follow apparently; thus Sasaki and Otsuka[16] state that *d*-tyrosin acted on by B. subtilis yields a *l*-oxy-acid; this suggests at first that the *d*-tyrosin is changed to *dl*-oxy-acid and the *d*-component is then completely destroyed, while the *l*-acid escapes. But this, they state, is not an adequate explanation, for this would account for an amount of *l*-oxy-acid corresponding to only half the original tyrosin, while they obtained a considerably larger proportion; they therefore infer that the bacillus first changes the whole of the *d*-tyrosin to some intermediate body from which the *l*-oxy-acid is subsequently formed directly.

In many instances the isomer which is more readily affected by an enzyme is that which occurs naturally, but this is by no means invariably the case, and as has been mentioned, the choice not infrequently differs according to the enzyme used.

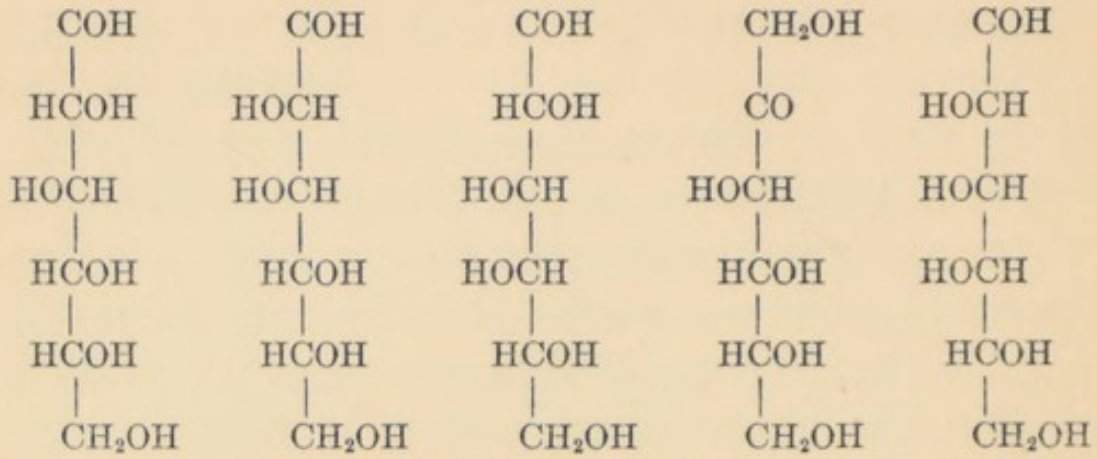
[16] Journ. Biol. Chem., **32**, p. 533, 1917.

And some isomers which probably do not occur in nature are also selected by the enzymes in the same way as others; thus Sasaki and Kinose[17] found that when *dl*-α-naphthylalanine was subjected to the proteus bacillus, the laevorotary form disappeared while *d*-α-naphthyl-lactic acid remained. Now none of these substances occur in nature, so that the same choice is made between isomers neither of which can have been met with previously, as between others which occur naturally and for which the enzymes might be expected to be prepared.

The reaction of a substrate to an enzyme is determined not by the previous relation between them but by their molecular configuration. If they are capable of entering into close relationship and the substratum contains an asymmetric carbon atom, one of the isomers is often but not invariably affected more than the other. It is impossible to state *a priori* which isomer will prove the more susceptible.

The importance of the configuration of the substratum in determining enzyme action has been demonstrated by Fischer's well-known analysis of the fermentation of the hexoses by zymase, which I may refer to for a moment.

[17] Biochem. Zeitschr., **121**, p. 171, 1921.



Here talose is not changed by the enzyme, though every feature of its structure is presented by some of the other four fermenting sugars, and though its whole molecule resembles the glucose type more closely than fructose does. It is obvious that very slight differences in the configuration are sufficient to determine the attack of an enzyme, and that apparently the general structure of the molecule is the final criterion rather than the presence or absence of single groups. But only when the general configuration of the substrate admits of its close contact with the enzyme, does the significance of the direction in which it rotates the plane of polarised light appear. The first test of susceptibility of the substrate to the ferment is its general configuration, but when this has been passed, a further qualification is the direction of rotation, for if this is unsuitable the rate of fer-

mentation may be infinitely slow. I may remind you that the direction of rotation in the four fermenting hexoses is not the same; for example *d*-glucose is dextrorotary, and its laevorotary isomer is unchanged by yeast, while *d*-fructose is laevorotary and its dextrorotary isomer is similarly little susceptible.

There is therefore no general rule in the matter, but the same variation occurs as in the case of the combination of asymmetric substances of known constitution. There we have seen that the qualities of the compounds differ in different cases. A laevorotary alkaloid may be separated from its mirror image by the insolubility of its salt with one optically active acid, while with another the solubility may be reversed, and with a third the difference may be too small to admit of their separation. In the same way a *l*-rotary substance may be destroyed by one enzyme in preference to the *d*-rotary isomer, while when another enzyme is employed, the sequence may be reversed, and with a third the two may be disintegrated equally rapidly, so that no separation occurs, and the reaction is said to be symmetrical.

DECOMPOSITION OF ISOMERS IN LIVING TISSUES

Similar changes occur in substances in their passage through the living tissues, and again

these arise for the most part from enzyme action. The first observations of the behaviour of the optical isomers in the higher organisms were those of Brion[18] on the assimilation of the tartrates by the dog, and resulted in his statement that the *l*-tartrates are oxidised more readily than the *d*-tartrates, as is shown by the escape of larger amounts of the *d*-isomer in the urine. These experiments are not convincing, since he finds that *dl*-tartrate is less assimilated than either of its active components. And Neuberg and Saneyoshi[19] found that Brion had not appreciated the variability of the assimilation of these bodies, and proved that the *l*- and *d*-tartaric acids were equally readily consumed in the tissues, which appear to be unable to differentiate between them. Thus when *dl*-tartrate is fed to a dog, the urine shows no change in its power of rotation of polarised light, indicating that *d*- and *l*- are equally oxidised in the tissues, and thus equal quantities appear in the urine.[20]

[18] Zeit. f. physiol. Chem., **25**, p. 282, 1898.

[19] Biochem. Zeitschr., **36**, p. 32, 1911.

[20] Here a curious observation made by Thunberg (Skand. Arch. f. Phys., **40**, p. 52, 1920) may be mentioned. As has long been known, living organs decolorise methylene blue through enzyme action, but muscle loses this property when thoroughly washed out, and regains it on the addition of lactic acid (Harden). According to Thunberg *l*-tartaric acid may be substituted for lactic acid but not

In some other instances the tissues also fail to differentiate between the isomers in the same way; thus when *dl*-saccharic acid or the corresponding mannose acid is given to rabbits, the *dl*-form reappears in the urine, and it is not unlikely that symmetrical oxidation is commoner than has been supposed.[21] On the other hand *dl*-malate injected subcutaneously in the rabbit gives rise to *d*-malate in the urine (Tomita[22]), showing that the laevorotary malate, which occurs in nature, is more readily attacked by the enzymes of the body than the artificial *d*-malate. The contrast between the behaviour of malic and tartaric acid in this relation is striking from their near structural connection:

$$\begin{array}{l} \mathrm{CHOH \cdot COOH} \\ | \\ \mathrm{CHOH \cdot COOH} \end{array} \quad \text{tartaric acid,} \qquad \begin{array}{l} \mathrm{CH_2 - COOH} \\ | \\ \mathrm{CHOH - COOH} \end{array} \quad \text{malic acid}$$

The arabinoses were examined by Neuberg and Wohlgemuth,[23] who found that in the rabbit less

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d-tartaric, and this receives some support from Lehmann (Skand. Arch. f. Phys., **42**, p. 266, 1922), who however finds the reaction rather inconstant and does not deny the property altogether to the *d*-tartaric acid, though he states that it possesses it in smaller measure than the *l*-isomer. Lipschitz and Gottschalk (Pflüger's Arch., **191**, p. 1, 1921) on the other hand found the *dl*-tartaric acid ineffective, and criticise the whole of the observations; in view of Lehmann's half-hearted support, Thunberg's differentiation between the tartaric isomers cannot be regarded as established.

[21] Färber, Nord u. Neuberg. Biochem. Zeitschr., **112**, p. 313, 1920.

[22] Biochem. Zeitschr., **123**, p. 231, 1921.

[23] Zeitschr. f. physiol. Chem., **35**, p. 41, 1902.

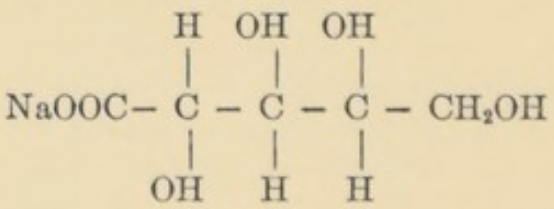
l-arabinose appeared in the urine than *d*-arabinose, when they were given by the mouth or subcutaneously, while there was less difference after intravenous injection, probably because they were eliminated too rapidly to allow of complete assimilation by the tissues. The amount of *dl*-arabinose excreted lay between the two active forms, as was to be expected. The actual percentages of the ingested arabinoses which were eliminated in the urine are as shown in table 1. Thus *l*-arabinose

TABLE 1

	GIVEN BY MOUTH	SUBCUTANEOUSLY
l-arabinose	14.5	7.1
d-arabinose	31.2	36.0
dl-arabinose	28.5	31.7

undergoes assimilation in the tissues more rapidly than the *d*-variety; it also leads to the formation of glycogen in the liver, while the *d*- and *dl*-arabinose do so to a less extent, if at all.

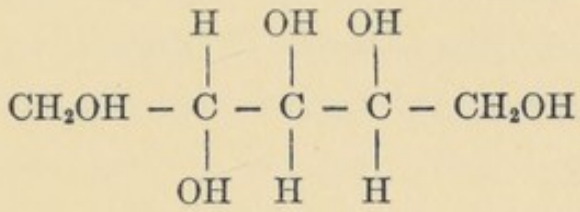
The three sodium arabonates



investigated in the same way also differ in the readiness with which they are destroyed, but here

the *d*-isomer is the more readily oxidised and less of it is excreted in the urine than of the *l*-form.

When *d*- or *dl*-arabite



is given to an animal, it leads to the appearance of some fructose in the urine, while this is absent after *l*-arabite. Of the mannose isomers, Neuberg and Mayer[24] found the *d*- more readily oxidised in the tissues of the rabbit, while more *l*- appears in the urine, when *dl*-mannose is given in excess; glycogen is formed from both forms, as also from both *d*- and *l*-glucose.

Mackenzie[25] states that *d*-β-oxybutyric acid is more readily decomposed in the tissues than *l*- and that when the *dl*-form is injected, both *l*- and *d*-varieties appear in the urine, but the *l*- in greater amount. Similarly[26] when sodium *dl*-phenyl-γ-oxybutyrate was given to a dog, the urine contained more of the *l*-isomer than of the *d*-,

[24] Zeitschr. f. physiol. Chem., **37**, p. 530, 1903.
Mayer. Cong. f. inn. Med., 1902, p. 486.
[25] Journ. Chem. Soc., **81**, II, p. 1409, 1902.
[26] Thierfelder u. Schempp. Pflüger's Arch. f. d. g. Phys., **167**, p. 280, 1917.

indicating that more of the latter undergoes oxidation in the tissues.

When *dl*-camphor is fed to dogs[27] or rabbits,[28] more of the *l*-isomer escapes as camphorol glycuronate than of the *d*-one, which thus appears to undergo combustion more readily in the tissues. There is less differentiation between *d*- and *l*-borneol,[29] though here also rather more of the *l*- appears in the urine as glycuronate.

Parnas[30] states that when lactates are injected hypodermically in the rabbit, over 95 per cent of the *d*- is oxidized, only about 2 per cent being recovered in the urine, while about 48 per cent of the *l*-lactate injected is recovered; after the injection of the racemic lactate there is an excretion of only about 6 per cent in the urine, most of this being *l*-; this seems to demand confirmation.

Similar differences are seen in the relative amounts of some of the isomers of the amino-acids which undergo destruction in the tissues and here, as might be expected, the one that is normally formed in the degradation of proteins, is the more quickly oxidized. Thus Wohlgemuth[31] found that

[27] Mayer. Biochem. Zeitschr., **9**, p. 439, 1908.
[28] Hämäläinen. Skand. Arch. f. Phys., **23**, p. 86, 1910.
[29] Magnus-Levy. Biochem. Zeitschr., **2**, p. 319, 1907.
[30] Biochem. Zeitschr., **38**, p. 53, 1912.
[31] Ber. d. d. chem. Gesell., **38**, p. 2064, 1905.

when the racemic form of tyrosin, leucin, aspartic or glutamic acids is ingested by rabbits, the naturally occurring component, *l*-tyrosin, *l*-leucin, *l*-aspartic and *d*-glutamic acid is excreted in the urine in smaller proportions than the foreign isomer; this was confirmed for leucin by Abder-

TABLE 2

	LAEVOROTARY	DEXTRO-ROTARY
Malate	+	
Arabinose	+	
Arabonate		+
Arabite	+	
Mannose		+
β-Oxybutyrate		+
γ-Phenyl-γ-oxybutyrate		+
Camphor		+
Borneol		+
Lactate		+
Leucine, tyrosin, aspartic acid	+	
Glutamic acid		+
Alanine		+
Hyoscyamine	+	
Hyoscine	+	

halden with Samuely[32] and Kautzsch,[32] Dakin[33] and others. When alanin was given in the racemic form to dogs by the mouth, *l*-alanin appeared in

[32] Zeitschr. f. physiol. Chem., **47**, p. 346, 1906.

Abderhalden u. Kautzsch. Zeitschr. f. physiol. Chem., **48**, p. 557, 1906.

[33] Journ. Biol. Chem., **8**, p. 25, 1910–1911.

the urine,[34] the dextrorotary isomer undergoing oxidation in the tissues more readily.

The results of these observations on the relative power of the tissues to assimilate the two active isomers are arranged together in table 2, in which + indicates the one more readily acted on by the organism.

There is thus, as in the enzymes and the substances of known chemical composition, a differentiation in the tissues between the two isomers; and again as in the unorganised agents, the choice is not always of the substance of the same sign of rotation. An alkaloid added to different racemic acids does not always form a less soluble compound with the *d*-isomer, and similarly the enzymes of yeast etc., and those which control assimilation in the tissues do not attack by preference bodies having one sign of rotation. It has been pointed out repeatedly that in the higher organisms the isomers occurring in nature are generally more readily destroyed than their counterparts which are formed artificially or at any rate occur more rarely. But the bodies hitherto examined in this

[34] Hirsch. Zeitschr. f. exp. Path. u. Ther., **1**, p. 141, 1905.
Abderhalden u. Schittenhelm. Zeitschr. f. physiol. Chem., **51**, p. 323, 1907.
Schittenhelm u. Katzenstein. Zeitschr. f. exp. Path. u. Ther., **2**, p. 560, 1906.
Plant u. Reese. Hofmeister's Beiträge, **7**, p. 424, 1906.

respect have in many instances been such as are habitually utilised by the tissues and it is not unlikely that further experience may afford more obvious exceptions to this rule than have been met hitherto.

PHARMACOLOGICAL ACTION OF OPTICAL ISOMERS

It remained to determine whether the functions of the cells which are not generally regarded as merely enzymatic in character also have this power of differentiating between the isomers. The earliest evidence was offered by Piutti,[35] who pointed out that *d*-asparagin has a sweet taste, while the natural *l*-isomer is insipid; it is of interest to find Pasteur[36] ascribing this difference to the presence of an optically active substance in the nervous mechanism of taste and pointing out the analogy with the ferments which he had demonstrated thirty years previously. Menozzi and Appiani[37] showed that *d*-glutamic acid has a characteristic taste which is not shared by its *l*-isomer and the dextrorotary isomers of serin,[38] histidin[39] and phenylalanin all are distinctly sweeter than the laevorotary or the racemic ones.

[35] Comptes rend., **103**, p. 134, 1886.
[36] Comptes rend., **103**, p. 138, 1886.
[37] Atti. R. Accad. d. Lincei [5], 1893, II, p. 421.
[38] Ehrlich. Biochem. Zeitschr., **8**, p. 438, 1908.
[39] Pyman. Trans. Chem. Soc., **111**, p. 1105, 1917.

Sternberg[40] states that the taste organs do not as a rule differentiate between the isomers qualitatively though it is possible that quantitative differences may exist.[41] Some statements are made as to the odours of the isomers differing, but these are qualitative and have not received much attention.

Many attempts have been made to compare the pharmacological and toxicological effects of pairs of optical isomers. Some of these have been done with more zeal than judgment, and unfortunately their erroneous results have been propagated by one writer after another, apparently without reading the original papers or at any rate without regarding them critically.

The Tartrates. Several such unsatisfactory papers concern the tartrates. Chabrie,[42] comparing the toxicity of *d*- and *l*-tartaric acid by injecting them into the peritoneal cavity, found them different; but death here was not due to the optically active portion, but to the hydrogen ion, which is common to both isomers, and the supposed difference in toxicity was purely casual. The same objection holds to Karczag's[43] experiments on the blood pressure done by the injection of the isomeric acids directly into the blood stream, or on the tortoise heart by direct application of

[40] Arch. f. [Anat. u.] Phys., 1898, p. 457.
[41] Werner u. Conrad. Ber. d. d. chem. Gesell. **32**, p. 3052, 1899.
[42] Comptes rend. de l'Acad., **116**, p. 1410, 1893.
[43] Zeitschr. f. Biol., **53**, p. 218, 1910.

the acids to it. Chio,[44] whose results have been cited recently, also compared the four tartaric acids as regards their effects on serum reaction, on blood clotting, and on toxicity when injected into the peritoneal cavity and the carotid artery. These oft quoted experiments are quite valueless, for here the essential tartrate action is complicated by the vastly more powerful one of the H-ion. And Salant and Smith[45] seem to have noted no significant difference in the toxicity of the sodium salts of the *d*- and *l*-tartaric acids in frogs and rabbits. On the other hand Salant, Mitchell and Schwartze[46] state that in experiments on the suspended gut of the rabbit and cat they found the *l*-tartrate increase the movement in a strength of N/200, while the *d*- acted only in N/25 solution; the *dl*-tartrate appeared to lie between the two active forms in effectiveness. These experiments need further confirmation in view of the absence of other differences in the effects of the tartaric isomers. Apart from them there appears to be little or no evidence of difference in the action of the *d*- and *l*-tartaric esters on the tissues or in their reaction to the destructive activities of the cells.

Hyoscyamines. The first definite example of difference in the pharmacological action proper between two optical isomers was offered[47] by *l*- and *dl*-hyoscyamine (atropine), which were kindly given me in pure form by the late Profes-

[44] Arch. Ital. de Biol., **60**, p. 283, 1913.
[45] Amer. J. Phys., **35**, p. 239, 1914.
[46] Journ. Pharm. Exp. Ther., **9**, p. 511, 1917.
[47] Journ. Phys., **30**, p. 176, 1904.

sors Prescott and Schlotterbeck of the University of Michigan. The two bases were found to be equally poisonous to many organs such as the heart, muscle and motor nerve ends in the frog, while atropine was more excitant in the central nervous system in these animals than *l*-hyoscyamine and this action lasted longer. In the mammals, many organs were unaffected by even large doses, and some others, such as the heart-muscle and central nervous system reacted to the same extent to the isomers, but in a number of instances I found that *l*-hyoscyamine was almost exactly twice as powerful as atropine. This was seen in the more specific actions of this group on the terminations of the autonomic nervous system, such as the myoneural junctions of the inhibitory fibres in the heart, of the motor oculi in the iris and of the secretory fibres in the submaxillary glands of the dog; the same ratio between the isomers holds for the intestine and other organs in which the action of atropine is not apparently exerted on the actual nerve terminations.[48] The experiments on

[48] Macht. (Journ. Pharm. and Exp. Ther., **12**, p. 255, 1919) states that *l*-hyoscyamine stimulates the contractions of the ureter, while *d*- has no stimulating properties. I am unable to accept this in view of the inconstancy of the stimulating effect of atropine on organs containing smooth muscle (See van Lidth de Jeude, Pflüger's Arch., **170**, p. 523, 1918; and Cushny, Journ. Pharm. and Exper. Ther., **17**, p. 41, 1921).

the eye and heart rate consisted in injecting the alkaloids on different days and comparing the amounts necessary to dilate the pupil or accelerate the heart to the full; in a cat for example the minimal hypodermic dose of *l*-hyoscyamine to cause distinct mydriasis was 0.025 mgm. while 0.06 mgm. of atropine was necessary; similarly 0.06 mgm. of *l*-hyoscyamine sufficed to paralyse the cardiac inhibition, while 0.12 mgm. of atropine was necessary.

The method used to compare the salivary secretion, which in my experience gives very accurate results, is as follows: A permanent fistula is established in the submaxillary duct, and the saliva secreted in each five minutes is collected on a weighed piece of cotton wool, which is then weighed again; atropine or *l*-hyoscyamine is injected hypodermically on successive days. The lowest dose necessary to arrest the saliva was found to be 0.25 mgm. of hyoscyamine or 0.5 mgm. atropine. Thirty minutes after the injection of these alkaloids, an injection of 5 mgm. of pilocarpine was made and the course of the secretion was followed for 20 to 60 minutes longer. The power of atropine and *l*-hyoscyamine to antagonise the secretory action of pilocarpine was thus compared and was again found to bear the ratio of 1:2 (figs. 3 and 4).

The same result is given by the experiments in

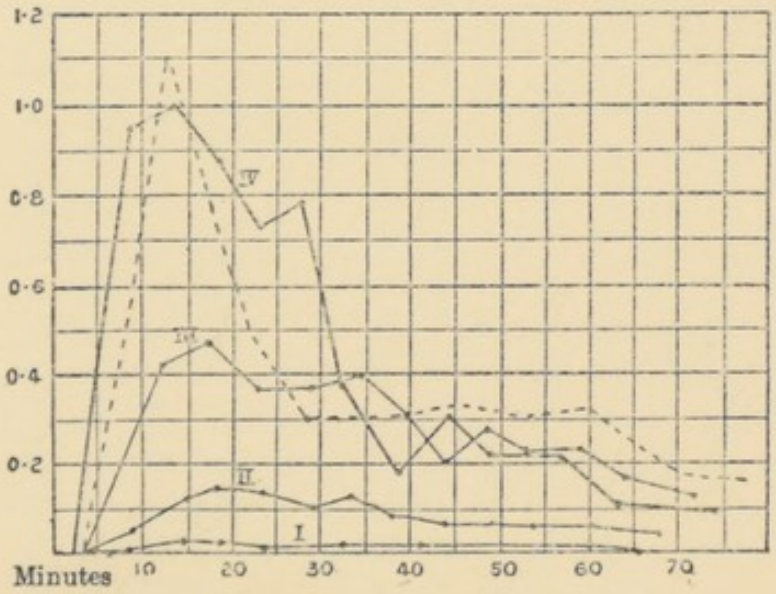


FIG. 3

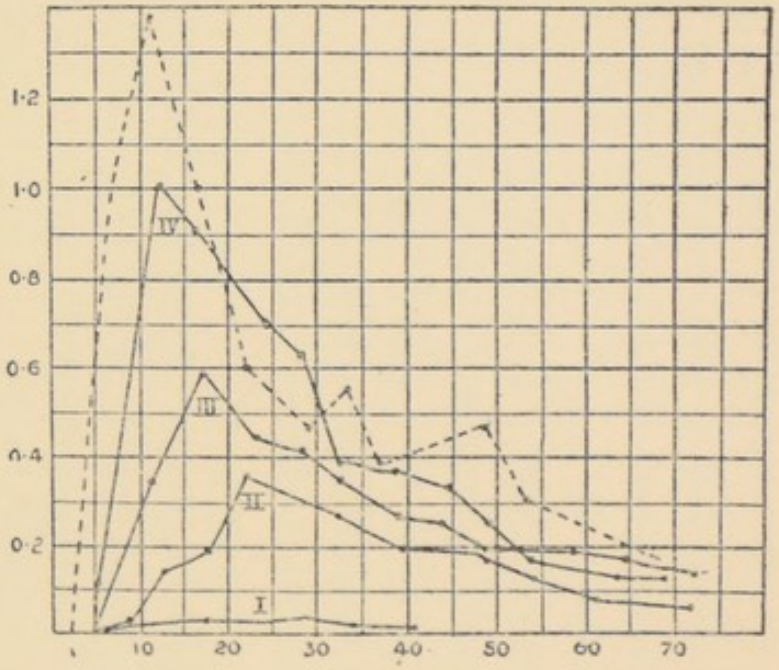


FIG. 4

FIG. 3. GRAPHS OF THE EFFECT OF PILOCARPINE (5 MGM.) ON THE SALIVARY SECRETION OF A DOG AFTER THE INJECTION OF ATROPINE IN DOSES OF 0.5 (I), 0.25 (II), 0.12 (III) AND 0.062 MGM. (IV)
The dotted line represents the secretion under pilocarpine without atropine. (Compare figure 4)

FIG. 4. GRAPHS OF SALIVARY SECRETION UNDER PILOCARPINE AFTER THE INJECTION OF *l*-HYOSCYAMINE IN DOSES OF 0.25 (I), 0.12 (II), 0.06 (III), AND 0.03 (IV)
Hyoscyamine reduces the secretion to the same extent as atropine, when given in half the dose

table 3 in which the pilocarpine secretion is reduced by hyoscyamine and atropine to practically the same extent when twice as much of the latter is given as of the former. The specific action of the *l*-hyoscyamine is thus twice as great as that of atropine as far as these experiments go. Now atropine is *dl*-hyoscyamine, that is two molecules

TABLE 3

DATE	DOSE IN MILLIGRAM AND ALKALOID	SALIVA IN FIRST 15 MINUTES AFTER PILOCARPINE	SALIVA IN 15 TO 30 MINUTES	SALIVA IN 30 TO 45 MINUTES	SALIVA IN 45 TO 60 MINUTES	TOTAL SALIVA IN 1 HOUR
January 19	Atropine, 0.125 mgm.	0.27	8.9	7.1	4.1	20.37
January 20	Hyoscy., 0.125 mgm.	0.26	1.71	2.50	2.03	6.5
January 21	Atropine, 0.25 mgm.	0.16	0.93	1.86	1.71	4.66
January 22	Hyoscy., 0.25 mgm.	0.03	0.47	0.64	0.53	1.67
January 23	Atropine, 0.5 mgm.	0.20	0.32	0.60	0.41	1.53

of atropine contain one of *l*-hyoscyamine and one of *d*-hyoscyamine.

$$2\,dl = l + d \text{ chemically}$$
$$= l \text{ in pharmacological action}$$

The inference drawn was that the *d*-isomer is practically devoid of action on the myoneural junctions of the autonomic system.

Later experiments in which the *l*- and *d*-isomers were compared directly, confirmed this in essen-

tials (table 4). Both isomers were found to act in the same way, but the laevo- about 12 to 20 times as strongly as the dextro-, except in the central nervous system in which the dextro- caused distinctly greater and longer excitation than the laevorotary. My best estimates[49] give the relative strength of 1:20 to *d*- and *l*-hyoscyamine.

TABLE 4

DATE	DOSE IN MILLIGRAMS	SALIVA IN 15 MINUTES AFTER PILOCARPINE	SALIVA IN 15 TO 30 MINUTES	SALIVA IN 30 TO 45 MINUTES	SALIVA IN 45 TO 60 MINUTES	TOTAL SALIVA IN 1 HOUR
February 4	Pilocarpine only	7.86	16.7	13.75	8.2	46.51
February 5	L, 0.125 mgm.	0.01	0.9	0.76	0.57	2.24
February 6	D, 1.1 mgm.	0.79	3.25	2.32	1.6	7.96
February 7	D, 3.5 mgm.	0.00	0.05	0.19	0.18	0.42
February 8	L, 0.25 mgm.	0.00	0.19	0.4	0.39	0.98
February 13	D, 2.0 mgm.	0.31	1.52	1.45	1.25	4.53
February 14	L, 0.125 mgm.	0.04	1.68	2.97	2.3	6.99
February 15	D, 1.5 mgm.	1.11	3.05	2.15	1.65	7.96

Laidlaw[50] found them vary from 1:25 on the cardiac vagus to 1:100 by direct application to the iris, but the latter method is not very accurate.

Hyoscines. Another series of observations[51]

[49] Journ. Pharm. and Exp. Ther., **15**, p. 105, 1920.

[50] See Barrowcliff and Tutin. Journ. Chem. Soc., **95**, II, p. 1966 (1909).

[51] Cushny and Peebles. Journ. Phys., **32**, p. 501, 1905.

was made in the same way on *l*- and *dl*-hyoscine and gave the same results on the peripheral nervous terminations, the *l*-base acting almost exactly twice as strongly as the *dl*-one on the cardiac inhibitory, the oculomotor and the secretory nerves to the salivary glands. Later experiments[52] in which the *l*- and *d*-hyoscines were compared directly, showed that the laevorotary acted 16–18 times as strongly as the *d*-base on these organs and also as antagonists to pilocarpine in the gut. As regards the action on other tissues the two isomeric hyoscines appeared to be equivalent except that the *d*- maintained its effect longer, probably from being excreted or destroyed more slowly. The effect on the human brain of *l*- and *dl*-hyoscines were compared without obvious differences being detected,[53] but more recently Dr. Moir in examining the efficacy of *d*- and *l*-hyoscine in "twilight sleep" found little or no effect from *d*-hyoscine, while the *l*-isomer was efficient, so that the *l*-hyoscine appears to act more strongly on the central nervous system.

The *Homatropines* are also optically active and in comparing the relative efficiency of the *d*- and *dl*-forms, I find that in action on the salivary nerve

[52] Journ. Pharm. and Exp. Ther., **17**, p. 41, 1921.

[53] Richards and Light: see Cushny and Peebles, Journ. Phys., **32**, p. 501, 1905.

ends the ratio of *dl*:*d* is about 5:8, so that the *l*-isomer is approximately twice as powerful as the *d*-.[54]

Nicotines. Mayor[55] states that *l*-nicotine, the natural alkaloid, is twice as poisonous as *dl*-nicotine; some further differences were observed between them, such as greater local pain and more violent convulsions from the laevorotary. But it would be desirable to repeat these experiments.

Epinephrines. On comparing the natural *l*-epinephrine with its synthetic *dl*-isomer, it was found to be nearly twice as strongly vasoconstrictor, and I therefore concluded[56] that the *d*-rotary base was nearly devoid of action on the sympathetic terminations in the arterioles. Later,[57] I had the opportunity of comparing *l*- and *d*-epinephrine directly, and found that the laevorotary base acts about 12 to 15 times as strongly as the dextrorotary on the sympathetic ends in the vessels and on those controlling the glycogenic function in the liver: the relation of the minimal lethal doses of the two isomers appeared to be of the same order of magnitude but could not be ascertained so accurately. These results were confirmed by ob-

[54] Journ. Pharm. and Exp. Ther., **15**, p. 105, 1920.
[55] See Pictet, Ber. d. d. chem. Gesell., **37**, p. 1234, 1904.
[56] Journ. Phys., **37**, p. 130, 1908.
[57] Journ. Phys., **38**, p. 259, 1909.

servations by Abderhalden and his pupils,[58] by Tiffeneau[59] and others.

Two isomeric *Isoadrenalines* (β-methyl-nor-adrenalines), $(OH)_2C_6H_3 \cdot CHOH \cdot CH(CH_3)NH_2$, have been compared by Tiffeneau,[60] who states that the laevorotary one is nearly thirty times as active on the blood pressure as the dextrorotary.

The *Cocaines* form two series of alkaloids, which are isomeric but differ spatially, and are known as cocaine and pseudo-cocaine. Each of these is asymmetrical and thus presents two optically active isomers; their efficiency as local anesthetics and their toxicity when absorbed into the contral nervous system have been compared by the late Prof. R. Gottlieb,[61] and this in fact formed the last of his many valuable contributions to science.

In the normal cocaine series,[62] *l*-cocaine, the ordinary alkaloid, proved slightly more powerful than its *d*-isomer in depressing nerve fibres when applied

[58] Zeitschr. f. physiol. Chem., **58**, p. 185, 1908–1909; **59**, pp. 22 and 129, 1909.

[59] Comptes rend., **161**, p. 36, 1915.

[60] Cong. de Physiol., Paris, 1920.

[61] Arch. f. exp. Path. u. Pharm., **97**, p. 113, 1923.
Zeitschr. f. physiol. Chem., **130**, p. 374, 1923.

[62] Ordinary cocaine and the d-cocaine compared by Poulsson (Arch. f. exp. Path. u. Pharm., **27**, p. 309, 1890) and Ehrlich and Einhorn (Ber. d. d. chem. Gesell., **27**, p. 1870, 1894) belong to the two different series and are not mirror images; the latter is the more powerful as a local anaesthetic.

directly to them, while it is distinctly superior in effect in infiltration anaesthesia and in application to the mucous membranes. The toxicity of *l*-cocaine on subcutaneous injection in mammals is 5 to 10 times as great as that of *d*-cocaine, but injected intravenously the two are practically equally poisonous. Gottlieb infers from this that *d*-cocaine is more quickly destroyed than its isomer; and he confirms this by comparison of their excretion in the urine, in which a larger proportion of the laevorotary cocaine injected reappears unchanged. Similar results were obtained later by Gruhn.[63]

In the pseudo-cocaine series the dextrorotary appears to be nearly twice as strongly anaesthetic as the racemic, and the laevorotary must thus be inferior to the *d*- and may indeed have little local anaesthetic action; but in some circumstances the difference does not seem to be distinct, since the *d*-ψ-cocaine may induce congestion and be more quickly removed by the blood current. The racemic form is more poisonous in cat experiments than the *d*-ψ-cocaine, so that again, as in the normal series, the *l*- appears more toxic than the *d*-. The dextrorotary form is rendered harmless in the tissues more quickly than the laevorotary.

[63] Arch. f. exp. Path. u. Pharm., **106**, p. 115 (1925).

Racemic *β-Eucaine* has been resolved into the component *d-* and *l*-isomers by King,[64] and their pharmacological properties have been compared by Burn, who finds that they are equally powerful as local anaesthetics on the rabbits' cornea and on the exposed sciatic nerve of the frog, but that the *l*-form is about twice as poisonous as the *d-* while the *dl-* is intermediate. A stereoisomeric form, iso-*β*-eucaine was also separated into *d-* and *l*-components, which were equal in anaesthetic action and also in toxicity.

The two known forms of *Coniine*, *d-* and *dl-*, induce identical symptoms in rabbits and mice (Ladenburg and Falck[65]).

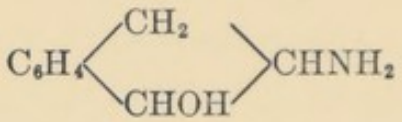
On comparing the effects of *d-* and *l-Tetrahydroquinaldine* ($C_6H_4 : C_3H_5 \cdot CH_3 \cdot NH$), Dale and Mines[66] found them identical on the frog's heart, while the laevo- was more poisonous to skeletal muscle, which shortens and loses its irritability; the *l*-base is about $1\frac{1}{2}$ times as toxic as the *d-*. The direct muscular excitability is reduced much more quickly by the *l-* and cannot be restored.

Camphors. Soon after *dl-* and *l*-camphor were put on the market, a number of papers appeared in which some minor differences were recorded

[64] Trans. Chem. Soc., **125**, p. 41, 1924.
[65] Annalen der Chemie, **247**, p. 83, 1888.
[66] Journ. Phys., **42**, Proc. Phys. Soc., xxxi, 1911.

between the action of the *l*- and *d*-isomers.[67] But such differences are very liable to occur in the comparison of substances which are so insoluble in the body fluids as the camphors unless great care is exercised and numerous experiments are made, and the elaborate researches of Joachimoglu and others[68] do not indicate any definite distinction between the action of these isomers. Thus they are equally toxic in rabbits and cats and on the frog's heart, and equally germicidal, and their action on suspended unstriated muscle is apparently identical.

The two optically active *Hydroxyhydrindamines*



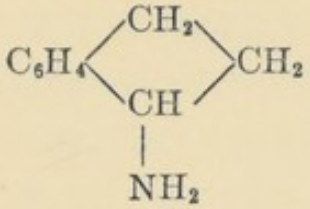
have been compared by Ikeda[69] and found to be weak poisons to striated, unstriated, and cardiac muscle and to micro-organisms, but seem to have no specific action on any one organ. The *d*-isomer is slightly more active than the *l*-.

[67] Langaard u. Maas. Ther. Mon., **21**, p. 573, 1907.
Hämäläinen. Skand. Arch. f. Phys., **21**, p. 64, 1909.
Grove. Journ. Pharm. and Exp. Ther., **1**, p. 445, 1909–1910.

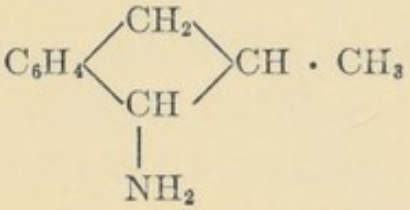
[68] Arch. f. exp. Path. u. Pharm., **80**, pp. 1, 259, 282 (1917); **88**, p. 364, 1920.
Dohrn. Arch. f. exp. Path. u. Pharm., **97**, p. 38, 1923.
Macht. Journ. Pharm. and Exp. Ther., **12**, p. 255, 1919.

[69] Journ. Pharm. and Exp. Ther., **7**, p. 121, 1915.

On comparing the action of *l*- and *d-hydrindamine*



and of *l*- and *d-methylhydrindamine*



on intact frogs, and on the isolated muscle and heart of the frog in my laboratory, Harrison did not observe any difference in the action between the isomers, which proved to be only feebly poisonous to the frog's heart and striated muscle.

Tetrahydro-β-naphthylamine has the same action whether given as the *d*-, the *l*- or the *dl*-form.[70] In frogs each causes paralysis along with dilation of the pupil. In rabbits the pupil is dilated and a rise of temperature occurs.

The *Canadine-metho-chlorides* have been examined by Laidlaw,[71] who points out that they exist in four different isomeric forms, inasmuch as an

[70] Cloetta und Waser. Arch. f. exp. Path. u. Pharm., **73**, p. 398, 1913.

[71] Journ. Pharm. and Exp. Ther., **4**, p. 461, 1912–13.

asymmetric carbon atom in the molecule is complicated by asymmetry in the nitrogen.[72] There are thus *d*- and *l*-α-canadine-metho-chlorides and *d*- and *l*-β-canadine-metho-chlorides. Only the *l*- and *dl*-forms were available and the action of the *d*-isomers was inferred by deducting the action of the laevorotary from the racemic form in each instance.

All four forms paralyse the terminations of the motor nerves in striated muscle in the frog, but in very different degree, as is shown in the following ratios of toxicity.

αl-canadine-methochloride	1
αd-canadine-methochloride	9

[72] The presence of an asymmetric N in a molecule also gives rise to isomers, but these are not so interesting as those arising from carbon asymmetry since they differ widely in their physical characters, such as solubility, melting points, water of crystallisation and crystalline form. They also differ in their pharmacological action in the few instances in which they have been examined. Thus Laidlaw finds that the β-*l*-canadine methochloride is twelve times as powerful as the corresponding α-isomer, while the β-*d*- is about three times as poisonous as the α-*d*-.

Hildebrandt (Arch. f. exp. Path. u. Pharm., **53**, p. 76, 1905) had previously shown that in a series of quaternary derivatives from coniine, such as ethyl-benzyl-coniium-iodide and others, the α-form is less poisonous than the β-form to frogs and mammals and he associates this with the higher melting point of the β-isomer; the difference in toxicity is comparatively small. The corresponding derivatives from conhydrine showed even smaller variation in activity and here the more poisonous was not always that with the higher melting point.

βl-canadine-methochloride 12
βd-canadine-methochloride 28

In each case the laevorotary alkaloid is weaker than the dextrorotary one, in the *α*-form the proportion being 1:9, in the *β*-form 3:7.

As far as simple diffusion is concerned optical isomers of course are identical in penetration, and in accord with this Dakin[73] finds that in absorption from the intestine of solutions of *dl*-bodies the isomers are taken up equally, so that the solution remains optically inactive. On the other hand Katake and Okagawa[74] state that when the isomeric oxyphenyl-lactates are exposed to red blood cells, only the *l*-form permeates freely into the cells, while the *d*- and *dl*- are taken up with difficulty or not at all. It seems unlikely that this can be correct, since the *dl*-contains the *l*-isomer, and further confirmation is required.

GENERAL ASPECTS OF THE PHARMACOLOGICAL ACTION

I have thought it advisable to enter upon this part of the subject in greater detail than the others, because it is more recent, the accounts of it are scattered through a large number of journals,

[73] Journ. Biol. Chem., **4**, p. 437, 1908.
[74] Journ. Biochem. (Japanese), **1**, p. 159, 1922.

and some inaccurate or unestablished general statements have been made.

In these pharmacological investigations the tissues sometimes prove able to differentiate between two optical isomers, while in other instances no distinction is made between them, each inducing the same effect in the same measure. There are of course in addition many tissues which fail to give any response to either isomer.

The results in the first class are in accord with those described in the earlier pages of these lectures; optical isomers differ in their pharmacological action proper, just as they differ in their reaction with enzymes or with substances of known structure such as alkaloids or acids. And they exhibit here also the same variability; for example, among the hyoscyamines and hyoscines, the laevorotary forms act about fifteen times as strongly as the dextrorotary in certain organs in which they are said to exert a "specific action," while in other positions such as in striated muscle or the nerve-ends supplying it, the isomers are equally and only feebly toxic. A parallel may be drawn with *l*- and *d*-lactates which differ in their reaction to penicillium, while they are equally responsive to some bacteria; and a further parallel exists between the behaviour of these isomers in the tissues and that originally observed by Pasteur

between the tartrates and the cinchona alkaloids. There can, I think, be no question that in all these three conditions the differences observed between the reactions of two isomers arise from the same fundamental cause and that for example *l*-adrenaline contracts the vessels of the conjunctiva more strongly than *d*-adrenaline from the same principle that makes *d*-tartrate more readily oxidised by

TABLE 5

ALKALOIDAL CATALYSTS ACTING ON THE CAMPHOR-CARBOXYLIC ACIDS	ENZYMES ACTING ON MANDELIC ACID	PHARMACOLOGICAL ACTION OF HYOSCYAMINES
Dextrorotary acid destroyed more readily by quinidine	Dextrorotary attacked by schizomycetes	Dextrorotary acts more readily on nerve cells
Laevorotary by quinine	Laevorotary by penicillium	Laevorotary on autonomic terminations

penicillium and less easily precipitated by cinchonine than the *l*-tartrate, that is because in the tissues, as in the test-tube, the isomers form compounds with some optically active substance and these compounds are no longer mirror images and no longer identical in their physical characteristics. For example, it may be supposed that *l*-adrenaline may cause a precipitate readily in the myoneural junction, while *d*-adrenaline does so only when it is present in much higher concentration, just as they differ in their reaction with camphor-sulphonic acid.

Fajans has used a very telling table (table 5) to illustrate that the catalysis induced in antipodal bodies in the presence of optically active alkaloids is of the same nature as that induced by enzymes, and I may borrow some of his items and supplement them by adding a column exemplifying the analogy of pharmacological action. In each case the more effective of the two isomers is given with the agent with which it reacts.

The parallelism is so striking that the suggestion is unavoidable that in all three columns the difference in the behaviour of the isomers is of the same origin. Now the reaction between the alkaloids such as quinine and camphor-carboxylic acid or the cinchonidine and tartaric acid of Pasteur partakes of the nature of such simple chemical combinations as that between sulphuric acid and barium and hence it has been inferred that in the action of these optical isomers in the living tissues the essential feature is a true chemical reaction differing in no essential features from those observed in the chemical laboratory.

It is true that some attempt has been made to show that in the reaction between two optically active substances such as tartrate and alkaloid, there is besides the simple reaction of acid and base another more mystical affinity (Erlenmeyer); one of the two isomeric acids is supposed to have a

special predisposition to unite with one of the two isomeric bases rather than with the other. But this has not been established and need not be taken into account at present.

On the other hand it has been claimed by Porter and Hirst[75] and more recently by Porter and Ihrig[76] that isomers may be separated by physical processes alone without true chemical combinations being formed: they found that some racemic asymmetric dyes can be analysed into their *d*- and *l*-components by soaking wool in them, the *d*-isomer being taken up with much greater avidity than the laevorotary one. On the tacit assumption that the dye is held by adsorption they conclude that chemical affinity can play no part in the separation. But it is generally held[77] that in adsorption the chemical processes are by no means excluded, so that their argument is not decisive.

In the comparison between the first and third columns of table 5, the analogy must not be pushed too far. In the first column in which the relations between definite known bodies endowed with optical activity is given, the combination is between

[75] Journ. Amer. Chem. Soc., 1919, **41**, p. 1264.

[76] Journ. Amer. Chem. Soc., 1923, **45**, p. 1990.

[77] For example Bayliss (1912) says " . . . . there is reason to believe that chemical relationship plays an important part in adsorption phenomena,"

an acid and a base. I do not wish to suggest that the relation between the enzymes and the mandelic acids of the second column or between the living tissues and the hyoscyamines of the third column also bear this simple relation: in fact it would be difficult to suppose that this can be the fact, for in the test tube experiments, a fairly strong acid combines with a base, and it is hardly likely that these conditions can be present in living tissues. What I suggest is that here some chemical combination occurs of such a nature that the isomers no longer form mirror images and these therefore differ in their physical properties and reactions. Whether this occurs through their forming esters with constituents of the enzymes and tissues or through some more occult combination is immaterial, so long as the chemical combination is formed.

Of recent years there has been a general tendency to attribute pharmacological action almost exclusively to physical changes wrought in the cells or tissues by the presence of poisons, which are supposed, for example, merely to change the mutual relations of the normal constituents by rendering some of them more soluble and therefore more mobile. Thus adherents of the Meyer-Overton theory of narcotic action have supposed that such a drug as chloroform accumulating in the nerve cell through its solubility in the lipoids renders

these more mobile and thus alters their relation to the proteins and other constituents, and that this change is the essential factor in the altered activity which we term narcosis and anaesthesia. And extremists have extended this physical view from the group of chloroform and its allies to other poisons, and have suggested that the altered activity of living tissues in the presence of poisons always arises from similar changes in the physical relations of the cell constituents rather than from actual chemical combinations being formed between these and the intruders; so that if the rates of diffusion of a drug in different media were known, its action could be inferred without reference to its chemical affinities.

The frontiers of physics and chemistry can no longer be drawn with the precision that was formerly supposed to be possible, and in every pharmacological reaction and physiological process such physical properties as diffusibility must play a large part. But in these optically active substances it is clearer than elsewhere that there are involved in addition processes which are essentially of the same nature as the reaction of an acid with an alkali and which must therefore be regarded as typically chemical. It may be surmised that in pharmacological activity the relation between the living cell and the drug is seldom purely chemical

or purely physical and that the proportion that these forces bear to each other varies from instance to instance.

The most pronounced differences in the behaviour of the isomers are confined to the three series which I was fortunate enough to meet early in my work—the hyoscyamines, hyoscines and adrenalines, and to the series studied by Laidlaw, in which the asymmetry of the carbon is complicated by that of the nitrogen. In some others while one isomer is distinctly more powerful than its opposite number, the ratio is not greater than 2:1. Such are the homatropines, cocaines, pseudo-cocaines, β-eucaines, iso-β-eucaines and tetrahydroquinaldines. And in some instances the two isomers have proved to exert the same effects on all the tissues examined—coniines, camphors, borneols, several hydrindamine compounds and tetrahydronaphthylamines. Difference in the pharmacological action of optical isomers is therefore by no means the rule; and this is still more obvious when it is borne in mind that in every experiment each isomer is exposed to many thousand different optically active reagents in the different tissues of the body, yet few tissues give any evidence of change at all and still fewer differentiate between the isomers.

The difference in the behaviour of isomers in the

tissues is strikingly illustrated by comparing the effects of the hyoscyamines on the myoneural junctions of the chorda tympani and on those of a motor nerve to striated muscle; the sign of rotation of the alkaloid determines its action on the first while it is indifferent as regards the second, and this suggests that the nature of the action which is exerted in those positions is distinct in character. Elsewhere, for example in the terminations of the cervical sympathetic, there appear to be almost no significant changes; yet the blood carries to all three sets of terminations the same proportion of poison. In discussing the relationship of the enzymes and isomeric substrates I have pointed out analogous qualities; thus the immunity of talose to fermentation by zymase may be compared with the failure of either of the hyoscyamines to act on sympathetic terminations, while apparently nearly related structures are readily affected; the equal reaction of the two lactates to certain micro-organisms finds its analogy in the equal reaction of the motor nerves to striated muscle to each of the hyoscyamines, while the great difference in their action on the terminations of the chorda tympani may be compared to the relative susceptibility of the two tartrates to the destructive action of penicillium. And a similar parallel may be drawn with the

reactions with pure substances, camphorsulphonic acid for example sometimes failing to precipitate either of two isomeric bases, precipitating them almost equally, or differentiating between the isomers readily.

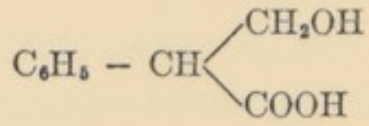
King has recently attributed the difference in the action of optical isomers to less of the one than of the other reaching the site of action owing to different amounts being intercepted on the way; he bases this view upon the observations of Porter that optically active dyes are differentially adsorbed by wool, so that an active isomer may be separated from a racemic solution by wool soaked in it. The two isomeric hyoscyamines must be present in the blood stream in equal amounts, for they have equal effects on the terminations of the motor nerves to muscle, and to explain the difference in their action in the salivary glands on King's view it would be necessary to assume that in their passage from the blood into the receptor on the myoneural junction about fifteen times as much of the dextrorotary isomer is detained by adsorption as of the laevorotary; in the brain of the frog on the other hand more of the laevorotary would be arrested in the interval between the plasma and the nerve cell. This view then relegates the differentiation between the isomers to the site at which they act, but regards the selection as

directed to the less powerful of the two, which is retained in the outer membrane, while the more natural view, I think, would be that the more powerful isomer is selected by the essential elements. It is further difficult to bring the physical retention of one isomer into relation with the phenomena seen in the experiments with enzymes or with simpler substances of known structure, with which they react in solution.

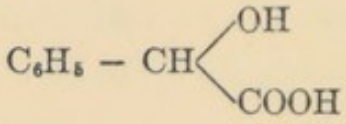
Gottlieb is disposed to attribute the differences in the local or systemic effects of the cocaine isomers to their varying susceptibility to destruction by enzymes, and in this particular case, it is possible that this plays some part; but this cannot easily account for their differing in their local anaesthetic action and cannot be applied as a general explanation for the difference in action of other isomers. For again the stronger action of *d*-hyoscyamine on the brain would on this view imply a more rapid destruction of the *l*-isomer, which would be incompatible with the stronger action of the *l*- on the nerve endings.

Considerable variations occur in the extent to which a tissue differentiates between the isomers; for example the specific action of *l*-hyoscyamine is fifteen times as powerful as that of its opposite, while the ratio between *l*- and *d*-homatropine is only about 2:1, although there can be no question

that these alkaloids act on the same receptors and their near relationship is indicated by their structural formula; in hyoscyamine tropine is attached to tropic acid



in homatropine to mandelic acid



The fact that one isomer acts more strongly than its counterpart on any tissue suggests that the latter is optically active but gives no clue to the direction of the rotation; curiously enough the receptors which respond so strongly to *l*-adrenaline or to *l*-hyoscyamine, also react strongly to their antagonists, dextrorotary and ergotoxine pilocarpin.

In the three instances in which the difference between the pharmacological action of isomers was first recognized, the predominance of the laevorotary observed in most tissues is interesting, and also the fact that this is the alkaloid occurring in nature, while the dextrorotary is less widely distributed or may occur only from artificial synthesis.

This has given rise to some speculation; for example, the greater action of the laevorotary component has been ascribed to some cosmic influence, and that of the natural form has been supposed to indicate some mystical harmony in nature, which is still beyond our ken. But the instances known are still far too limited in number for such generalisations, and of those known, the laevorotary "natural" isomer is not always the more powerful. Its effects on the autonomic system are so striking that they have diverted attention from the symptoms of central nervous excitation by hyoscyamine, but here the action seen in the frog is elicited only by the dextrorotary "unnatural" isomer; and among the pseudococaines also the laevorotary is not the stronger in local action, while the natural dextrorotary base is less toxic than the unknown laevorotary one. A still more striking instance is seen in the canadine methochlorides, in which the natural laevorotory α-l-form, which is found in Xanthoxylum, is nine times weaker than the synthetic dextrorotary one. Different tissues select different isomers in the same way as among the enzymes, of which one destroys the laevorotary, another the dextrorotary one; and the reason for selection of the one or the other is at present equally obscure in the living tissues, in enzymes and in pure substances.

We can only point out the analogy and leave the final explanation to future research.

In the instances in which two isomers showed the more extreme divergence in action, the question arose whether the weaker really possessed any action in itself. It appeared possible that *d*-hyoscyamine might be devoid of effect on the nerve ends, but that some of it might be rapidly racemised and that the slight activity might arise from the *l*-hyoscyamine thus formed. But the examination of a large number of tropeines proved that the specific action is inherent in many of them, which exhibit a definite chemical configuration, although it is always in lower measure than in hyoscyamine; some of these contained no asymmetric carbon and thus possessed no optical activity.[78] It would thus appear that the slight specific action of the *d*-isomer is not derived from the formation of the *l*-form, but is present in the weaker isomer from the beginning. A somewhat analogous question has been discussed in regard to the enzymes; when it was found that opposite isomers are digested though in unequal degree, the suggestion was made that two antipodal enzymes were present; this has now been abandoned, the view being generally held that a single enzyme

[78] Journ. Pharm. and Exp. Ther., **15**, p. 105, 1920.

acts upon both of the antipodes, though the sign of rotation determines largely the velocity of the reaction. In pharmacological effects there can hardly be any question that the action is on a single receptor, which embraces both isomers, though with unequal warmth.

INFLUENCE OF CONFIGURATION ON PHARMACOLOGICAL ACTION

Since atropine was shown to be tropyl-tropeine, the effect of substituting other acids for tropic acid in the molecule has been investigated repeatedly, the most extensive series being those of Jowett and Pyman[79] and Pyman[80] who formed altogether 45 tropeines and had their mydriatic effects tested by Marshall and Dale by instillation in the cat's eye. Dr. Pyman afterwards sent me some of these for examination by the salivary-fistula method, by which the relative potency could be ascertained more exactly.[81] The doctrine had received a wide currency that only tropeines with a benzene acid containing an alcoholic hydroxyl group possess the characteristic action; this generalisation is often associated with

[79] Seventh Internat. Congress of Applied Chem., London, 1909, IV A, **1**, p. 352.

[80] Trans. Chem. Soc., 1917, **111**, p. 1103.

[81] Journ. Pharm. and Exp. Ther., **15**, p. 105, 1920.

Ladenburg's name but is disclaimed by him (Pyman). The earliest statement in regard to the hydroxyl group occurs in Pictet's "Pflanzenalkaloide" (1891, p. 134). Both qualifications have been shown to be too absolute by Jowett and Pyman, who found that a pyridine nucleus could be substituted for benzene without the activity being destroyed, while benzoyl tropeine contains no alcoholic hydroxyl and yet has the mydriatic and other effects, though it is much less active than atropine. There is evidence that some of the compounds of tropine with an aliphatic acid also have a very feeble atropine action as shown by Gottlieb for acetyl-tropine[82] and by Marshall[83] for two other more complex members.

Among the tropeines containing an aromatic acid, I found a remarkable increase in toxicity in passing from those containing the simpler acids to the homatropine isomers (table 6) and this is the more interesting in view of the comparatively slight difference in formula, for the change from phenylacetyltropine to homatropine involves only the substitution of CHOH for CH_2 in the side chain of the acid; yet it increases the action seven times.

[82] Arch. f. exp. Path. u. Pharm., **37**, p. 218, 1896.

[83] Marshall. Arch. f. exp. Path. u. Pharm. Schmiedeberg's Festschr., p. 389, 1908.

Similarly, in a long series of bodies related to epinephrine which were examined by Barger and Dale,[84] there are a considerable number of amines which possess the characteristic action on the sympathetic myoneural receptors, but this is greatly reinforced in certain of them in which

TABLE 6*

TROPEINES	PHARMACOLOGICAL ACTIVITY
l-hyoscyamine, Atropine, d-hyoscyamine: $C_6H_5-CH<^{CH_2OH}_{COOT}$	600, 300, 15
l-homatropine, dl-homatropine, d-homatropine: $C_6H_5-CHOH-COOT$	14, 10, 7
Phenylacetyltropeine $C_6H_5CH_2COOT$	1
Benzoyltropeine C_6H_5COOT	1
Oxybenzoyltropeines $C_6H_4OHCOOT$	½–1

* T indicates tropine.

OH is substituted for H and one carbon is thus rendered asymmetrical. For example, *dl*-aminoethanol-catechol, $(OH)_2C_6H_3CHOH\cdot CH_2NH_2$, is no less than fifty times as powerful as aminoethyl-catechol, $(OH)_2C_6H_3CH_2CH_2NH_2$.

In each of these series, a considerable number of compounds are shown to possess the characteris-

[84] Journ. Phys., **41**, p. 19, 1910–1911.

tic effect to some extent, but individual members stand out as possessing this property greatly intensified. And these exceptional individuals in the series examined are distinguished by the possession of an asymmetric carbon. The temptation was great to ascribe their special pharmacological activity to this chemical feature, and to ascribe to the asymmetric carbon a high power of intensifying the pharmacological action of the molecule. I was careful to point out[85] that this was not by any means proved, for that in all the instances known, the asymmetry arose in the presence of an hydroxyl group in the side chain, and that there was some evidence that this rather than the asymmetric carbon was the essential factor in the augmentation of the activity.[86] Thus Lewin and Guillery[87] had stated in a rather unconvincing way that when the OH of atropine is substituted by Cl or NH_2 the asymmetry of the carbon atom is preserved, but the pharmacological action is much reduced; this suggests that the

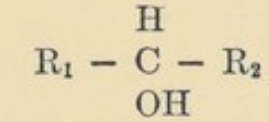
[85] Journ. Pharm. and Exp. Ther., **15**, p. 105, 1920.

[86] Others have been more daring; for example Hjort and Kaufmann, finding that phenyl-methyl-carbinol is more strongly narcotic than some of its near relatives, ascribe this to its possessing an asymmetric carbon (Journ. Pharm. and Exp. Ther., **15**, p. 129, 1920).

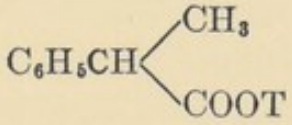
[87] Die Wirkungen von Arzneitmitteln und Giften auf das Auge, Berlin, 1905.

presence of OH enhances the power more than that of such substitutes as Cl or NH_2.

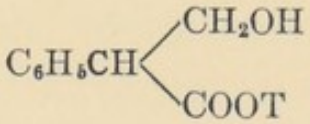
The same fact emerges more or less distinctly from a comparison of some other compounds closely related to atropine; though the exact ratio is generally not known, it may be inferred that while the presence of the asymmetric carbon may be of significance in intensifying the action of this group, as is shown by the comparison of *d*- and *l*-hyoscyamine, its full effect is obtained only when it coincides with the presence of OH. In the optically isomeric bodies which occur in nature, a large proportion owe their asymmetry to the presence of hydroxyl, the common group being



I have been able to test this view quite recently through the kindness of my colleagues, Professor Barger and Dr. Silberschmidt, who prepared for me a small quantity of hydratropyltropine; this substance,



resembles atropine



in possessing an asymmetric carbon and differs from it only in being devoid of an OH group, the CH_2OH of atropine being replaced by CH_3 in the hydratropyl base; its pharmacological activity has not been examined previously. The racemic form was that put at my disposal and it was compared with atropine in its action in arresting the

TABLE 7

DATE	DOSE OF TROPEINE	SECRETION OF SALIVA UNDER 5 MGM. PILOCARPINE			
		0 to 15 minutes	15 to 30 minutes	30 to 40 minutes	Total for 40 minutes
		cc.	*cc.*	*cc.*	*cc.*
March 31	Atropine, 0.12 mgm.	0.48	0.37	0.04	0.89
April 7	Hydratropyl-tropine, 6 mgm.	1.93	3.66	1.50	7.09
April 8	Atropine, 0.1 mgm.	0.13	1.78	0.46	2.37
April 9	Hydratropyl-tropine, 12 mgm.	0.50	2.96	1.59	5.05
April 14	Hydratropyl-tropine, 20 mgm.	0.02	0.88	0.76	1.66
April 16	Atropine, 0.1 mgm.	0.04	1.42	0.64	2.10

salivary secretion and antagonising pilocarpine in a dog with a salivary fistula in the way I have used so often (see table 7). I found that 20 mgm. of the new tropeine was required approximately to equal the effects of 0.1 mgm. of atropine. Thus the pharmacological activity of hydratropyltropine, possessing asymmetrical carbon but

no hydroxyl, is of the same order as that of such bases as benzoyltropine and phenacetyltropine which possess neither asymmetric carbon nor OH in the side chain, while atropine, which possesses both, is about two hundred times as powerful. It is obvious that the activating factor in the atropine molecule is not the asymmetric carbon but the OH. The asymmetric carbon and the optical activity with which it endows the molecule, thus does not lend any specific action in itself nor does it necessarily intensify the reaction possessed by related but symmetrical bodies except under definite conditions.

One of these conditions in the case of the tropeines, and apparently also in the epinephrine series, is the presence of OH in the side chain. This may be attached directly to the asymmetric carbon, as in homatropine, or indirectly as in atropine. In either case the molecule is greatly increased in pharmacological activity compared with nearly related compounds which have no hydroxyl even though they contain an asymmetric carbon, and the two optical isomers often differ greatly in power;[88] the hydroxyl must probably be alcoholic in character, for salicyltropeine is not appreciably more powerful than benzoyltropeine in spite of its containing OH.

[88] I have not as yet had an opportunity of determining whether the optical antagonists differ in their action in such tropeines as hydratropyl-tropine, which are devoid of hydroxyl in the side chain.

In considering the specific activity of such a drug as hyoscyamine then, three factors have to be taken into account: (1) The general structure of the molecule, which is a tropine ester, preferably of an aromatic acid, and which gives a slight activity as a general rule; as a type phenacetyltropine may be taken, (2) the presence of alcoholic hydroxyl in the side chain of the acid, which intensifies the activity to a pronounced extent; for example, atropine, possessing OH, is some 200 to 300 times as powerful as hydratropyltropine which is devoid of it, and (3) the presence of an asymmetric carbon, which gives optical rotation to the molecule, and may vary its activity about 15 to 20 times according to the direction of that rotation.

The first of those, the general structure, is strictly analogous with the configuration of the molecule which, as Fischer pointed out, is a necessary preliminary to the onset of enzyme action; the fact that in his instances it is the configuration of the whole molecule rather than the presence of individual radicles that is the determining factor, may perhaps suggest that the influence of this feature may be rather physical than chemical, if it is possible to distinguish these.

Given this general molecular configuration, the second factor, the presence of alcoholic OH in the side chain of the acid is of signal significance;

without this, atropine would be neither a poison nor a drug of importance; it increases the activity 200 to 300 times, as is shown by comparison of the tropyl and the hydratropyl tropeines. The influence of this individual group, as distinct from the general configuration of the molecule, does not appear to have an analogy in the relations between the enzymes and substrates, so far as they have been analyzed. It is possible that the presence of hydroxyl may lend certain physical properties to the molecule which promote its reaction with the receptors of the tissues, for the same effect is seen in comparing the action of phenols or alcohols with hydrocarbons, and here there can be little question that the greater solubility of the former in the tissue fluids is the main factor.

The effect of the third quality, the direction of rotation, is almost certainly due to a chemical combination being formed between receptor and drug, as I have already explained. It thus seems probable that in the case of this group, and presumably in that of others, the pharmacological action may involve both chemical and physical processes as far as these can be differentiated at the present time. The extent to which each of these determines the effect may vary in different instances, the physical sometimes prevailing, as perhaps in the action of chloroform, the chief rôle being chemical in other instances.

This analysis of the reactions of enzymes and substrates, of receptors and drugs, far from clarifying the relation of living matter and chemical constitution, indicates that more factors are involved than are generally recognised and that very slight changes in chemical structure may alter enormously the reaction between them. Such investigation discourages such crude rules of the relation of chemistry and therapeutics as have often been laid down and shows how far we have still to travel before we begin to understand the chemistry of life.

I have dwelt at what may have appeared to you prodigious length upon the properties of optical isomers in living tissues, because it seems to me that here we have a clearer connection between the behaviour of substances in the test tube and in the living tissues than is often met with. For I think it is beyond question that the differences in the reaction of the two components of atropine on the salivary glands on the one hand and to such substances as camphor-sulphonic acids on the other are of the same essential nature, each depending on the union of two optically active substances. We have seen that they are conditioned in part by the asymmetry, in part by some specific configuration including the presence of HO; of these, asymmetric carbon is

present in every organ and cell, but the configuration necessary for the development of its results is limited to very few organs. The reaction is so definite when it is present that it may be taken as a test for the presence of this configuration in the tissues.

Pharmacological investigation may thus be regarded as a method of applying a qualitative test for the presence of certain chemical groupings in living matter, and joins the other biochemical methods in its object. And viewed in this way as tests for specific combinations in living matter, drugs assume a new interest, which is only limited by our ignorance of the exact points on which they act in many cases. In the instances of which I have treated chiefly this reacting substance or receptor has been met only within very narrow limits, for the most part confined to the terminations of nerves in unstriated muscle and analogous organs. But even in these instances the occurrence of a substance reacting to atropine in the central nervous system is of interest as suggesting that there is a grouping common to the central synapse and the extreme terminations of the autonomic nerves. Under other drugs such analogies between organs which apparently have very little in common are more frequent. For example, the most susceptible receptor for the bodies of the digi-

talis group is the heart muscle, but the reaction of the inhibitory centre suggests that it also contains a receptor of a somewhat similar configuration. Again the action of morphine, common to the respiration, the sense of pain, and the movement of the intestine, would suggest that the respiratory centre, the pain path and the intestine contain elements with common chemical features. Further it may be argued that these receptors are not identical, for some are more easily affected by the poison than others; but this fascinating subject cannot be entered upon further at present; the recognition of the presence of chemical substances in the living tissues by the use of drugs as reagents for these must remain for the distant future—perhaps the final phase of pharmacology.

When a biological phenomenon has been shown to be of the same nature as others observed in unorganised bodies by the chemist and physicist, the next advance must be expected from the latter. And the pharmacology of the optical isomers seems to have reached this point; we may multiply examples occurring in different tissues or from different enzymes, but the fundamental theory seems unlikely to be advanced until we know the conditions which govern the difference in the reaction of these bodies in the test tube and recognise why one isomer is precipitated or destroyed more

readily by one active acid than by its counterpart. The methods required are those of chemistry rather than those of biology.

In this relation I have often been asked how research in such fundamental questions as I have been discussing is to be justified; of what profit is it to us, and how does it benefit medicine? And I must admit that it is of minor practical importance, it has little direct application to the use of these remedies in therapeutics to know that the natural adrenaline is twice as powerful as the synthetic, for this can be compensated by doubling the concentration of the latter. I do not suppose that it is necessary to justify to my present audience investigations which apparently have no direct practical bearing on disease and its treatment. But if our faith in such "theoretical" work needs strengthening, I would refer doubters to the quite unpractical observations of v. Mering and Minkowski, who first showed the relation of the pancreas to the carbohydrate metabolism, a "theoretical" observation which after lying fallow for so many years led to the introduction of insulin. The history of therapeutics in the last eighty years is full of similar instances, in which a casual observation made in the course of a purely theoretical research has had the most important results in practical medicine. As among the earliest exam-

ples, I may cite the story of the introduction of amyl nitrite by Brunton, which was based on his noting its effects on the blood-pressure in experiments by Gamgee designed to elucidate the formation of methaemoglobin. Again, Liebreich, working upon the question of the powers of the tissues to decompose organic substances, injected chloral, previously untried, observed the resulting effects on the brain and introduced chloral hydrate into therapeutics; this led to the study and to the use of the whole group of modern hypnotics. Saul was not the last who, going forth to seek his father's asses, found a kingdom, and it would seem that casual observations correctly appreciated may be of as great profit to therapeutics as investigations aimed directly at some definite phenomenon in disease.

Sans Tache

Sans Tache

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