OPTICAL ACTIVITY AND LIVING MATTER

by

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Judging by the bibliographical list of publications dealing with optical activity in biological material, one has the impression that this subject did not arouse among American scientists the same degree of interest as did many other problems of biophysics and biochemistry. It is thought that this review will contribute to focus the attention of more of the so active investigators of this country on the important role of *asymmetry* in the building stones of protoplasm.

In presenting "*Optical Activity and Living Matter*" by G. F. Gause to scientists at large, the object of the editor of this series of monographs is to bring to the fore a subject which seems to be of fundamental significance in the problem of *the structure and the mechanism of action of living matter*.

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The Editor.

*Saint Louis, Missouri, May 1941.*
PREFACE

Although the study of the asymmetry of protoplasm was begun by Louis Pasteur about a century ago, it did not receive from the biologists the attention which it deserves. The observations on that subject are scattered and need to be brought together into a separate division of experimental biology. The author of the present monograph, who has for several years been engaged in experimental studies of the structure and of the activity of living systems as related to the asymmetric configuration of their constituents, intends to review here this scattered literature and to discuss the various problems which the subject involves.

Pasteur would, no doubt, rejoice in the importance that a number of questions related to the asymmetry of protoplasm have acquired in the development of medical sciences. The recent findings on anthrax, a subject to which Pasteur has contributed so much, illustrate this point. Bruckner and Ivanovics showed in 1937, in the laboratory of Professor Szent-Györgyi, that the unnatural optical isomer of glutamic acid, which was not found anywhere before in organic nature, enters into the composition of the capsules which enclose the anthrax bacilli. The capsules are responsible for the virulence of the bacilli, and the investigators just mentioned suggest that the protective role of the capsules is due to the unnatural configuration of glutamic acid.

The author wishes to express his thanks to Professor W. W. Alpatov (Moscow) and to Professor W. J. Vernadsky (Moscow) for their aid in the course of the preparation of this work, and to Professor B. J. Luyet (St. Louis) for the revision of the manuscript.

G. F. Gause.
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1. Dissymmetry and Asymmetry. A survey of the literature on optical activity of protoplasm reveals some confusion in the terminology. Terms such as dissymmetry and asymmetry, which are so often used, are not always clearly defined. Some preliminary definitions are, therefore, necessary.

Dissymmetry is a property of the individual components of a system, that is, in the cases to be considered here, a property of molecules, while asymmetry refers to an aggregate of molecules.

The term dissymmetry was used in this sense for the first time by Pasteur in the classical paper that he wrote in 1848 on the relations between crystalline form, chemical composition and optical rotation and that he summarized in the two well-known lectures delivered in 1860 before the Paris Chemical Society on the molecular dissymmetry of natural organic products. Pasteur says that when we study material objects of whatever nature, as regards their form and the repetition of their identical constituent parts, we soon recognize that they fall into two large classes which present the following characters. Those of the one class, placed before a mirror, give images which are superposable on the objects themselves, while the images of the others are not superposable on the objects. A cube, straight stairs, a branch with opposite leaves, the human body—these are of the former class; an irregular tetrahedron, winding stairs, a hand—these belong to the second group. The latter are
dissymmetric,¹ and are defined as objects possessing non-superposable mirror images.

Dissymmetric objects can exist in two forms, right and left.

When the two forms of dissymmetric molecules are represented in equal concentrations (racemic mixture),² the aggregate of molecules is symmetric. When they are represented in unequal concentrations, the aggregate is nonsymmetric. There may be a predominance of the right forms (dextrality) or of the left forms of molecules (sinistrality). Pasteur did not propose any special term for such a deviation of the molecular aggregate from the racemic state. His views on this subject were somewhat uncertain.³

Following Emil Fischer (1894) and Japp⁴ (1898), we shall designate this condition by the term asymmetry.

¹ In the German (1891) and in the English (1897) translations of Pasteur's work (lectures of 1860), the word dissymmetry was arbitrarily replaced by the word asymmetry.

² Recently Findlay (1937) pointed out that the term acide racémique, as applied to tartaric acid, was due to Gay-Lussac (1828), but its use in the sense accepted at present originated with Pasteur (1861). Pasteur wrote in 1860: "We still need a word in chemical terminology to express the fact of a double molecular dissymmetry concealed by the neutralisation of two opposite dissymmetries, the physical and geometrical effects of which compensate each other exactly."⁵

³ Pasteur did not distinguish sharply the dissymmetry of individual molecules from the asymmetry of their aggregates in the sense given above. For him the molecules acquire dissymmetry by receiving a "twist" in living organisms or in contact with products of living organisms and they lose their dissymmetry by being untwisted. In 1860 he wrote that "the twisted organic group can be untwisted and so assume the ordinary character of artificial and mineral substances." The "twisting" was considered as characteristically "vital" and destructible by energetic chemical reactions. According to modern views, these reactions, instead of "untwisting" the molecules, produce a racemisation or an equalization in the concentrations of the right and left forms of a substance. While Pasteur is the discoverer of the fact that "Racemic tartaric acid of chemists, inactive as to the optical rotatory power, consists of two acids, the rotations of which mutually neutralize each other, as one of them rotates to the right and the other to the left, and both in the same degree," (Pasteur, 1848, p. 458) he thought that the molecules of the racemates were symmetric by their very nature, and that they became dissymmetric in their separation from the racemate by crystallization of the antipodes under the action of some dissymmetry forces which might be furnished by "organic dissymmetric particles on the surface of the crystallization dish."⁶
From what has been said, it follows that dissymmetry, or non-superposable ability of mirror image on the original object, can exist without any asymmetry, as in racemic mixtures. Dissymmetric molecules have the possibility of forming symmetric or asymmetric aggregates; asymmetry is the realization of one of these two potentialities. It is, therefore, obvious that dissymmetry represents a necessary pre-requisite condition for any asymmetric state.

2. Optical and Geometrical Asymmetry. Asymmetry as defined here should be distinguished from geometrical asymmetry. A geometrically asymmetric figure is one which possesses no element of symmetry, that is, no center, no axis and no plane of symmetry, while dissymmetric figures (in the sense of Pasteur) might possess a complex system of axes of symmetry, although they cannot possess

(Pasteur, 1884). According to our views, one half of a racemic aggregate consists of the right and the other of the left form of molecules, before, as well as after, crystallization.

That Pasteur was mistaken in this particular point is evidenced by the following investigations. Ostwald (1889) has shown by electric conductivity methods that, in dilute water solutions, racemic tartaric acid does not exist as such but is entirely dissociated into its dextrorotatory and laevorotatory components. Raoult reached the same conclusion by cryoscopic methods. Wyrouboff (1884), Jungfleisch (1884), and Errera (1898) pointed out that the separate crystallization of antipodes from racemic tartrate depends on the relative solubilities of the individual components and of the mixture. These solubilities, in their turn, are controlled by the temperature. Thus, at ordinary room temperature the antipodes are less soluble than the racemic mixture, and they crystallize separately, while, at temperatures above 26° C., the order of solubility is reversed, and the racemate crystallizes.

Emil Fischer (1894) introduced the concept of asymmetric synthesis, that is, of the production of molecules which exhibit a rotation of a given sign with full or partial exclusion of the antipode. But the term asymmetry for expressing the properties of aggregates of molecules was employed—for the first time, it seems—by Japp (1898) in his well known address, "Stereochemistry and Vitalism," which was followed by an interesting discussion in "Nature." Japp wrote that the simultaneous production of two opposite asymmetric halves is equivalent to the production of a symmetric whole, whether the two asymmetric halves be actually united in the same molecule, as in the case of meso-tartaric acid, or whether they exist as separate molecules in the left and right constituents of racemic acid. This statement shows quite clearly that the author conceived asymmetry as the property of the aggregate of molecules and not as the configurational character of the individual molecules (the term enantiomorph was used in this latter sense).
PRINCIPLES AND DEFINITIONS

a plane, a center or an alternating axis of symmetry (cf. the definition of Lowry, 1935), these elements being incompatible with the non-superposability of the image. So, dissymmetric molecules are not necessarily asymmetric in the geometrical sense.

3. Dissymmetric Structure as a Basis of Optical Activity. The fact that the rotation of the plane of polarized light is caused by a dissymmetric structure of molecules leaves no place for doubt, but the problem of the physical mechanism by which this is done did not yet receive a definite solution. Two models proposed by Pasteur—irregular tetrahedron and spiral line—have formed the basis for further theories. We shall consider separately the case in which optical activity is due to a dissymmetric spatial distribution of atoms as found in entire crystals and the case in which it is due to a dissymmetric structure of isolated molecules.

It is known that the optical activity of quartz depends on the structure of the crystal itself, since the rotation of the plane of polarized light disappears with the crystalline state. The optical effect also diminishes, and at last vanishes when a plate cut out from a crystal of quartz passes from a position perpendicular to the direction of the ray to an inclined position. Consequently, the fundamental difference between the dissymmetry of quartz and the molecular dissymmetry of organic substances lies in the fact that in the former case the crystal as a whole is anisotropic, i.e., possesses different properties in different directions, while, in the latter, as it was ascertained by Pasteur, dissymmetry represents a property of the separate molecules independent of their relative position in space. A substance in which one of the two possible dissymmetric forms of molecules, right or left, predominates, will possess optical activity.

It was Fresnel (1824) who suggested for the first time, that the structural dissymmetry of quartz may be explained on the basis of the spiral distribution in space of the molecules of silicon. In one of the two optical anti-
podes of quartz, these spirals would turn from right to left and, in the other, from left to right. This view was adopted by Pasteur (1860), and, about a hundred years after its formulation by Fresnel, it received full confirmation in the X-ray analysis of quartz made by Bragg (1913, 1925). This investigator showed that crystals of quartz can be considered as giant molecules in which the constituent units build up a three-dimensional network, where every atom of silicon is linked to four atoms of oxygen, whilst every atom of oxygen unites two atoms of silicon. The complex aggregate thus formed has a spiral structure which is shown in Fig. 1. The lines uniting the centers of the atoms are spirals, and these spirals are twisted in opposite directions in dextrorotatory and in laevorotatory quartz. (For further details on the coordination of separate spirals in the so-called $\alpha$ and $\beta$ form of quartz, cf. Bragg.) Let it be noticed, then, that it is the spiral type of structure which prevails in the dissymmetric spatial distribution of elements in crystals of quartz.

What is the structure of dissymmetric organic molecules and its relation to optical rotation? Modern theories, a detailed account of which may be found in the excellent monograph by Lowry (1935), consider the irregular tetra-

---

**Fig. 1.** Spiral structure in a crystal of quartz. The silicon atoms are represented by solid black circles, the oxygen atoms by lighter and larger circles. Three atoms of silicon form a spire. Each atom of silicon is in the center of a tetrahedron at the apices of which are 4 oxygen atoms; only 2 of the latter are represented in the figure.
hedron with four different radicals situated in its corners as the basis for the explanation of the origin of optical activity. This structure accounts for both the existence as well as the approximate value of optical rotation in the simplest dissymmetric molecules. It should be noticed that a tetrahedric molecule presents a spiral type of distribution of its atoms. In Fig. 2 (a) is represented an

![Diagram](image_url)

**Fig. 2.** Dissymmetric configuration of organic molecules; a) l-isomer, b) d-isomer.

irregular tetrahedron in the corners of which are placed four different groups. In the order of diminishing magnitude these groups can be arranged in the following manner: \( R_1 > R_2 > R_3 > R_4 \). By joining the centers of these groups in the order just given a spiral is obtained. If the largest group \( (R_1) \) is placed nearest to a hypothetical observer, the spiral represented in Fig. 2 (a) will appear to rotate counter-clockwise. According to Boys (1934), such a structure would correspond to the left absolute configuration of the molecule. If we interchange the groups \( R_2 \) and \( R_3 \), we obtain a figure which is the mirror image of the preceding one; the spiral twist will now assume a clockwise direction and the molecule will possess the right configuration.

Recently an attempt has been made to adapt the concept of absolute configuration to the definition of the configurati-
tion of natural \( \varepsilon \)-amino-acids (see Rainey, 1937). We shall also mention as related to this problem the geometrical investigations of Study (1913) on the right and left structures in a system of points. Finally we wish to point out again that the spiral distribution of elements appears as basic in the mechanism of optical rotation in molecules as well as in crystals.

4. "Relative Configuration" and "Biological Series" of Optical Isomers. Emil Fischer (1894) drew attention to the necessity of distinguishing the relative configuration of a substance from the sign of its optical rotation, there being substances which possess the same relative configuration but rotate the plane of polarized light in opposite directions. The importance of this remark became more evident in the subsequent developments of stereochemistry. Changes in temperature, solvent, concentration, etc., are often accompanied by a change in the sign of the optical rotation. As Lowry (1935) pointed out, these changes make it impossible to judge the configuration of a substance by the sign of its rotation. This may be demonstrated by the following example. Let us consider an optically active compound

\[
\begin{align*}
\text{CH}_3 & \quad \text{X} \\
\text{C} & \\
\text{C}_2\text{H}_5 & \quad \text{Y}
\end{align*}
\]

containing a single asymmetric carbon atom, linked to methyl and ethyl and to two other radicals, X and Y. No matter what the influence of temperature and of solvent is, the sign of the rotation will be reversed but its magnitude will be unaltered if the methyl and ethyl radicals are interchanged, \( i.e., \) if usual optical inversion takes place. The rotation will disappear completely if methyl is replaced by a second ethyl radical, or conversely, since then the plane of symmetry will appear in the molecule. If methyl is replaced not by ethyl but by propyl, it is gen-
Generally admitted (Lowry, 1935) that the sign of the rotation will be reversed, i.e., that the molecules

\[
\begin{align*}
\text{CH}_3 & \quad X \\
\text{C}_2\text{H}_5 & \quad Y
\end{align*}
\quad \quad \quad \text{and} \quad \quad \quad
\begin{align*}
\text{C}_3\text{H}_7 & \quad X \\
\text{C}_2\text{H}_5 & \quad Y
\end{align*}
\]

will have opposite rotations, although the position of the univalent radical \(\text{C}_2\text{H}_5\text{CXY}\) is identical, and although there has been only a substitution of one chemical group in the molecule by another. Such possibilities render illusory any conclusion as to the configuration of a substance on the basis of the direction of its rotation.

To clarify this situation, Fischer (1894) proposed to take as a prototype of configuration that of a specific isomer of some definite substance and compare to this prototype the optical isomers of other substances. In this manner a series of optical isomers of different substances can be established, all the members of this series possessing the same relative configuration. Wohl and Freudenberg (1923) suggested that the members of one such series be designated by the letter \(d\) and their antipodes by the letter \(l\), while the sign of their optical rotation would be indicated by (+) for a rotation to the right and by (−) for a rotation to the left. The decision as to which one of the two series should be marked by the letter \(d\) is, of course, arbitrary, the absolute configuration of the substance being unknown. According to this system, a substance belonging, for example, to the left steric series, but rotating the plane of polarized light to the right will be marked by \(l\) (+). Fischer, furthermore, suggested to take as a standard of comparison dextrorotatory glucose, conventionally taking it as a \(d\)-form. He proposed that, in writing the formulas, the aldehydic or ketonic group of sugars and the carbonyl group of monobasic acids be put on top and the chain of carbon atoms in a downward direction, the hydroxyl of the fifth carbon atom being to the right. If one figures out,
on the basis of what is known on chemical structure, which isomer of fructose presents the same position for the fifth carbon atom as d-glucose, one finds that it is laevorotatory fructose. Thus \( d\) (+) glucose and \( d\) (-) fructose possess the same relative configuration in spite of their rotation in opposite directions. Both these isomers are found in living organisms and belong to the same "biological series."

Wohl and Freudenberg (1923) proposed to take glycerine aldehyde and not glucose, as a standard of comparison, conventionally considering the dextrorotatory form as a member of the \( d\)-series and attributing to it such a structure that the hydroxyl of the fifth carbon atom be again written to the right.

**SUMMARY**

1. Dissymmetry is the property of molecules of possessing non-superposable mirror-images. Dissymmetric molecules can exist in two forms, right and left. 2. Asymmetry is the property of molecular aggregates of presenting a predominance of the right or the left form of dissymmetric molecules. 3. Optical asymmetry is to be distinguished from geometrical asymmetry. 4. Optical activity is attributed to the spiral arrangement of atoms, either in entire crystals, as in quartz, or in single molecules, as in some organic compounds. 5. If, besides the sign of the optical rotation of a substance, one considers the configuration of its molecules, one can classify the optical isomers into "biological series" as found in living organisms.

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--------, Rev. Scientif. iii, 4, 2, 1884.
CHAPTER I

OPTICAL ACTIVITY OF BIOLOGICAL MATERIAL

1. Dissymmetry in Organic and in Inorganic Nature. It has been repeatedly pointed out that all physiologically important substances possess a dissymmetric structure. This is precisely what Pasteur meant when he wrote: "On trouve la dissymétrie établie notamment dans les principes immédiats essentiels à la vie." But is there any essential relation between dissymmetry and life, in the sense that one is a necessary attribute of the other? Dissymmetry is certainly much more general than life. We know that the dissymmetric structure exists in crystals of quartz. The same is true of several metallic compounds (cf. Lowry, 1935). Recently Jaeger (1919), after having investigated a great number of inorganic compounds, came to the conclusion that the dissymmetric structure might be much more general than we usually assume, but that, in inorganic nature, the existence of dissymmetry is often difficult to establish, there being no method for separating the anti-podes. Vernadsky (1934) made a similar remark. In such cases at least, dissymmetry has no obvious relation to life. But, if dissymmetry exists without life, life might not exist without dissymmetry. The possibility that life be the attribute of systems built of substances of such a level of complexity that dissymmetry is the very condition of their existence is not excluded. A suggestion which was recently made by Ackermann (1935), and which is practically identical with that of Pasteur (1884), is that dissymmetry is characteristic of the basic components of protoplasm, whilst such products of metabolism as urea, uric acid, creatinin and hippuric acid are devoid of dissymmetry and their molecules are structurally inactive. The simplest amino-acid of the protein molecule, glycocoll, is
the only one devoid of dissymmetry, and, in metabolic processes, it is less important than the other amino-acids which are dissymmetric.

It is of interest to mention here that the elements of which optically active compounds consist include twenty-one of them, as follows (Lowry, 1935):

Non-metals
B  C  N  Be
Si  P  S  Al
As  Se  Te

Metals
Cr  Fe  Co  Ni  Cu  Zn
Ru  Rh  Ir  Pt

2. Asymmetry as a Specific Property of Protoplasm. It is generally established that all the substances which are produced in the laboratory or in nature, without the action of living organisms, have right and left forms represented in equal concentrations, the formation of both being equally probable. It has never been observed, for example, that in any quartz bed the right or the left crystals would predominate to any extent (Tromsdorff, 1937; Lemmlein, 1938). There is, of course, dissymmetry in individual components, but no asymmetry in their aggregation.

On the other hand, all basic chemical substances of which living systems are made up or which are formed in connection with the activity of living systems, deviate from the racemic state and are represented mainly by one antipode. In other words, asymmetry is a specific attribute of living systems and an essential feature of their organization. This is one of the most significant principles of experimental biology; it is based on a large number of observations accumulated within the last hundred years, since the pioneer work of Pasteur.

We shall study here, in some detail, which parts of living systems consist of racemic compounds and which parts deviate from the racemic state and in what direction. It will appear that the asymmetric state of protoplasmic components is directly related to the role played by these components in metabolic activity.
3. Asymmetry of Primary Constituents of Protoplasm.

From the view-point of their asymmetric molecular aggregation, the substances which enter into the composition of living systems may be divided into two groups. Physiologists have for a long time been accustomed to call these two groups, respectively, the primary and the secondary constituents of protoplasm. To the group of primary substances belong the proteins and the lipoids which form together the so-called lipoprotein complexes, and the carbohydrates which functionally are closely related to them. These primary substances, except for some stored carbohydrates and proteins, build up protoplasm itself and preside over the fundamental living processes. To the group of secondary constituents belong various products of transformation of the primary substances, which represent either storage material or excreta.

We shall study, to begin with, the asymmetric structure of primary substances, and we shall consider, first, the degree in which they deviate from the racemic state, or, in other words, their optical purity.

As far as proteins are concerned, the optical activity of which was already known to Pasteur, Emil Fischer was the first to express the idea that their constituent amino-acids are always found in protoplasm in the optically pure state, and that, when a total or partial racemisation occurs, it is due to the application of too coarse methods of isolation. For instance, serine from silk was known for a long time only in the form of a racemic compound, as it is rather easily racemised in the process of protein hydrolysis. However, Fischer (1907) succeeded in isolating from silk optically active serine, of which the specific rotation (in hydrochloride solution, at 18°) was $+11.6^\circ$, while the rotation of optically pure laevorotatory serine prepared synthetically (i.e., crystallized from racemate with alkaloids) was $+14.4^\circ$ (at 20°). On the ground of these experimental data alone it is, of course, impossible to conclude that serine in silk is optically pure, and that it is partially racemised

1 The hydrochloride of laevorotatory serine is dextrorotatory.
in the process of isolation, but we shall see below that the principle itself is definitely established.

Pringsheim (1910) had shown on asparagine that the optically pure form is gradually racemised by boiling with water, a step in the isolation procedure.

With appropriate treatment, amino-acids both of vegetable and animal origin, always prove to be optically pure, that is, one isomer only of each amino-acid is present, its antipode being completely absent. In the case of leucine, this was established by the elaborate investigations of Ehrlich and Wendel (1908), the results of which are given in Table 1.

<p>| TABLE 1 |</p>
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<th>Specific Rotation of Preparations of Leucine of Different Origin, in Water at 20° (Ehrlich and Wendel, 1908)</th>
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<tbody>
<tr>
<td>Synthetic, optically pure preparation</td>
</tr>
<tr>
<td>From egg-white (chicken)</td>
</tr>
<tr>
<td>From casein (cow's milk)</td>
</tr>
<tr>
<td>From yeast (Saccharomyces cerevisiae)</td>
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The optical purity of tyrosine remained for a long time questionable as a result of a number of old contradictory observations (Lippman, 1884). But Schulze and Winterstein (1905) have definitely shown that, after careful preparation from vegetable material, one obtains always optically pure substances and that racemisation and the consequent decrease of rotatory power are the result of the application of coarse methods of isolation (Table 2).

<p>| TABLE 2 |</p>
<table>
<thead>
<tr>
<th>Optical Rotation of Preparations of Tyrosine of Different Origin, in Hydrochloride Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic, optically pure preparation</td>
</tr>
<tr>
<td>From Cow's Milk; hydrolysis of casein by HCl</td>
</tr>
<tr>
<td>From the bulbs of Dahlia variabilis; 80% boiling alcohol was used in the isolation procedure</td>
</tr>
<tr>
<td>From embryos of Lupinus albus; autolysis</td>
</tr>
</tbody>
</table>
The preparation from cow's milk and that from the bulbs of *Dahlia* show a weak racemisation as a result of the treatment, while the preparation from the embryos of *Lupinus* is optically pure; the autolysis procedure used in this last case prevents racemisation.

One can, at present, consider as an established fact that all amino-acids entering into the composition of protoplasmic proteins are optically pure; not a single exception is known.\(^1\)

The fats or lecithins, which contain nitrogen and phosphorus, and which are considered integral constituents of the fundamental units of protoplasm, are also optically pure, as it was, for instance, established by the investigations of Mayer (1906).

Among primary substances, the carbohydrates, as well, are for the most part optically pure. Brown and Morris (1893) have shown in an extensive investigation that glucose and other sugars are found in the optically pure form in the leaves of the plant *Tropaeolum majus*.

An interesting exception to the general rule has been observed in sugars. Neuberg (1900) found in the human organism optically inactive, racemic sugar under pathological conditions. Salkowsky (1892), who had discovered that in this case a pentose (arabinose) is excreted in urine, instead of glucose as it happens in glucosuria, called the disease pentosuria. Neuberg established that the arabinose excreted in urine is optically inactive. These observations were later confirmed by a number of other physiologists. In what relation the inactive arabinose stands to the active arabinose entering into the composition of the nucleo-proteids of our body is at present unknown.

Racemic sugar, dl-galactose, was also found in plants. Oshima and Tollens (1901) isolated it from the Japanese marine alga, *Porphyra laciniata*.

The presence of racemic sugars in plants and animals is

\(^1\) It seems preferable, for the present, to suspend judgment on the recent data of Kögl and Erxleben (1939) concerning partial racemisation of some amino-acids in proteins of malignant cells.
very rare but it is particularly significant. Since sugars do not racemise when boiled in water, it seems that the racemic state does not result from the process of isolation but that the optically inactive forms actually enter into the composition of living systems. The origin of racemic sugars in living organisms is by no means clear. Neuberg (see Färber, Nord and Neuberg, 1920) remarks that it might not be a mere accidental fact that the two racemic sugars found are just arabinose and galactose.

To conclude, among the primary substances, all the amino-acids, the lecithins and the majority of important sugars such as glucose, fructose and many others are always present in protoplasm in the optically pure state.

4. Asymmetry of Secondary Constituents of Protoplasm. As one passes from primary to secondary substances, the optical purity loses its obligatory character. This is particularly evident in organic acids which represent intermediate products of metabolism. Their origin and their signification is still a source of controversy, especially in plants. Whether, in the latter, the formation of organic acids is related to the metabolism of the amino-acids, or whether they represent a stage in the carbohydrate cycle cannot be decided. When the organic acids begin to appear, they are optically pure, as if bearing some birth marks from the primary substances, but as soon as they separate from the primary asymmetric system, beginning perhaps to play the role of storage material, they assume the character of racemic compounds.

The experimental data on which these conclusions are based are principally those of Ruhland and of his school. Ruhland and Wetzel (1929), and later Schwarze (1932) observed that, in the leaves of different plants, malic acid is found especially in the two forms: laevorotatory and racemic (Table 3).

1 The data of the Leipzig school and, particularly, the analytical part of the work were severely criticized by Bennet-Clark (1937). But, as far as optical activity is concerned, Ruhland’s data are reliable. Enzymatic racemisation of malic acid in plants, according to Bennet-Clark, was observed also by Naylor (unpublished Thesis, Manchester University, 1935).
TABLE 3

Content of Optically Active and of Racemic Malic Acid, in ml. of Molar Acid Solution per gr. of Dry Weight, in Leaves of Different Plants (Schwarze, 1932)

<table>
<thead>
<tr>
<th>Plant</th>
<th>l-malic acid</th>
<th>dl-malic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotiana tabacum</td>
<td>0.422</td>
<td>0.163</td>
</tr>
<tr>
<td>Pelargonium zonale</td>
<td>0.133</td>
<td>0.150</td>
</tr>
<tr>
<td>P. peltatum</td>
<td>0.869</td>
<td>0.485</td>
</tr>
<tr>
<td>Rubus idaeus</td>
<td>0.067</td>
<td>0.208</td>
</tr>
</tbody>
</table>

According to Ruhland and Wetzel, the newly formed malic acid is always optically active and only later does it pass into the racemic form. In Rheum hybridum, laevo-rotatory acid was found to be racemised after the newly formed portions of it had penetrated into the roots.

Bendrat (1929) observed that all malic acid, in the plant Sempervivum glaucum, is in the racemic form in the evening, that it increases during the night, and that, after this increase one can find some laevo-rotatory acid, in the morning (Table 4). It seems, then, that the optically active form appears in metabolic processes and that it is racemised later.

TABLE 4

Content of Total and Laevo-rotatory Malic Acid, in ml. of Molar Acid Solution per gr. of Dry Weight, in the Middle Leaves of Sempervivum glaucum (Bendrat, 1929)

<table>
<thead>
<tr>
<th>Time</th>
<th>Total malic acid</th>
<th>1-malic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evening</td>
<td>0.140</td>
<td>0</td>
</tr>
<tr>
<td>Morning</td>
<td>0.194</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Data on other organic acids, though incomplete, agree in general with the observations just mentioned. Thus it was known to Pasteur that d-tartaric acid as well as dl-tartaric acid are present in grape juice (see Thiele, 1911).

Inactive lactic acid has been found in the leaves of the common ash, Fraxinus excelsior (Gintl, 1869) and in a number of other plants (Stoklasa, 1907).
Katagiri and Katahara (1937) have shown that, in bacteria, optically pure lactic acid is formed first and that it racemises later under the influence of some environmental conditions.

Inactive lactic acid was also recorded in comparatively rare post mortem observations in animals (Morishima, 1900).

As is well known, dextrorotatory lactic acid is found in vertebrates and in different organs of invertebrates and racemisation is rare. The tendency has been, for a long time, to explain the presence of this racemic lactic acid (especially in the case of bacterial fermentation) by the inactivity of the intermediate product, methylglyoxal, which has no asymmetric carbon atom and from which racemic lactic acid could be formed without the participation of an optically active enzyme. But, at present, methylglyoxal is no longer considered an intermediate product in the transformation of the carbohydrates, and, besides, the thorough investigations of Katagiri and Katahara (1937) have demonstrated an initial formation of active lactic acid, which racemises later.

Racemisation of the secondary substances after they are detached from the primary asymmetric complex takes place also in the glucosides which, in plants, play the part of storage material. The nitrile of mandelic acid which is enzymatically synthesised in plants in the relatively pure dextrorotatory form is subsequently racemised, and in the leaves of Prunus laurocerasus, a glucoside of racemic dl-nitrile is found (Kuhn, 1936; this subject will be examined in detail elsewhere).

The terpenes which, in general, represent vegetable secretions but on whose origin and physiological function much remains to be investigated are also often found in plants in the racemic state. For instance, racemic limonene or dipentene has been observed in Pinus silvestris, Laurus camphora, Valeriana officinalis and many others (Bartelt, 1910, names 16 of them). But optically active limonene as well is found in the same or similar kinds of
plants; consequently, the secondary origin of the racemic form from initially active limonene appears to be probable. The same could be said also of racemic borneol.

The last group of secondary substances to be considered is that of the alkaloids. They seem to represent some modified fragments of protein molecules which perhaps are some end products of metabolism. The question of the optical purity of the alkaloids in plants has been repeatedly and extensively discussed. Apparently in a great number of cases racemisation results from the process of isolation. This seems to hold, in particular, for optically inactive atropine, which represents the product of racemisation of the laevorotatory hyoscyamine, the latter being found in plants in the optically active state (McKenzie and Wood, 1919; Hess and Weltzien, 1920). It is known that hyoscyamine is very easily racemised by weak alkalis at room temperature. Some alkaloids, however, it was suggested, might be present in plants in the racemic state, for instance, coniine and scopoline. Since the racemisation of these alkaloids proceeds very slowly even at high temperatures and pressures, an artificial racemisation in the process of isolation seems excluded (Hess and Weltzien, 1920). The origin, in the plant, of racemic coniine and scopoline is therefore still a mystery.

In spite of the presence of a number of racemic forms of alkaloids in plants, the majority of them are found in the optically pure state, for instance, nicotine, anabasine, etc. The alkaloids constitute, therefore, an exception among the secondary substances which have severed their connection with the primary complex. It is probable that, owing to peculiarities of chemical structure, the mobility of some groups in the molecule of several alkaloids is exceptionally low; their optical purity would be due, then, to a too slow racemisation. In fact, it has not been possible to attain racemisation of the alkaloid heliotridane by any of the means employed successfully in other cases (Menshikov, 1937).

5. Exclusiveness of the Asymmetry-Sign in Primary
Substances. As has been said, the primary organic substances are obligatorily asymmetric and the secondary substances are optionally asymmetric. To this characteristic property one should add another which might be called the "replaceability" or "non-replaceability" of a given optic isomer by its antipode. Substances possessing obligatory asymmetry are found in nature in the form of one only of the two optical isomers, whilst the secondary substances are found as well in the dextrorotatory as in the laevorotatory form, often as inactive racemates. We shall describe this property as exclusiveness or non-exclusiveness of the asymmetry-sign.

Exclusiveness of the asymmetry-sign in primary substances is a well established fact. In amino-acids, no exception has ever been recorded. Only dextrorotatory alanine, laevorotatory leucine, dextrorotatory valine, laevorotatory histidine, laevorotatory aspartic acid, etc., have been isolated from animal or plant tissues. All apparent exceptions to this rule could be traced to some experimental error as shown by Pringsheim (1910).

The same holds true for the carbohydrates which possess obligatory asymmetry. Only dextrorotatory glucose, laevorotatory fructose, etc., can be found in living material.

The isomer which is present in the biological material is often called "natural," whilst its antipode which is prepared synthetically is considered unnatural, but it is evident that the term "natural" as a synonym of "biological" is somewhat improper.

6. Non-Exclusiveness of the Asymmetry-Sign in Secondary Substances. Turning now to the substances in which optical purity is not obligatory, we find that one optical isomer is found in one species of plants and its antipode in another.

Let us consider first the optionally asymmetric carbohydrates. Arabinose, which is found in organic nature in the racemic state, can also be present in the form of the relatively pure dextrorotatory and of the relatively pure laevorotatory isomers. The left form is the most widely
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spread; it was found, for example, in the leaves of *Adonis vernalis* (Ekenstein and Blanksma, 1908), entering in the composition of their glucosides. The right arabinose was found in the glucoside from *Barbados*, the so-called barbaloine (Léger, 1910).

Similar findings were recorded in alkaloids. The laevorotatory alkaloid sparteine, for instance, is widely spread in plants; it was repeatedly isolated from *Spartium scoparum* and *Lupinus luteus*. Recently Orechoff, Rabino-witch and Konovalowa (1933) discovered the dextrorotatory isomer of sparteine in *Sophora pachycarpa*, a plant from Middle Asia.

Blockmann and Roth (1935) reported to have isolated and obtained in a chemically pure state laevorotatory alcainine, a red dye found in the roots of *Alkanna tinctoria*, a South-European species; the dextrorotatory isomer of the same substance was obtained from the roots of the Japanese plant, *Lithospermum erythrorhizon*.

The terpenes were recorded often as dextrorotatory in one species and laevorotatory in another (Oudin, 1932; Branke and Parishev, 1937). We tabulated below (Tables 5 and 6) some data on the distribution of the optical isomers of the two most important terpenes, borneol and limonene.

It is clear, then, that in secondary substances, both optical isomers participate in the composition of living sys-

### TABLE 5

**The Distribution of the Optical Isomers of Borneol in Different Plants (Bartelt, 1910)**

<table>
<thead>
<tr>
<th>1-Borneol</th>
<th>d-Borneol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus maritima</em> (Belloni, 1906)</td>
<td><em>Amomum cordamomum</em> (Schimmel, 1897)</td>
</tr>
<tr>
<td><em>Thuja occidentalis</em> (Wallach, 1901)</td>
<td><em>Dryobalanops sp.</em> (Schimmel, 1905)</td>
</tr>
<tr>
<td><em>Andropogon nardus</em> (Schimmel, 1899)</td>
<td><em>Lavandula spica</em> (Boucharat, 1893)</td>
</tr>
<tr>
<td><em>Artemisia canadensis</em> (Power and Lees, 1902)</td>
<td><em>Salvia officinalis</em> (Schimmel, 1895)</td>
</tr>
<tr>
<td><em>Bhumea balsamifera</em> (Haller, 1886)</td>
<td></td>
</tr>
<tr>
<td><em>Pyrus communis</em> (Schimmel, 1894)</td>
<td></td>
</tr>
<tr>
<td><em>Tanacetum vulgare</em> (Schimmel, 1895)</td>
<td></td>
</tr>
</tbody>
</table>
7. Relative Configuration of Biological Material. The results of numerous investigations undertaken to establish the relative configuration of organic substances may be summarized as follows. All biological isomers of amino-acids possess the same relative configuration. Fischel and Raske (1907) observed that from biological (−) serine can be obtained biological (+) alanine and biological (−) cys-
The suggestion that the relative configuration in all biological isomers of amino-acids is identical was made by Clough (1918); it received confirmation from the work of a number of later investigators (Freudenberg and Rhino, 1924; Langenbeck, 1925; Karrer and Ehrenstein, 1926; Levene and Mardaszew, 1937; Pfeiffer and Christeleit, 1937). In general, it is established that the primary substances, although they rotate the plane of polarized light in different directions, possess the same relative configuration and form a definite "biological series" of optical isomers. Their antipodes are excluded from participation in living processes. Not all the potentialities of dissymmetric configuration, therefore, are employed in the organization of living systems.

8. Asymmetry as a Criterion of the Organic Origin of a Substance. From what has been said, it follows that every deviation from the racemic state, that is, every asymmetry of molecular aggregates represents actually a specific attribute of biological systems, and we do not know a single case when it would take place outside of living organisms or of the products of their activity. Consequently, optical activity can be used as a criterion of the biological origin of such natural products as petroleum. Two theories of the origin of petroleum are generally held, one attributing it to an inorganic, the other to an organic source. The first suggestion concerning the inorganic, volcanic origin of this so-called mineral oil is due to Humboldt (1804). The theory of its organic origin is still older (Lemery, 1675; Lomonosoff, 1761; Spielmann, 1774). In its more modern form (see, e.g., Engler, 1906), this theory implies that the fat contained in the dead bodies of fishes, molluscs and other sea animals, and especially the stable palmitic, stearic and oleinic acids are the ancestors of petroleum. As a result of a breaking down of the chains of carbon compounds under high pressure, hydrocarbons with comparatively low boiling point could arise, a polymerization of which, during geological periods, resulted in our present-day oil. It is also possible that in some cases the initial substance was of vegetable origin.
The theory of the organic origin of oil entered a new phase when Tschugaeff and Walden (1900) pointed out the significance of the forgotten observations of Biot (1835) on the optical activity of oil as a criterion of its origin (see for further confirmation of these views Vernadsky, 1934). Since the asymmetry of molecular aggregates and their optical activity represent an attribute of the material of living systems only, the theory of the organic origin of oil can be considered as based on solid ground.

The genesis of the optical activity of oil is far from clear. Natural fats or glycerides, except lipoids of the lecithin type and fats with active acid radicals, are optically inactive and do not possess any structural dissymmetry. Neuberg (1907) outlined the following scheme for the transformations undergone by these structurally inactive fats in the process of oil formation. Inactive trioleine, which constitutes a considerable part of vegetable and animal fats, would be the original source. By oxidation or hydration, the structurally inactive free oleinic acid would be transformed into a dissymmetric racemic body, for instance, into dioxystearic acid. If now the racemic trioleine with oxidized or hydratated radicals is subjected to the asymmetry-producing action of the fat-splitting enzymes, optically-active fatty acids would arise. Neuberg and Rosenberg (1907) performed all these transformations experimentally; after having obtained optically active fatty acids out of structurally inactive material they transformed these active acids into optically active oil. According to another suggestion of Neuberg (1906), supported by Trask (1937), the active constituents of oil may result from the transformation of proteins of dead bodies. In putrefaction and in autolysis, the transformation of amino-acids into corresponding fatty acids is possible; dextrorotatory isoleucine, for instance, has been transformed into optically active capronic acid. The latter could, by further condensation, give the numerous optically active hydrocarbons of oil.
SUMMARY

1. Dissymmetric molecules are found in inorganic nature where they have evidently no relation to life, but it is questionable whether life is possible without dissymmetric molecules.

2. In inorganic nature, the two forms of dissymmetric molecules are always represented in equal concentrations and the aggregate of molecules thus formed is symmetric (racemic mixture). 3. Asymmetry of molecular aggregates is a specific property of protoplasm and of living systems.

4. Primary constituents of protoplasm, such as the amino-acids, the lecithins and the majority of the important sugars are present in protoplasm in the form of only one of the optical isomers: they are obligatorily asymmetric. 5. In these substances, the sign of the optical activity is not replaceable by the opposite sign. 6. The primary constituents of protoplasm are structurally related to each other and form "biological series" of optical isomers.

7. The secondary constituents of protoplasm which, functionally, represent storage material or excreta are not obligatorily asymmetric; they are sometimes found in living organisms in the racemic state. 8. The sign of their optical activity is replaceable by the opposite sign, so that one optical isomer is sometimes found in one species and its antipode in another.

9. The optical activity of mineral oil lends support to the theory of its organic origin.

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CHAPTER II
THE ORIGIN AND MAINTENANCE OF OPTICAL ACTIVITY IN LIVING MATTER

There are, in living matter, some components which constantly produce optically active substances while other components always racemize. The mechanism which insures the constant production of asymmetric substances needs an explanation. It is somewhat surprising that, for a long time after optical asymmetry had been recognized as a characteristic of some constituents of living matter, the problem of the origin of this asymmetry and of the maintenance of the optical purity of protoplasm was so little investigated.

1. The Transmission of the Asymmetric State by Asymmetric Synthesis. Before studying how the asymmetric purity of the primary components of protoplasm is maintained, let us consider how the asymmetric state is transmitted from one aggregate of molecules to another or, in other words, how the asymmetry of protoplasm is "multiplied." To answer this question, Pasteur (1860) suggested that every asymmetry owes its existence to some asymmetric forces operating at the moment at which the asymmetry appeared. In this manner, one asymmetric substance would bring into being another in the same way as life produces life. The principle "Omne vivum ex vivo" would be paralleled in the transmission of asymmetry.

Pierre Curie (1894) expressed the same fundamental principle as follows: "If a phenomenon possesses a definite asymmetry, the same asymmetry can also be detected in the causes which have given rise to the phenomenon." Curie admits that a given asymmetry gives birth to an-
other asymmetry of the same order of magnitude, i.e., possessing the same degree of optical purity. We shall refer to this view as Curie's principle.

Emil Fischer (1894), after having stated that "one active molecule gives birth to another," illustrates his statement by the following concrete example: "The formation of sugar in plants, according to the observations of physiologists, takes place in chlorophyll grains, which themselves consist of optically active substances." (The optical activity of chlorophyll has been demonstrated recently by Stoll and Wiedemann, 1933.) "I assume," continues Fisher, "that, in the formation of sugar, there is a combination of carbon dioxide or of formaldehyde with these substances of the chlorophyll grains and that the subsequent production of sugar proceeds asymmetrically on account of the presence of the asymmetric molecules of chlorophyll." Thus Fischer considered chlorophyll to be an asymmetric catalyst, the asymmetric state of which is transmitted to the molecules of the organic substance undergoing synthesis and these are, therefore, represented by only one optical isomer. The notion of asymmetric synthesis was thus introduced.

Somewhat earlier, Fischer (1890) had proposed another explanation for the origin of the asymmetric state. He assumed that, in plants, as in the laboratory, racemic compounds would appear first and that these would subsequently be split up by the plant itself into their optical antipodes.

A few years after Fischer had expressed this opinion, Brown and Morris (1893), in a thorough study of sugar metabolism in plants, could find in them neither racemic nor laevorotatory glucose. This finding caused Fischer to abandon his previous idea.

It is now well known that laevorotatory glucose does not occur in living organisms, that it practically does not ferment and that it is not used as food by plants or animals. Furthermore, a great deal of experimental data has been accumulated which shows that, in different enzymatic re-
actions, no intermediate racemic glucose is formed, but the optically active product is obtained immediately (cf., Tomiyasu, 1937).

The catalytic transmission of the asymmetric state was later considered by Strong (1898) in a well known discussion on asymmetry and vitalism.

Asymmetric syntheses were soon experimentally realized in the laboratory. Marekwald (1904) synthesized optically active valerianic acid from structurally inactive methylethylmalonic acid in the presence of active brucine. He defined asymmetric synthesis as a process "in which optically active substances are obtained from symmetric compounds through the intermediary of optically active substances."

The same year McKenzie also realized some asymmetric syntheses.

Since these pioneer investigations, the literature on this subject has expanded considerably and the synthesis of optically active compounds from structurally inactive material has been carried out by a number of other chemists. These researches have been well reviewed by McKenzie (1932, 1936) and by Ritchie (1933) to whom we refer the reader.

But, on the question of the fundamental physical mechanism by which the asymmetric state of the catalyst is transmitted to the substance acted upon, there are only some still incompletely shaped theories, for example, the theory of the so-called asymmetric induction (see Ritchie, 1933).

To summarize, the authors whose views have been described in this section admitted, generally, that some optically active, relatively simple compounds appeared once in nature and that, by asymmetric syntheses, the asymmetric state has been transmitted to other compounds more and more complicated in structure.

2. The Transmission of Asymmetry, from the Thermodynamic and Kinetic Point of View. Recent investigations of the kinetics of asymmetric synthesis have con-
siderably modified our ideas concerning the maintenance of the asymmetric state.

Among the first observations on this subject, one should mention those of Bredig and Fajans (1908) and those of Fajans (1910) on the asymmetric splitting of racemic camphorocarbonic acid into camphor and carbonic acid in the presence of various catalysts.

Almost simultaneously, Rosenthaler (1908) began his studies on the asymmetric synthesis of the nitrile of mandelic acid, which were later on repeated and extended by a number of other investigators and which constitute, at present, the basis for the general theory of asymmetric synthesis. He observed that, by combining the symmetric molecule of benzaldehyde with the symmetric molecule of hydrocyanic acid under the action of the asymmetric catalyst emulsine, one obtains an optically active nitrile of mandelic acid. A considerable excess of dextrorotatory over laevorotatory nitrile was recorded. Rosenthaler also pointed out that the optical activity of the product synthesized by emulsine reached a maximum value after a certain time and then decreased (cf. Table 7).

**Table 7**

**Change in Optical Activity during the Enzymatic Synthesis of the Nitrile of Mandelic Acid (Rosenthaler, 1908)**

(The numbers give the optical rotation of the synthetic product)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time from the beginning of the synthesis:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>25° C.</td>
<td>1.8</td>
</tr>
<tr>
<td>30° C.</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Other observations on the change of optical activity during asymmetric synthesis were made by Nordefeldt (1922). The optical activity was found to tend asymptotically to zero (cf. Fig. 3).

Bayliss (1913), Kriible (1913) and Nordefeldt (1922) showed the important fact that the synthesis of racemic mandelo-nitrile takes place in the absence of enzymes and
that the addition of the latter accelerates the reaction and gives to it an asymmetric character but does not influence its equilibrium constant.

These observations made it possible for Werner Kuhn (1936) to undertake the theoretical analysis of the principles of the asymmetric synthetic action of enzymes. We shall summarize here his more important conclusions.

It should be pointed out, first, that the separated active components of a given organic substance and the equimolecular mixtures of these components (racemates) are not equivalent from the thermodynamic point of view. The mixing of the components into a racemate liberates energy, while their separation requires an expenditure of work. Consequently the optically active state is not a state of equilibrium as compared to the racemic state. The question arises, then, as to the manner in which such conditions of thermodynamic disequilibrium can be realized in catalytic reactions in living matter. One might first inquire whether such reactions are true catalytic reactions or not.

Let us consider the characters of a true catalysis leading to the formation of an asymmetric compound. Inasmuch as the preparation of the left and that of the right antipode of a given substance in equal concentrations are equivalent from the standpoint of energy expenditure, the
constant $K_i$ of equilibrium between the $l$-antipode of the final substance and the initial product must be equal to the constant $K_d$ of equilibrium between the $d$-antipode of the final substance and the initial product. If $c$ is the concentration of the initial substance, $c'_i$ the concentration of the $l$-antipode and $c'_d$ the concentration of the $d$-antipode of the final substance, one has

$$c'_i/c = K_i = c'_d/c = K_d$$

(1)

Condition (1) characterizes a true catalysis. If this condition is not fulfilled, the initial substance will be simultaneously in equilibrium with different concentrations $c'_i$ and $c'_d$ of the two antipodes and the final product will be partially optically active. But this is thermodynamically impossible in the case of true catalysis.

Another character of true catalysis is that the value of the equilibrium constant in equation (1) is the same irrespective of whether a catalyst is used or not. The velocity constant $k_d$ in the formation of the $d$-antipode from the initial material and the velocity constant $k'_d$ in the reverse conversion are increased by the catalyst to the same degree. If the addition of an enzyme would influence the two velocity constants differently and change the equilibrium constant, the reaction would not be a true catalysis and the final product would be optically active.

Experimentally, as has been said above, it was found that, in the synthesis of the nitrile of mandelic acid, the use of the catalyst does not change the equilibrium constant.

That both velocities $k_d$ and $k'_d$ are accelerated to the same extent by the catalyst has been proved in optically non-specific enzymatic reactions (cf. Borsook, 1935).

Furthermore, Nordefeldt (1922) has observed that if in the synthesis of mandelo-nitrile, one adds emulsine when the reaction has already proceeded for a while, the enzyme does not change anything in that which has already been transformed, it exerts its asymmetrical effect only on the material yet to be transformed. In a system which has reached the state of equilibrium without enzymes, the ad-
dition of an enzyme does not change anything either. This does not leave any doubt that, in the case of these isolated enzymatic transformations, we are dealing with true catalysis.

Finally, in true catalysis, the optical activity of the substance being formed represents only a temporary phenomenon which gradually disappears. This will be clearer when we have examined the dynamics of the two ways in which optical activity could be obtained in biochemical reactions, namely, the splitting up of racemates and asymmetric synthesis.

In the splitting of a racemate consisting of two antipodes, \( A_l \) and \( A_d \), which change respectively into \( B_l \) and \( B_d \), one can represent the process as follows:

\[
\begin{align*}
A_l & \xrightarrow{k_1} B_l \\
& \xleftarrow{k'_1} \\
A_d & \xrightarrow{k_d} B_d \\
& \xleftarrow{k'_d}
\end{align*}
\]

(2)

If the left initial product \( A_l \) is transformed into \( B_l \) with a velocity constant \( k_1 \), different from the constant \( k_d \) with which \( A_d \) is transformed into \( B_d \), there results optical activity. If \( k_1 = k_d \) the racemate will be split up symmetrically.

In asymmetric synthesis, a symmetric initial substance \( A \) is transformed with different velocities, \( k_1 \) and \( k_d \), into, respectively, \( B_l \) and \( B_d \), according to the diagram

\[
\begin{align*}
A & \xrightarrow{k_1} B_l \\
& \xleftarrow{k'_1} \\
& \xrightarrow{k_d} B_d \\
& \xleftarrow{k'_d}
\end{align*}
\]

(3)

Kuhn integrated the systems of differential equations
corresponding to these two cases and studied the dynamics of the change of optical activity in terms of time.

In the case of the splitting up of a racemate (2), if \( k_i/k_d >> 1 \) and \( K >> 1 \), the substance \( B_i \) will be obtained almost exclusively at the beginning; its concentration might approach \( c_i \) (if \( c_i \) is the concentration of the initial substance); later, \( A_d \) will be transforming itself into \( B_d \) till, finally, the concentrations \( B_i \) and \( B_d \) are equalized. At the initial and final states the solutions will be optically inactive.

In the case of asymmetric synthesis (3), assuming again that \( k_i/k_d >> 1 \) and \( K >> 1 \), there will be, at the beginning, an accumulation of the \( l \)-form, \( B_l \); the whole initial material \( (c_i) \) will be practically transformed into this \( l \)-antipode, since the velocity constant \( k_d \) is supposed to be very low as compared to \( k_i \). The concentration of the initial substance \( A \) will approach \( c_i \cdot K \). \( A \) will also change very slowly into \( B_d \) and, as a result of this change, its concentration will be reduced and the equilibrium between \( A \) and \( B_i \) will be disturbed: consequently, a certain quantity of \( B_i \) will be transformed into \( A \). This will cause a further transformation of the initial substance into \( B_d \). The process will continue as long as the initial substance \( A \) is in equilibrium simultaneously with \( B_i \) and \( B_d \) or, in other words, until the racemic state is obtained. So the same catalyst which, at first, brought about the transformation of \( A \) into practically pure antipode \( B_i \) later causes a complete racemization of the product.

It is of interest to inquire what is the difference in the stability of the temporary state of optical activity in the case of the splitting up of a racemate and in that of asymmetric synthesis. Kuhn showed that the ratio \( H \) between the time \( T_i \) necessary for racemization and the time \( T_d \) necessary for the attainment of maximal activity is \( H = k_i/k_d \cdot K/2 \) in the case of asymmetric synthesis and \( H = k_i/k_d \) in the case of the splitting of the racemate. The factor \( K/2 \) is absent in the second equation. Since the constant \( K \) is large, it is evident that the stability of the
optically active state will be considerably greater in asymmetric synthesis than in the splitting of racemates.

The experimental data reported above, concerning the temporary character of optical activity (cf. also Bredig and Fajans, 1908; Bredig and Minaeff, 1932; Nordefeldt, 1922) are in agreement with Kuhn's calculations. The conclusion to derive from this agreement is that true catalysis occurs in the isolated enzymatic systems considered.

Furthermore, it should be noticed that asymmetric synthesis, which seems to take place in protoplasm rather than dissociation of racemates, is precisely the process which secures a longer duration of the state of optical activity.

The maintenance of asymmetry in mineral oils is probably to be explained by the extreme slowness of the transformations which take place in them.

3. Maintenance of Optical Purity by the So-Called "Stereo-autonomic Substances." If, in enzymatic systems, one has to do with true catalysis, and in true catalysis there is a gradual decrease in optical activity, one might expect that a substance formed in an asymmetric synthesis be optically less pure than the compound from which it originates (Langenbeck and Triem, 1936). Curie's principle, postulating that any asymmetry originates from another asymmetry of the same order would not hold then. So the presence of protoplastic components in the form of pure optical isomers for an indefinitely long time still lacks an explanation. The features which, in the organization of protoplasm, are responsible for the maintenance of optical purity are still to be found.

Kuhn (1936) showed that, in some cases, the maintenance of optical purity in a system, despite the gradual decrease of optical activity in a single synthetic enzymatic process, can be explained by the behavior of some substances that he called "stereo-autonomic." It is known that the right nitrile of mandelic acid, when synthesized
by the plant, is stored not as such but combined with gentiobiose in the form of β-glucoside (natural amygdaline). The latter easily crystallizes from water solutions, while the glucoside which consists of gentiobiose and of the left nitrile of mandelic acid possesses such a high solubility that it does not, in general, crystallize from water solutions (Walker and Krieble, 1909; Krieble, 1912). 1 So, the fact that pure natural amygdaline is deposited in the plant does not necessarily postulate the existence of an optically specific enzyme, synthesizing only the right nitrile of mandelic acid. The right and the left nitriles may be produced; then the right component precipitates in the form of gentiobioside; the excess left nitrile can thereupon be racemized according to the requirements of true catalysis; the right nitrile originating from this process is again bound to gentiobiose and the process continues until all the nitrile is converted into the less soluble gentiobioside of the right nitrile, i.e., into pure natural amygdaline. The same final state would evidently ensue no matter whether the enzyme possesses the capacity of preferential synthesis of the right nitrile or if it would synthesize racemic nitrile. In the latter case, however, the gradual catalytic transformation of the left component into the initial substance (benzaldehyde and hydrocyanic acid) and the resynthesis of the right component would demand a long time, which is evidently spared by the utilization of an optically specific enzyme. Natural optically active gentiobiose is a stereo-autonomic substance in the sense that it conditions the stable optical purity of the synthetic product. Kuhn sees a confirmation of his views in the fact that, in the fruits of Prunus laurocerasus, one finds a gentiobioside of the pure right nitrile of mandelic acid, while, in the leaves of the same plant, one finds a glucoside of the racemic nitrile. It is probable that the difference in solubility brought about

1 Similar differences are found, in general, in diastereomers, that is, in substances consisting of one antipode of a substance A combined with either of the two antipodes of a substance B. For example, $A_1B_1$ and $A_1B_d$ are two diastereomers.
by glucose as a component of the glucoside and by gentiobiose as a component of the gentiobioside is responsible for the fact that they are found in nature as indicated.

4. Procedures Used by Nature for Maintaining Optical Purity and Establishing a "Fixed Internal Milieu." The evolution of living beings has consisted in a gradual increase of the number of fixed parameters of the internal milieu. For example, in the transition from poikilothermic into homoiothermic animals, the body temperature has been fixed at a constant value. The non-dependence on the temperature of the external medium has given the homoiotherms important advantages over the cold-blooded animals in natural selection.

Considering such cases of specific fixity acquired by the internal medium, Claude Bernard made his famous statement: "La fixité du milieu intérieur est la condition de la vie libre" ("The fixity of the internal medium is the condition of independent living").

The elaboration of mechanisms in living matter to maintain optical purity evidently contributes to the fixity of the internal milieu. The spatial parameters which determine the asymmetry of a substance are fixed in primary constituents of protoplasm in such a way that optical purity is maintained.

Barcroft (1934) notes that two methods are used by nature to secure the constancy of internal medium, the method of evasion and the method of correction.

a) Widely Different Values in the Formation of the Two Optical Isomers. As a mechanism of evasion nature uses a high ratio of the reaction velocities, $k_1/k_2$. While, in the cases of catalysis more commonly encountered, this ratio is of the order of 1 to 2, in enzymatic reactions the ratio reaches 100, 1000, or even greater values. This ratio evidently determines the degree of predominance of the right or of the left isomer in the substance synthesized. A great difference between the velocity constants, $k_1$ and $k_2$, makes that
the non-utilizable isomer appears only in insignificant concentrations in the first stages of synthesis. A large value of the constant of equilibrium $K$, on the other hand, assures a more lasting stability of the active state. Both these factors contribute toward having the system "evade" for a time the effects of inevitable racemization.

b) Langenbeck and Triem's Mechanism. Recently, Langenbeck and Triem (1936) have shown that an increase of optical purity can be obtained in interrupted reactions between two optically impure substances. If an optically impure enzyme is acting upon an optically impure substance and if the reaction is not allowed to proceed to the end, the optical purity of the system may be increased. Let us suppose that two substances, optically active but not optically pure, $A$ and $B$, combine to form $AB$. Let us assume also that the laevorotatory isomers, $A_l$ and $B_l$, predominate over their antipodes, $A_d$ and $B_d$. The following partial reactions will take place

$$A_1 + B_1 \rightarrow A_1B_1$$
$$A_d + B_d \rightarrow A_dB_d$$
$$A_1 + B_d \rightarrow A_1B_d$$
$$A_d + B_1 \rightarrow A_dB_1$$

Since $[A_1] > [A_d]$ and $[B_1] > [B_d]$, we shall have, if we interrupt the reaction before it is completed,

$$\frac{[A_1B_1]}{[A_dB_d]} > \frac{[A_1]}{[A_d]} \text{ and } \frac{[A_1B_1]}{[A_dB_d]} > \frac{[B_1]}{[B_d]}.$$

If, for instance, the concentrations of the initial substances, $A_l$ and $A_d$, are in the ratio 2:1, and if the concentrations of $B_l$ and $B_d$ are identical respectively to those of $A_l$ and $A_d$, i.e., are also in the ratio 2:1, a time will come at which the ratio of the concentrations of the enantiomorphic products, $A_lB_l$ and $A_dB_d$, will be the product of the ratios, $\frac{2 \times 2}{1 \times 1}$, that is, 4:1. If the reaction is interrupted at that time, the optical purity of the transformed material will be increased. (It is certain that, simultan-
eously, the remainder of the untransformed substances will undergo a corresponding decrease in optical purity.)

Langenbeck and Tricem (1936) proved experimentally that the optical activity can be increased in reactions of this type. They synthesized \( \text{l-tyrosine anhydride} \) from \( \text{\text{l-tyrosine methyl ether}} \) and observed a concentration of 30.8% of \( \text{l-tyrosine} \) in the final product while the initial substance contained only 27.4%.

It is possible that such processes have taken place in the enzymatic origin of ferments, that is, when one ferment has been synthesized with the aid of another optically active ferment. Then the necessary decrease of optical purity of the initial material is of no importance since only the newly formed ferment, in the interrupted reaction, will transmit to some other substance its increased optical purity.

It should be noted, in relation with the reactions described in this section, that the succession of synthetic processes which take place continuously in living systems might in itself be an important factor in the evasion of the effects of racemization. The incessant reconstruction of living matter should then, perhaps, be considered as an indispensable condition for the maintenance of the optical purity of stereo-autonomic substances.

It is usually thought that, though nature might evade for a time the effects of racemization, finally the latter will inevitably set in and that nature does not possess any method of correction by which it would remove the unnatural isomer and actively resist racemization. Kuhn (1936) not only accepted the idea of the absence of such active resistance, there being no enzyme known for performing this function, but he thought that the racemization which finally takes place might constitute, in part, the process of ageing.

The fact that, when animals and plants are fed with racemic amino-acids, they principally consume the natural isomers of the left steric series and leave the other isomer intact, has been, in general, considered as proving that the
organisms are devoid of enzymes suitable for catalyzing transformations of the unnatural isomers.

Schulze and Bosshard observed this selective action of one isomer in lower organisms already in 1886 and their data were later confirmed by a great number of authors and especially by Pringsheim (1910).

Many similar observations were made on mammals. A dog which receives a racemic preparation of leucine (Abderhalden and Samuely, 1906) or of alanine (Abderhalden and Schittenhelm, 1907) consumes preferably the natural isomers and excretes in its urine a large portion of the unnatural amino-acids. The same was observed later by Abderhalden and Tetzner (1935) in rats, rabbits and dogs fed with racemic alanine.

But another series of facts points out the possibility of the presence, in living protoplasm, of an active mechanism contributing, by a method of correction, toward maintaining optical purity and thus toward securing the fixity of the internal medium.

(c) Krebs' Mechanism. Krebs (1933) who has undertaken extensive investigations on the oxidative deamination of different amino-acids by tissue slices of liver and kidney from rat, pig, cat, dog and rabbit, discovered the very important fact that, while both optical isomers of amino-acids are deaminated, the unnatural forms of the right steric series are almost always deaminated much more rapidly than the natural ones (cf. Table 8).

These observations were soon confirmed by Kisch (1935) and by Neber (1936). Some data of Kisch are given in Table 9.

Krebs (1935) assumes that there are two different enzymatic systems one of which catalyzes the deamination of the right and the other that of the left amino-acids. This assumption follows, in particular, from the fact that the deamination of the left amino-acids is inhibited by octyl alcohol, while that of the unnatural isomer of the right series is not affected by octyl alcohol of the same concentration. He further points out that the data con-
### Table 8
**Deamination of Optically Active M/20 Amino-acids by Slices of Rat Kidney (Krebs, 1933)**

<table>
<thead>
<tr>
<th>Amino-acid</th>
<th>ml. of ammonia</th>
<th>Ratio of the velocity of deamination of the unnatural to that of the natural isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>$l(+) \text{alanine}$</td>
<td>3.36</td>
<td>11.3</td>
</tr>
<tr>
<td>$d(-) \text{alanine}$</td>
<td>37.80</td>
<td>2.6</td>
</tr>
<tr>
<td>$l(+) \text{valine}$</td>
<td>3.86</td>
<td>14.9</td>
</tr>
<tr>
<td>$d(-) \text{valine}$</td>
<td>57.60</td>
<td>2.8</td>
</tr>
<tr>
<td>$l(-) \text{leucine}$</td>
<td>6.68</td>
<td>5.2</td>
</tr>
<tr>
<td>$d(+). \text{leucine}$</td>
<td>34.90</td>
<td>7.4</td>
</tr>
<tr>
<td>$l(-) \text{phenyl-alanine}$</td>
<td>10.4</td>
<td>7.4</td>
</tr>
<tr>
<td>$d(+). \text{phenyl-alanine}$</td>
<td>77.0</td>
<td>1.2</td>
</tr>
<tr>
<td>$l(-) \text{histidine}$</td>
<td>3.18</td>
<td>3.1</td>
</tr>
<tr>
<td>$d(+). \text{histidine}$</td>
<td>9.75</td>
<td>3.1</td>
</tr>
</tbody>
</table>

### Table 9
**Deamination of M/50 Amino-acids by Slices of Liver and Kidney of Different Animals (Kisch, 1935)**

(The velocity of deamination is expressed in ml/5000 of NH$_3$ per gram of fresh weight of tissue in 2 hours)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Organ</th>
<th>Number of experiments</th>
<th>Amino-acid</th>
<th>Natural isomer (\text{I})</th>
<th>Unnatural isomer (\text{d})</th>
<th>Ratio of the velocity of deamination of the unnatural to that of the natural isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Kidney</td>
<td>3</td>
<td>Alanine</td>
<td>15.7</td>
<td>56.8</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Luecine</td>
<td>3.9</td>
<td>115.6</td>
<td>29.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Isoleucine</td>
<td>16.2</td>
<td>141.1</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>2</td>
<td>Alanine</td>
<td>1.9</td>
<td>11.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Sheep</td>
<td>Kidney</td>
<td>2</td>
<td>Alanine</td>
<td>13.5</td>
<td>81.6</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Luecine</td>
<td>1.2</td>
<td>24.9</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Isoleucine</td>
<td>13.2</td>
<td>118.9</td>
<td>9.0</td>
</tr>
<tr>
<td>Pig</td>
<td>Kidney</td>
<td>2</td>
<td>Alanine</td>
<td>16.9</td>
<td>222.2</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Luecine</td>
<td>2.8</td>
<td>52.0</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>Isoleucine</td>
<td>12.5</td>
<td>136.5</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>5</td>
<td>Alanine</td>
<td>7.1</td>
<td>23.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>
cerning the oxidative deamination do not contradict the fact of a more rapid consumption by the whole organism of natural isomers of amino-acids. Natural amino-acids can evidently be consumed without deamination; consumption and deamination need not to coincide.

From the standpoint of the problem of the maintenance of optical purity in protoplasm, Krebs' results are very significant. The study of catalysis has shown that in protoplasm composed of optically pure left isomers of amino-acids the appearance of small quantities of the right forms is inevitable. Krebs' data suggest that the organisms have developed a mechanism for removing the isomers of unsuitable spatial configuration. This mechanism would consist in a deamination of the inappropriate right forms as soon as they appear. The right isomers would be transformed into structurally inactive keto-acids identical with those which can be obtained from the left amino acids. In this manner, the organisms would by no means be so helpless in regulating the optical purity of their protoplasm as was assumed by Kuhn and they would possess an active method of correction for securing the fixity of their internal medium.

It is to be noticed that Ritchie (1933), before any of the researches that we mentioned on oxidative deamination had been made, admitted a priori the possibility of the existence of such a method of correction. He wrote that, while one of the antipodes participates in cell metabolism, the other, which is formed simultaneously but at a much lower rate, almost certainly is removed as soon as it is formed. Ages of evolution would be responsible, according to him, for the development of such a physiological regulating system.

One might, at first, be inclined to consider the existence of a special enzymatic system acting on unnatural isomers of amino-acids, as a chance happening without particular significance. But, then, what sense is there in talking of a "specificity" of any enzymatic reactions, and in explaining this specificity as a result of a long process of natural selection? (Cf., Eric Holmes, 1937.)
Krebs (1936) proposed another interpretation of his data. Following Emil Fischer’s somewhat archaic views on a possible synthesis in protoplasm of racemic amino-acids and of their subsequent splitting into optical isomers, he considered the deamination by a deaminase specific for right amino-acids as a process by which the organism decomposes the racemate and obtains the left amino-acids required. But it has been seen above that the experimental data available do not speak in favor of the hypothesis of a primary symmetric synthesis.

5. Biological Advantages of Optical Purity. In the study of the methods used by nature to maintain optical purity, some authors have considered the advantages for living organisms of working with asymmetric material. Mills (1932) has attempted to show that living systems consisting of substances in the asymmetric state are more efficient than their hypothetical racemic competitors would be. On the basis of what is known on the stereo-specificity of the action of, for example invertase, in the hydrolysis of saccharose one can expect that the common invertase activate only the dextrorotatory saccharose and that the optical isomer of this invertase act only on the left saccharose. Let us consider the initial stage of the reaction, when, with small concentrations of saccharose, the velocity of hydrolysis is approximately proportional to the concentration of the enzyme and to the concentration of the substance acted upon. In an experiment with optically pure saccharose and corresponding invertase, every molecule of saccharose coming in contact with the enzyme will be subject to activation, while in an experiment with a racemic saccharose and a $d/l$-invertase only one-half of the collisions will be effective. Consequently, the reaction in the racemate will take place at a considerably lower rate than that in the optically active system.

It should be noticed that, in the case of two enantiomorphic systems of transformations working side by side (racemic material), the velocities of many processes might be decreased when the two dissymmetric substances inter-
act. Consequently, the synthesis of the components of new tissues and the growth of the latter will proceed more rapidly with asymmetric than with racemic material.

If the fundamental physiological processes are more intense in asymmetric systems, the passage from racemic to optically active protoplasm was a significant physiological advance which contributed to the survival of asymmetric protoplasm in the process of natural selection. Besides, the development of asymmetry, by contributing to the fixity of the internal medium, increased the possibility of independent life for any given organism, in the sense of Claude Bernard.

6. The Origin of the Asymmetry of Protoplasm. Assuming that the asymmetry of protoplasm is maintained by some mechanism devised by nature, a fundamental problem still remains to be solved, that of the origin of the initial inequality of the right and the left components of protoplasm.

In the study of the causes of the initial asymmetry, the authors have followed two directions. Some have attempted to correlate the origin of the asymmetry of matter with the asymmetric influence of terrestrial magnetism; others have considered asymmetry as originating in a deviation from a statistical average.

Since Cotton (1896) had shown that solutions of optically active substances possess different coefficients of absorption for the right and the left circularly polarized light, it has been thought that the action of such light might furnish a promising method of obtaining active compounds from racemic ones.

It is known that the circularly polarized light is found in nature, for instance, when the plane-polarized light from the sky is reflected on the surface of the sea. Byk (1904) suggested that, because of the rotation of the plane of polarization of light by terrestrial magnetism, there must be, in the total quantity of light circularly polarized at the surface of the earth, a predominance of one of the two forms of light. This predominant form acting for
long periods of time on racemic compounds would initiate optical activity.

More recently Kuhn and Braun (1929) and Kuhn and Knopf (1930) have shown that, in laboratory experiments, when circularly polarized light is used in the photochemical decomposition of racemates, it causes the appearance of optically active isomers.

Ritchie (1933) and later Langenbeck and Triem (1936) supported the hypothesis just described.

The second explanation of the origin of optical asymmetry (cf., Pearson, 1898; Fitzgerald, 1898; Bartrum, 1898; Errera, 1898; Kipping and Pope, 1898; Byk, 1925; Mills, 1932) is based on the assumption that the equality of the right and left components represents a statistical mean value around which fluctuations occur. Kipping and Pope (1898) observed, for example, that, while the occurrence of either right or left component, in crystallization experiments, furnished a mean value of 50.08% ± 0.11, the proportion varied from 24.14% to 77.36% in separate experiments (46 of them). An inequality of the right or the left form of a substance might have originated accidentally in this manner when some living systems were in formation and this inequality might have spread by asymmetric catalysis (Strong, 1898).

Lately Spiers (1937) supported the chance deviation hypothesis of the origin of asymmetry.

7. General Survey of the Problem of the Origin and Maintenance of Optical Asymmetry. The various stages in the development and maintenance of the asymmetric state are represented diagramatically in Fig. 4.

Let us note, first, that there are two levels of stability for the state of symmetry or asymmetry: 1. the level of thermodynamic stability which characterizes the racemic state; 2. the level of protoplasmic stability which is maintained by living matter. In inorganic nature, the racemates are stable because they possess the least amount of free energy. In living nature, optically pure forms are stable because they are the most advantageous in natural
selection. So the racemic state is stable in inorganic nature and unstable in living matter.

For living systems to pass from the level of thermodynamic to that of protoplasmic stability and to stay at the latter level requires a series of mechanisms of which two have been described: the Langenbeck and Triem mechanism by which the optical purity is increased in a series of interrupted reactions (upward arrows in plain lines, \( A \), in the diagram) and the Krebs mechanism by which the unnatural optical isomers are removed (upward arrows in dotted lines, \( B \)).

Since the racemic state represents a state of thermodynamic equilibrium, the initial asymmetry will have a tendency to disappear (Kuhn's mechanism, downward arrows, \( C \), in the diagram).

The effect of circularly polarized light in inducing some asymmetry will probably not be sufficient to maintain the high degree of optical purity exhibited by protoplasm.

It is possible also that the Langenbeck and Triem mechanism, which probably is not efficient enough to maintain the almost absolute purity of protoplasm as we know
it at the present time, was involved in the early stages of natural selection while Krebs' mechanism, which is more highly efficient, was developed only later in evolution.

The asymmetric state of protoplasm and its maintenance by regulative mechanisms appear, then, as a heritage of countless ages of transformations, and as a result of the elaboration by nature of systems which seem to tend to some sort of physiological perfection.

**SUMMARY**

1. According to the earlier authors, asymmetry, once originated, has been transmitted from one substance to another, as life is transmitted from one living being to another.

2. Emil Fischer suggested that asymmetric catalysts synthesize asymmetric compounds from symmetric ones. Such *asymmetric syntheses* were soon realized in laboratory experiments.

3. It was then observed that, in an asymmetric synthesis, the optical activity reaches a maximum and then decreases, and that the enzyme does not influence the equilibrium constant of the reaction. These observations have been the basis of theoretical investigations by Kuhn on the thermodynamics of asymmetric synthesis.

4. Kuhn pointed out that the separation of two optical isomers requires an expenditure of work, while their mixing into a racemate liberates energy, the optically active state being a state of disequilibrium. He further showed that the characters presented by enzymatic reactions are those thermodynamically expected in true catalysis.

5. Optical purity might be conditioned in some cases by the behaviour of "stereo-autonomic substances," *i.e.*, of substances whose properties, such as solubility, maintain one isomer in solution while the other separates out.

6. To maintain the state of disequilibrium inherent in optical purity, nature, it seems, has developed regulating mechanisms, such as: (a) The use of widely different velocities in the formation of the two optical isomers in
asymmetric syntheses; (b) The succession of reactions which are interrupted before the optical activity has had time to disappear (Langenbeck and Triem's mechanism); (c) The more rapid deamination of the unnatural isomer which then separates out (Krebs' mechanism).

7. Optical purity seems to impart to protoplasm some advantages in natural selection, in particular, it seems to increase the reaction rate and the growth activity. So, the establishment of a state of optical purity can be considered as a method used by nature to stabilize the internal milieu.

8. The origin of asymmetry has been ascribed by several authors to the influence, on some reactions, of circularly polarized light, which would be predominantly right or left on account of its rotation by terrestrial magnetism; other authors think that the equality of right and left isomers is a statistical mean value and that the inequality resulted from fluctuations from the mean.

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CHAPTER III

HEREDITY AND THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE OPTICAL ACTIVITY OF BIOLOGICAL MATERIAL

Pasteur (1860) wondered how living beings would differ from what they are if the basic chemical substances which compose them would change the sign of their optical rotation. Emil Fischer (1890) attempted to show how this question can be answered experimentally. "If it proves possible", he writes, "to feed plants, moulds or yeasts with unnatural optical isomers of different substances, should one not expect that such a change in the constructive material would result in a modification of the delicate molecular architecture and of the entire structure of organisms? The biologists have not yet studied this question because the chemists have not given them the substances necessary for such experiments". At present, fifty years after Emil Fischer made this statement, we know that such transformations of biological structures are impossible, as the following data will show.

1. The Impossibility of Inverting the Optical Properties of the Primary Constituents of Protoplasm. It is now well established that, if one gives to microorganisms unnatural food material as, for instance, laevorotatory leucine, they are simply unable to make use of such food as they do not possess the suitable enzymatic outfit. This was soon ascertained by Fischer himself who found that laevorotatory glucose is practically not fermented by yeast cells (Fischer and Thierfelder, 1894).

In the light of modern knowledge one would not expect that a simple change in nutrient conditions would deter-
mine the sign of asymmetry of protoplasm, which is, according to all evidence, the result of a long evolutionary development.

Among more recent investigations on the inefficacy of culture media on the sign of optical activity of primary constituents of protoplasm, we shall mention those of Gause and Smaragdova (1938). These authors have studied the effect on the yeast, Torula utilis, of a prolonged cultivation in the optical isomers of leucine. The yeast was cultivated at 27°C on pure left leucine in one series and on pure right leucine in another. During the 1½ months that the experiment lasted, 14 passages were made. At the end of this period the rate of growth in both series was measured. A similar procedure was followed for a study of the action of right and left valine. It was found that, though growth of Torula proceeds much more rapidly on the biological forms of these amino-acids (laevorotatory leucine and dextrorotatory valine), there is some growth on their optical antipodes. It should be noticed that the majority of other yeasts cannot grow at all on the unnatural isomers of amino-acids. The very possibility of a weak but unlimited growth of Torula utilis on the right leucine and on the left valine is correlated with the fact that these substances are first deaminated by yeast (leucine, according to Ehrlich, 1906, is transformed into iso-amyl alcohol), and the ammonia thus formed proves to be a sufficient source of nitrogen for the unlimited growth in Torula utilis. But the supply of nitrogen in the form of ammonia alone is not sufficient for most of the other species of yeast and a prolonged growth on the unnatural isomers of amino-acids cannot take place in them.

As was to be expected, a prolonged cultivation of Torula on such nonbiological isomers had no essential influence on the asymmetric properties of the protoplasm of these yeasts, so that growth always remained more rapid on the natural amino acids. Structurally inappropriate isomers
cannot be directly involved in metabolism. The slight growth observed on amino-acids which can be deaminated and thus transformed into material deprived of dissymmetry, from which subsequently molecules of suitable properties are built, even confirms this view. This conclusion is in accord with a number of recent observations (Conrad and Berg, 1937; Du Vigneaud et al., 1939; Ratner, Schoenheimer and Rittenberg, 1940).

2. The Impossibility of Modifying Protoplasm so as to Cause it to Invert the Optical Properties of the Products of its Metabolism. That some products of metabolism, that is, some secondary constituents of living matter may be generated in either of the two optically isomeric forms, under the influence of external conditions, has been admitted for a long time by a number of investigators. The case of lactic acid fermentation by some microbes is classical in this respect. Concerning this case we shall mention first the fundamental facts on the specificity of each bacterial strain in the production of one type of lactic acid and then we shall review the more important investigations on the effect of external conditions on such production.

Nencki (1891) showed that the optical form of the lactic acid produced in microbial fermentation is specific for the kind and strain of microbes. Some species produce the pure dextrorotatory isomer, others give the pure laevorotatory one and still others produce a form of lactic acid which is either totally or partially racemic. The sign of the asymmetry of the secondary substances seems, therefore, to represent a stable hereditary characteristic of the physiological organization of the cell which has produced these substances and Nencki even proposed to employ it for the identification of bacteria. These observations were subsequently confirmed by Currie (1911), Pederson, Peterson and Fred (1926) and by Katagiri and Kitahara (1937).
As to the problem of the possibility that different isomers of lactic acid be produced when conditions of cultivation are changed, the data of the literature have for a long time been somewhat contradictory.

a. Influence of General Culture Conditions. It was Péré (1893) who claimed for the first time that one and the same line of *Bacterium coli* in different culture conditions can produce the two inverse forms of lactic acid (cf. also Pottevin, 1898, and Péré, 1898). But to what extent the strains of microbes used by them were bacteriologically pure is not clear.

Pederson, Peterson and Fred (1926) showed that in mixed cultures of microbes consisting of producers of the right and left isomers of lactic acid, one can often observe a change in the form of the lactic acid produced when the temperature, for example, is changed. However, in such cases, there is evidently no inverting mechanism in protoplasm. The species and strains of microbes which produce the right and the left isomers of lactic acid possess different temperature optima of growth and metabolism and consequently, in some conditions of cultivation, the strains which produce the right isomer and, in other conditions, those which give the left isomer of lactic acid predominate. With microbiotic material of a guaranteed purity having originated from one cell, the possibility of inverting the lactic acid produced has not been observed even in the most different culture conditions, though the only isomer produced may subsequently undergo racemisation to various degrees.

b. Influence of the Culture Medium. In the literature the work of Kayser (1894) is often referred to as confirming Péré's data, whilst, as a matter of

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1It is to be pointed out that *Bacterium coli* does not cause a pure lactic acid fermentation. Approximately half of the sugar is fermented into lactic acid, the remainder is transformed into acetic acid, ethyl alcohol, carbonic acid and hydrogen (Neuberg and Gorr, 1925). After this was shown, the investigators began to work with true lactic-acid-producers such as *Lactobacillus*.
fact, the results of these two authors do not agree. Kayser studied the formation of the optical isomers of lactic acid by different strains of microbes cultured on various sugars. He used 14 bacterial species or strains, with which he performed 61 experiments. His data, which are summarized in Table 10, show that there are strains which always produce the laevorotatory lactic acid, others which always produce the dextrorotatory isomer and finally some which yield a racemic mixture. Kayser’s results (1894), therefore, coincide not with Péré’s (1893) but with the data of recent investigators, particularly with the thorough observations of Katagiri and Kitahara (1937). In only one of the 61 experiments of Kayser (the last one marked with an asterisk in the table) was dextro-rotatory lactic acid produced on one sugar (glucose) and laevorotatory lactic acid on another (maltose). Since this single exception might have resulted from an insufficient purity of the bacterial culture employed (cf. Pederson and his collaborators, 1926), one comes to the

### Table 10

**Influence of Various Sugars in the Nutritive Medium on the Optical Properties of the Lactic Acid Produced by Different Bacteria**

(Kayser, 1894)

(The letters a, b, c, ... refer to the species and strains of bacteria, most of which were not completely identified; the letters l, d, and dl indicate the optical rotation of the lactic acid produced; the figures (1) and (2) after Maltose and Lactose mean that two media of different composition were used with each one of these sugars.)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>s</th>
<th>g</th>
<th>h</th>
<th>l</th>
<th>m</th>
<th>n</th>
<th>o</th>
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</table>
conclusion that Péré's results are by no means confirmed by Kayser's.

This same table shows that racemization is greatly influenced by the culture medium. A given strain of bacteria, when cultured on a definite sugar, forms an almost optically pure lactic acid (Pederson and collaborators, 1926, had shown that it is never entirely optically pure), while on another sugar, it forms a racemic mixture. This is the case, for instance, in the strains g, m, n, o, p. Pederson and his collaborators (1926) made further observations on this point.

There are also the recent researches of Tatum and his coworkers (1932) in which 4 strains of lactic acid bacteria producing laevorotatory acid, 3 strains producing the dextrorotatory acid and 13 different strains of \textit{Clostridium acetobutylicum} were used. These authors found that lactic acid bacteria produce the optically pure form of lactic acid when grown separately and the racemic form when grown in association with the microbe causing acetonebutylic fermentation (\textit{Clostridium acetobutylicum}).

At first Tatum (1932) interpreted his results in the light of the hypothesis of Orla-Jensen (1919) according to which there are in the bacterial cell two independent enzymes, one of which produced the right and the other the left lactic acid. In consequence of the association of lactic acid bacteria with the butylic bacteria the metabolism of the former would change in such a manner that both optical isomers of lactic acid would start to be produced. However, in his later work (1936), Tatum showed that, in the association of the two types of bacteria, lactic acid is always initially formed in the optically active state by the lactic acid bacteria, and that \textit{Clostridium} is only responsible for the subsequent racemization. (It is interesting to note that racemization takes place in the presence of antiseptics, therefore it is of enzymatic nature.) The investigations were continued by Katagiri
and Kitahara (1937) who established that the lactic-acid bacteria which produced racemic acid are also capable of racemizing a ready-made active acid. This is performed by a special enzyme, called by these authors racemiase. Those species and strains of bacteria which, in fermentation, form as a final product optically active lactic acid cannot racemize a ready-made active acid, they have no racemiase. The formation of racemiase is closely dependent on the culture conditions.

The results of the experiments of Katagiri and Kitahara (1937) are represented in Table 11. They show clearly that, with various strains of *Leuconostoc mesenteroides* and *Lactobacillus sake*, a change of the conditions of cultivation never brings about a change in the type of the lactic acid formed; a greater or lesser degree of racemization of the substance produced accounts for all the observed facts.

c. Influence of Temperature. Similar results were reported also in investigations on the in-

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<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Xylose</th>
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fluence of nitrogen nutrition and of temperature upon the optical form of the lactic acid obtained in bacterial fermentation. The effects of various incubation temperatures are presented in Table 12. One sees that the temperature does not influence the sign of the optical rotation, but that the degree of racemization of the acid regularly increases with the rise of temperature.

Thus the old data of Kayser (1894) become clear. In different conditions of culture, the lactic acid formed by a specific strain of bacteria possesses a different degree of optical purity depending on the quantity of racemiase contained in the bacterial cells.

Recently Kopeloff (1937) has shown that racemiase is sometimes lost in the transition of the R-forms of lactic acid bacteria into the S-forms.

To summarize, the production of a specific optical isomer, in the case of such secondary substances of the protoplasm, or products of metabolism, as lactic acid, represents a fixed hereditary character which is not dependent on the conditions of cultivation. It is only such processes as the velocity of a catalytic racemization of secondary substances initially formed in the optically pure state and the formation of racemiase which are dependent on the culture conditions. The hereditary char-

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Temperature</th>
<th>30°C</th>
<th>20°C</th>
<th>60°C</th>
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<td>No. 42</td>
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<td>dl</td>
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<td>dl+dl (17%)</td>
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<tr>
<td>No. 24</td>
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<td>dl+dl (39%)</td>
<td>dl+dl (59%)</td>
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<tr>
<td>No. 45</td>
<td></td>
<td>dl+dl (10%)</td>
<td>dl+dl (47%)</td>
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</tbody>
</table>

*Table 12: Influence of the Temperature of Incubation upon the Optical Form of the Lactic Acid Obtained in Fermentation (Katagiri and Kitahara, 1937)*

(The letters l, d and dl indicate the optical rotation.)
acter of optical activity in secondary constituents of protoplasm and its independency on the external conditions indicates that some physiological mutations peculiar to some specific strains of bacteria must have occurred some time in the past in those of them which produce optically unusual isomers.

Let us now attempt to penetrate into the nature of the process by which a given isomer arises instead of its antipode. This problem is directly related with the study of some of the basic principles which underly the formation of physiological mutations.


a. Production of Dissymmetric Substances from Symmetric Phenyl-Glyoxal. The observations of various authors concerning the transformations of phenyl-glyoxal, a substance deprived of dissymmetry, into mandelic acid which possesses an asymmetric carbon atom, are important in the study of the question here discussed. These transformations are catalysed by enzymes known generally as ketonaldehydemutases. Starting from a symmetric initial product these enzymes synthesize directly, without any intermediate racemic stage, the optically active mandelic acid. Furthermore, ketonaldehydemutases of different species of bacteria synthesize from the same initial product substances which are optically inverse, as the results reported by different authors and represented in Table 13 show.

The action of the ketonaldehydemutases is probably to be attributed to the asymmetric state of these enzymes.

There are many observations more or less directly related to those just given. Neuberg and Simon (1926), for example, found that an acetic-acid bacterium, B. ascendes, produced laevorotatory amyl alcohol from a racemic valeric aldehyde, while another bacterium of acetic acid
fermentation, *B. pasteurianum*, formed dextrorotatory amyl alcohol (an excess of 5 to 18 per cent) from the same initial aldehyde. Analogous results were also obtained with *Bacterium pasteurianum* in acetone preparations.

b. Production of Optical Isomers by Esterases. The data of Willstätter and his collaborators on the stereochemical specificity of esterases, the enzymes which catalyse the hydrolysis of the ethers of different organic acids, are of special interest in the present problem. Some of these data are presented in Table 14, but for more complete information we refer the readers to the review by Rona and Ammon (1933).

In the majority of cases the esterase from liver and the esterase from pancreas catalyze the hydrolysis of optically inverse forms in initial racemic substrates.

c. Production of Optical Isomers by Optically Active Alkaloid Catalysts. Bredig and Fajans (1908) and Fajans (1910), in their classical investigations on the decomposition of racemic

| Table 13 |
| Optical Activity of Mandelic Acid Produced by Various Microorganisms from Phenyl-Glyoxal |
| Organism | Initial Substance | Mandelic Acid Obtained | Author |
| 1. *Bacterium ascenden* | Phenyl-glyoxal | d(−) about 100% | Mayer, 1926 |
| 2. *Lactobacillus 48* | " | l(+) " 84% | " |
| 3. The same; Acetone preparation | " | l(+) " 82% | Neuberg and Simon, 1927 |
| 4. *B. delbruecki* | " | l(+) " 10% | " |
| 5. *B. lactis acrogenes* | phenyl-glyoxal | d(−) " 95% Hayashi, 1929 |
| 6. *B. proteus* | " | d(−) " 78% | " |
| 7. The same; Acetone preparation | " | d(−) " 43% | " |
| 8. *B. fluorescens* | " | d(−) " 37% | " |
| 9. *B. pyocyaneum* | " | d(−) " 87% | " |
| 10. *B. prodigiosum* | " | d(−) 68 to 75% | " |
| 11. *B. coli* | phenyl-glyoxal | d(−) about 100% | Binder-Kotrba, 1926 |
| 12. Parts of green plants | " | " | " |
camphoro-carbonic acid into camphor and carbonic acid under the influence of catalysts (optically active alkaloids), have established that laevorotatory quinine catalyses a more rapid decomposition of the left camphoro-carbonic acid, while dextrorotatory quinidine causes a more rapid decomposition of the right camphoro-carbonic acid.

TABLE 14

<table>
<thead>
<tr>
<th>Initial racemic substrate</th>
<th>Esterase from Pig's Pancreas</th>
<th>Esterase from Pig's Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandelic acid—methyl ether</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>&quot; &quot; ethyl ether</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>&quot; &quot; monoglyceride</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>Phenylmethoxyacetic acid—methyl ether</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>Phenylchloroacetic acid—methyl ether</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Phenylaminoacetic acid—propyl ether</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Tropic acid—methyl ether</td>
<td>(+)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

A similar condition has been observed in the synthesis of optically active substances from structurally inactive material. Bredig and his collaborators, in their study of the synthesis of nitriles from hydrocyanic acid and different aldehydes with the aid of optically inverse catalysts of known chemical constitution, have obtained the results reported in Table 15.

So the optically inverse catalysts, quinine and quinidine, bring about the synthesis of optical antipodes of the nitrile of mandelic acid from initial structurally inactive material. Quinine behaves in this respect analogous to emulsin, while quinidine has the properties of the antipode of emulsin. If the optically active catalyst, therefore, is of a given sign, it affects in a definite direction the products of the catalyzed reaction.

d. Production of a Given Optical Isomer by a Chemical Alteration of the Catalyst. In the cases studied in the preced-
ing pages substances of different optical signs result from the action of optically active catalysts of different signs. One of the two inverse catalysts might have originated by an inverson from the other, but another possibility is that the original catalyst has been changed chemically so as to produce an optically inverse substance without being itself inverted. The following case illustrates this last possibility. In the study of the enzymatic hydrolysis of racemic ethyl mandelate by the esterase of the human liver, it has been found that if one adds some strychnine, one obtains a strongly laevorotatory mandelic acid instead of the usual dextrorotatory one (Bamann and Laeverenz, 1930). Strychnine does not influence the velocity of hydrolysis of the right ether, but it strongly increases that of the left ether thus causing the formation of an excess of laevorotatory material (for further details see Rona and Ammon, 1933). There was no optical inversion of the enzyme but the chemical properties of the latter have been changed by combination with strychnine.

c. Control of the Production of Optical Isomers by Intermediate "Pathways". Let us now consider in greater detail the case

<table>
<thead>
<tr>
<th>Initial aldehyde</th>
<th>Catalysts</th>
<th>Synthetic nitrile</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>Emulsin</td>
<td>d—nitrile</td>
<td>Rosenthaler,</td>
</tr>
<tr>
<td></td>
<td>Quinine</td>
<td>d—nitrile</td>
<td>1908</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bredig and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fiske, 1912</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
<td>l—nitrile</td>
<td></td>
</tr>
<tr>
<td>Cinnamic aldehyde</td>
<td>Emulsin</td>
<td>d—nitrile</td>
<td>Rosenthaler,</td>
</tr>
<tr>
<td></td>
<td>Quinine</td>
<td>d—nitrile</td>
<td>1909</td>
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<td></td>
<td>Quinidine</td>
<td>l—nitrile</td>
<td>Bredig and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Minaeff, 1932</td>
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</table>

TABLE 15
Optical Properties of the Nitriles Synthesized from Hydrocyanic Acid and Various Aldehydes under the Action of Different Organic Catalysts
so much investigated of the production of one optical form of lactic acid by one kind of organism and of the production of the other isomer of lactic acid by other organisms.

An important contribution to the study of this phenomenon has been brought forward by Embden, Baldes and Schmitz (1912) who discovered that in the transformation of glucose into lactic acid by different animal tissues dextrorotatory lactic acid is produced exclusively.

Another important advance was the finding of Neuberg (1913) that extracts of animal tissues transform ethylglyoxal, a structurally inactive body, into laevorotatory lactic acid. A number of papers were then published on the methyl-glyoxal reactions. It was found that in all cases when methyl-glyoxal is converted into optically active lactic acid the latter is laevorotatory. This was observed, in particular by Neuberg and Kobel (1927) with the yeast, Saccharomyces cerevisiae, by Neuberg and Simon (1928) with Mucor javanicus and by Widmann (1929) with Bacterium fluorescens.

From these observations Embden, Deuticke and Kraft (1933) drew the important conclusion that since in tissues of higher animals pure dextrorotatory lactic acid is always formed and since the same tissues transform methyl-glyoxal into laevorotatory lactic acid, methylglyoxal cannot be the precursor of the dextrorotatory lactic acid which appears in normal metabolism. Embden then developed his theory of glycolysis in muscle which received general acknowledgment. But, so far as we are concerned in the present review, the essential fact is that both optical isomers of a certain substance can appear in metabolism when different intermediate substances are involved. The left isomer of lactic acid is obtained from glucose if the intermediate is methyl-glyoxal, and the right isomer of lactic acid if the intermediate is, according to current views, pyruvic acid. Embden suggested that one or the other of these "pathways" could
be followed in the cells of different organisms and then the production of the right or the left isomer of lactic acid by various types of bacteria or tissues would be accounted for. A physiological mutation which brings about an optical inversion of the secondary protoplasmic constituents may consequently consist in a change of the intermediate pathways in the transformation of these substances.

It appears then that the right and the left form of a substance should not be considered so fundamentally opposed as far as their production is concerned, since one has only to change the path followed in the transformations to obtain one or the other.

What happens when a right or left isomer originates might also happen when the *relative configuration* of optically active organic substances is concerned. In the majority of cases optical isomers entering into the composition of living systems possess the same relative configuration. Thus the configuration of natural alanine is the same as that of natural ephedrine (Freudenberg and Nikolai, 1934); the configuration of natural proline is the same as that of natural nicotine according to Karrer (see Pfeiffer and Christeleit, 1937). But there are also cases in which the substances which constitute living systems belong to different series. Thus dextrorotatory lactic acid, which is so generally found in the tissues of higher animals, possesses the same relative configuration as the unnatural laevorotatory tartaric acid, which is never found in organic material. (It also has the same configuration as natural dextrorotatory alanine; cf. Freudenberg and Rhino, 1924, and Freudenberg, Brauns and Siegel, 1923). The unity or the diversity of the relative configuration of organic substances, as well as the character of being dextro- or laevorotatory, might depend only on the biochemical path followed in the formation of the substance.
f. Control of the Production of Opti-
tical Isomers by an Inversion of the Walden Type. Walden (1905) has shown that some optically active substances, when subjected to a series of substitution reactions, come out inverted. For example, dextrorotatory alanine treated with bromides forms l-bromopropionic acid and, upon reaction with ammonia, alanine is again obtained, but laevorotatory alanine. Such a process is known as the "Walden inversion" and, according to Emil Fischer, it is "the most remarkable finding in the field of optical activity since the fundamental investigations of Pasteur".

The mechanism of this inversion is far from clear. In reactions of a certain type the asymmetric carbon atom must be acted upon in such a manner that the configuration of the molecule is inverted. In the example given above one cannot even say if this is accomplished in the transformation of alanine into bromopropionic acid or in the transformation of bromopropionic acid into alanine.

Mills (1932) had attempted to explain the Walden inversion on the basis of some peculiarities of substitution reactions. According to Levene, Rothen and Kuna (1937), there does not seem to be any general agreement on this point.

Some have assumed that in biochemical reactions the presence of a special enzyme, the waldenase, would be responsible for the Walden inversion of some amino-acids. This assumption, however, does not seem to stand a critical study.

Walden inversion might perhaps play a role in some biochemical processes, such as in the formation by some bacteria of laevorotatory lactic acid from dextrorotatory glucose (see Freudenberg, Brauns and Siegel, 1923), while other bacteria form dextrorotatory lactic acid. This assumption, however, is not in the trend of current biochemical theories. It is generally supposed that, in the
formation of the left lactic acid, the structurally inactive methyl-glyoxal represents an intermediate stage and, according to Embden’s scheme, in the formation of dextro-rotatory lactic acid, the structurally inactive pyruvic acid is the intermediate stage. The dissymmetric configuration of the molecules is believed to disappear in the intermediate stages of transformation and then to reappear again. In this interpretation one assumes that the asymmetry of molecular aggregates has disappeared in the intermediate stages because of the loss of dissymmetry of the molecules.

However, it is not necessarily so and Neuberg (1913), questioning such necessarily so, brought forward the theory of "temporary dissymmetric substances." If, for instance, in the intermediate stages of transformation, methyl-glyoxal possesses some H and OH groups attached to it, the dissymmetric configuration of molecules will not disappear nor the asymmetric structure of molecular aggregates; then it would be possible that in the production of one of the two optical isomers of lactic acid from an initial active glucose by one type of microbes a Walden inversion of the configuration of molecules takes place.

The various questions studied in the last two sections suggest the two following generalizations which may be of significance in understanding the basic principles of vital activity: 1. The impossibility of altering the optical properties of the primary substances of protoplasm fits in with the assumption that the activity of the fundamental physiological systems is based upon the principles of "fixed pathway", i.e., all the intermediate transformations in these physiological systems would proceed along definitely fixed paths. On the contrary, the optical properties of the secondary protoplasmic substances can be altered to a certain extent. Consequently, the formation and the transformations of the secondary substances are not bound by the principles of fixed pathway. 2. Fur-
thermore, in the fundamental protoplasmic systems, there are devices to avoid racemization, which were discussed previously; similarly it seems that there are devices to avoid inversion, such as those regulated by the "principle of fixed pathway". Neither of these devices operates in the transformations of secondary protoplasmic constituents.

**SUMMARY**

1. It is not possible to invert, by external influences, the asymmetric structure of the primary constituents of protoplasm which is the result of a long evolutionary process.

2. As to secondary substances of protoplasm such as products of metabolism (for example, lactic acid in fermentative processes), the sign of their optical activity also represents a fixed hereditary character of the species or strain which elaborated them, but external influences can affect the catalytic racemization of these products which were initially formed in the optically pure state.

3. Concerning the mechanism by which the production of a given optical isomer is controlled in metabolic activities, one should note that: (a) From the same symmetric initial substrate, enzymes of different organisms can synthesize optically different substances; (b) It is often through optically inverse catalysts that optical antipods are synthesized; (c) Some catalysts can, after chemical alterations which do not constitute an inversion, synthesize substances in a form which is the optical inverse of the form that was synthesized before the alteration of the catalyst; (d) The sign of the optical rotation of the final product of a series of metabolic reactions may depend on the "pathway" followed in intermediate reactions; (e) Inversions of the Walden type (transformation of one optical isomer into its antipod in a series
of chemical transformations) may play a role in biological phenomena.

4. Physiological mutations which, in the evolutionary development of an organism, bring about optical inversions of the secondary protoplasmic constituents may consist in a change of the intermediate "pathways" in a series of reactions.

5. Protoplasmic systems are provided with mechanisms by which "pathways" that would lead to an inversion of the asymmetric structure are avoided. Primary constituents of protoplasm are then regulated by a principle called here: "Principle of fixed pathway". Such mechanisms do not operate in the transformations of the secondary substances of protoplasm.

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CHAPTER IV

ON THE RELATION BETWEEN THE INVERSION OF SPIRALLY TWISTED ORGANISMS AND THE MOLECULAR INVERSION OF THEIR PROTOPLASMIC CONSTITUENTS

1. Morphological Dissymmetry and Morphological Inversion. The attention of biologists has for a long time been attracted by the existence of dextral and sinistral spirally twisted forms in some animal or plant populations. Ludwig (1932, 1936) published two extensive reviews in which he summarized a large number of scattered observations on this subject. These reviews show that practically all the studies of dextrality and sinistrality in plants and animals consist in descriptions of the morphological aspects of the phenomenon and that the physiological mechanism which underlies the morphological processes has been left almost untouched.

One of the basic attributes of spiral structures is their ability to undergo genotypic inversion. The work of Boycott, Diver, Hardy and Turner (1929) on the heredity of sinistrality in the mollusc Limacca peregra has shown that the usual twist of the coil of this mollusc to the right (clockwise) is determined by a dominant gene, while the twist to the left is controlled by the recessive gene, and that the sinistral mutant individuals appear in the population from time to time. Consequently, in almost all the cases in which some experimental work was carried out with organisms possessing a spiral form, it was possible to detect among the usual, typical individuals a few hereditary inverse specimens. (We shall,
hereafter, call these two kinds the typical and the inverse individuals.)

It may be supposed that the direction of the spiral is determined by some dissymmetric substance which is labile, in the sense that it can undergo an inversion of its molecular configuration with comparative ease, and that by means of such a mutation, the form of the organism can change. Such an idea is due to Koltzoff (1934) and to Needham (1934), according to whom the origin of dextrality and sinistrality, as observed in the eggs of certain snails and later in their body, is connected with the stereo-chemical properties of some of their component protein molecules. But, at present, these relations are very obscure.

Koltzoff expresses himself as follows: "Particularly interesting is the case when in a pond-snail, Limnaea rubella, in one and the same species genotypes are found which are characterized by either the left or the right spiral types of shell. The cleavage of the ovum in those genotypes proceeds, correspondingly, according to the right or left spiral types. Here, already at the first division of the ovum, the difference between both types is marked in a sufficiently distinct manner by the position of spindles. It is very probable that the right and the left types are distinct already in the unfertilised ovum, because they can be detected in the relative position of both directing bodies. Hybridological analysis shows that this character is determined by one pair of allelomorphs. The right twist of the spiral is determined by the dominant gene, the left twist by the recessive one. The mother homozygous as to the recessive gene can itself have the right spiral (because the ovocyte from which it evolved could be heterozygous), but all its eggs develop according to the left type, even if they were fertilized by the sperm of the homozygous dextral father. This shows that genotypical peculiarities of the male nucleus are not manifest on this stage. On the
other hand, one can see here the proof of the fact that the basic features of the whole plan of structure of an organism can be the result of the action of a single gene.

"What kind of influence this gene exercises on the structure of the ovum, we certainly do not know. As a hypothesis I can express the suggestion that this or other type of cleavage is here determined by the presence in the protoplasm of the ovum of the right or of the left optical isomer of some organic substance. This substance goes out of the nucleus of the ovum during the ripening of the latter, forming itself preliminarily in connection with corresponding genes of the chromosome apparatus of the oocyte. Hence it may be inferred that both genes of a given couple of allelomorphs are optical isomers in respect to each other."

Koltzoff's hypothesis is, of course, not the only possible interpretation of the observed facts. The optical inversion of genes is certainly possible, but a change of their chemical properties, without the inversion of their configuration, may also be supposed. In the latter case the optical inversion of some organic substance determining the structure of the animal would take place only in a subsidiary reaction. The facts discussed in the preceding chapter would confer about the same degree of probability on the hypothesis of gene inversion and on the hypothesis of a chemical modification of the genes without inversion.

The solution of some basic biological problems depends on the answer to this question. According to the first interpretation, the substance of the genes which determine the morphological structure of an animal would belong to the group of secondary protoplasmic constituents, those which play the role of storage substances or of products of metabolism. These products would then be quite important in the mechanism of evolution.

2. Morphological Dissymmetry and Morphological Inversion in Bacillus Mycoides. The typical strain of Bacil-
Bacillus mycoides, when grown on the surface of agar peptone medium, produces colonies spirally twisting to the left, i.e., counter-clockwise (according to the terminology adopted by Ludwig, 1932). After one has introduced a small quantity of inoculating material in the centre of a Petri dish of agar-peptone, one soon sees it grow; the thin filaments of the growing culture begin to deviate to the left (cf. Fig. 5).

![Fig. 5. Dextral (D) and sinistral (L) spiral twisting of the growing filaments of colonies of Bacillus mycoides, as observed on peptone agar, in Petri dishes.](image-url)

The inverse form of this organism, growing in dextral coils, rarely occurs. It was first recorded by Gersbach (1922), who described this interesting case as an “isomerism in bacteria”. He further established that the dextral and sinistral strains are entirely identical in all their properties.

Later a single dextral strain among a great number of sinistral ones was observed by Oesterle (1929).

Lewis (1932) isolated several dextral strains in Texas.

In an extended series of investigations with Bacillus mycoides at the Microbiological Institute of the Academy
of Sciences in Moscow, the dextral form was found only three times, though numerous isolations from different soils were made.

The dextrality and sinistrality in Bacillus mycoides is a hereditary feature. Dextral forms are always obtained from dextral forms, and sinistral from sinistral ones.

It can be shown that the spiral form of the colonies of this organism is a secondary feature which is the result of the primary spiral structure of the growing cells which constitute the filaments. If one stains the filaments on the surface of the agar with neutral red or with toluidin blue (1:5000) and examines them under the microscope, one can occasionally observe the twisting of two filaments which have encountered each other. The motion of the growing filament consists of two components: an elongation and a rotation around the axis of the filament, these will result in a spiral motion. Similar observations have been made also by Stapp and Zycha (1931) and by Roberts (1938). If during the free growth of a filament on the agar surface, the filament rotates around its longitudinal axis counter-clockwise, the interaction of the firm surface of the agar and of the growing filament will cause the latter to follow a spiral path in a counter-clockwise direction. Consequently, the secondary sinistral coil of the growing colony of bacteria will arise as a result of the primary sinistral spiral growth of the cells of the filament. This is confirmed by the fact that a certain consistency of culture medium is necessary for the typical spiral growth of colonies (Pringsheim and Langer, 1924; Hastings and Sagen, 1933). The latter authors state that on agar of usual strength the growth of Bacillus mycoides spreads from the place of inoculation in the form of coarse filaments which twist counter-clockwise, forming a symmetrical pattern. On less consistent agar this pattern does not appear or is diffuse.

3. Morphological Dissymmetry and Morphological Inversion in the Snail, Fruticicola lantzi. We shall consider
next the morphological dissymmetry of an animal which, in the natural classification, stands far from the bacteria, namely, the land snail, *Fruticicola lantzi*. In this animal the typical individuals are dextrally spiralled as is the case in the majority of species of snails. Numerous observations have led to the conclusion that, in snails, the sinistrally twisted individuals are ecologically weaker than the dextral forms. In joint occurrence of the two forms the sinistral ones often disappear in a rather short time. Zvetkov (1938) has recently shown, in a study of the distribution of *Fruticicola lantzi* in Middle Asia, that most of the populations consist of typical forms, dextrally spiralled. Populations consisting almost exclusively of inverse, sinistrally spiralled forms were found only in some districts separated from the remaining area by mountain barriers. Such isolated colonies of inverse forms in both bacteria and hermaphroditic molluses, it is thought, have originated from a single inverted ancestor.

   a. *Action of temperature.* Recent experiments made by Gause (1939) have shown that there is a difference in the action of temperature on the growth of the dextral and on that of the sinistral strains of *Bacillus mycoides*. Three series of experiments were performed as follows: A small quantity of inoculating material was placed in the center of the Petri dish, on agar, in the form of a circle 0.5 mm. in diameter. Twenty hours after growth had started the diameter of the colony was about 6 mm. at 20°C and about 25 mm. at 32°C. Taking then the diameter of colonies, either dextral or sinistral, growing at 20°C, as a unit, curves of growth in terms of temperature were constructed. Such curves are represented in Figure 6. If the rate of growth of the usual sinistral strain is normal, the inverted dextral strain presents the phenomenon of "heat injury."
While the preceding experiment was made on the rough form (usually designated as the R-form) of bacteria, we repeated it with the smooth form (S-form). By "disso-

Fig. 6. Growth-temperature relation in dextral (D) and sinistral (L) strains of *Bacillus mycoides* (R type) grown on solid medium. The three sets of curves represent three series of experiments. The size of the colonies grown at various temperatures is indicated in terms of the size of the colonies grown at 20° as a unit. (From Gause, 1939.)
ociation" both dextral, DR, and sinistral, LR, strains of *Bacillus mycoides* develop into smooth ones, which form, on growing on solid medium, flat spherical colonies, LS and DS (for literature see Arkwright, 1930). The study of the action of temperature was made according to the previous plan. It was found that the phenomenon of heat injury characteristic of the dextral strains was about as marked in the S-forms as in the R-forms.

It was decided then to try the experiment with the DS and LS strains in liquid medium. The rate of growth was now determined by a bacterial count in a Thoma chamber, under the microscope, forty hours after the inoculation. The relation of growth to temperature is represented in Figure 7. One sees that in the dextral strain the characteristic heat injury appears in the range of temperatures extending from 24° to 28°C.

Similar results were thus obtained with the R-forms, with the S-forms and on liquid as well as on solid culture

![Figure 7](image_url)

*Fig. 7. Growth-temperature relation in dextral (DS) and sinistral (LS) strains of *Bacillus mycoides*, grown on liquid medium. Abscissae: Temperature in degrees C; Ordinates: Number of cells per 1/160 cc. (From Gause, 1939.)*
media. There is definitely in the dextral form a special sensitivity to heat injury at the temperatures indicated. It is interesting to compare this characteristic weakness of the inverted dextral form with the following property pointed out by Lewis (1933) and observed again by Gause (1939).

b. Enzymatic properties. Lewis, working with dextral and sinistral strains of *Bacillus mycoides*, reported a specific physiological difference between them. It is known that this bacillus possesses the ability to decompose glucose and saccharose and to acidify the medium. On glucose the acid production is similar in sinistral and in dextral strains but not on saccharose. According to Lewis the formation of acid on saccharose is rapid with the left spiralled strain and it ceases with the right-spiralled type.

Gause (1939) repeated these experiments using the S-form of *Bacillus mycoides*. Two per cent saccharose and a little quantity of a weak (0.02 per cent) solution of phenol red (according to Clark) were added to an agar-peptone medium of usual composition. At pH 7.7, which is the optimal hydrogen ion concentration for the growth of *Bacillus mycoides*, phenol red has an orange tint. The culture was kept at 28°C. Nineteen hours after the beginning of the experiment the color of the sinistral strains differed very sharply from that of the dextral. The former showed a rapid production of acid, the pH of the colony being about 6.8. The dextral strains showed no production of acid. The reaction of the colony was alkaline, its pH being about 8.4. Lewis' results were therefore confirmed with the S-form of the bacillus.

It might be worth mentioning the following detail. While the dextral strains are, without exception, unable to produce acid on sucrose, the sinistral strains were all able to form acid except in two cases, one mentioned by Lewis and another (doubtful) observed by Gause.
Lewis' reaction might be considered as a fermentative deficiency of the dextral strain.

c. Growth on two optical isomers. It is of interest to know how an organism which presents a morphologically dissymmetric structure (right or left spiral) behaves when grown on a medium which is molecularly dissymmetric. The dextral and sinistral strains of *Bacillus mycoides* were grown on the optical isomers of arginine (Gause, 1939). The results are summarized in Tables 16 and 17.

A safe conclusion that one can draw from these data is that both the dextral and the sinistral strains of *Bacillus mycoides*, in both the rough and the smooth forms, grow

**TABLE 16**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Number of cells in LR</th>
<th>Ratio of growth on d-arginine to growth on dl-arginine</th>
<th>Number of cells in DR</th>
<th>Ratio of growth on d-arginine to growth on dl-arginine</th>
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<td>19.7</td>
<td>1.59</td>
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<td>13.2</td>
<td>1.61</td>
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**TABLE 17**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Number of cells in LR</th>
<th>Ratio of growth on d-arginine to growth on dl-arginine</th>
<th>Number of cells in DR</th>
<th>Ratio of growth on d-arginine to growth on dl-arginine</th>
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<td>dl-arginine</td>
<td>23.3</td>
<td></td>
<td>33.0</td>
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</table>
better on natural d-arginine than on the racemic dl-arginine. Therefore, the dextrally and the sinistrally twisted organisms are alike in the optical properties of their basic protoplasmic constituents, since nutritive substances of the natural configuration are more favorable for both of them.

*d. R e s p i r a t i o n.* The oxidation of glucose by the dextral and sinistral strains of *Bacillus mycoides* was determined by the Warburg technique at three temperatures: 22°, 25° and 28° C. As is characteristic of biological processes generally, the velocity of respiration rose exponentially with the rise of temperature, and practically at the same rate in the sinistral and in the dextral strains of the bacillus. Consequently the phenomenon of heat injury, which was characteristic of the growth of the dextral strain, was not observed in respiration on glucose.

The general conclusions to draw from these investigations is that the inverse, dextral strains of *Bacillus mycoides* are weaker than the typical, sinistral ones. This was observed in the rate of growth at 24° to 28° and in the deficiency of an enzymatic action.

5. **Some Physiological Properties of the Dextral and of the Sinistral Strains of the Snail, Fruticicola lantzi.** *A n a b o l i c g a i n, r e s i s t a n c e t o s t a r v a t i o n, m o r t a l i t y r a t e.* The results of the investigations on *Bacillus mycoides* are paralleled by the data obtained with *Fruticicola lantzi.*

Gause and Smaragdova (1940) made a comparative study, under well controlled laboratory conditions, of the physiological behavior of the dextral and sinistral individuals of this snail. They investigated (1) the velocity of anabolic assimilation as judged by the change in weight when the snails were fed for a long time on carrots; (2) the velocity of the catabolic loss as determined by the decrease in weight when the animals were kept in a moist chamber without food; (3) the mortality rate in the second group of experiments.
It was found that after having been fed for a prolonged time on carrots the typical, dextrally twisted individuals practically did not change their weight, while, under perfectly identical conditions, the sinistrally twisted snails considerably decreased in weight (cf. Figure 8).

![Graph showing the change in weight of dextral and sinistral forms of the snail Fruticicola lantzi](image)

Fig. 8. Change in weight observed in the dextral and sinistral forms of the snail Fruticicola lantzi when they were fed for a long time on carrots. Abscissae: Time in days; Ordinates: Weight in milligrams. (From Gause and Smaragdova, 1939.)

In the study of the behavior of Fruticicola in starvation it was observed that the sinistral individuals lost weight more rapidly and died off more quickly than the dextral forms (cf. Figure 9).

The relative weakness of inverse left spiralled individuals is evident in the three series of experiments. Similar results were obtained also in the study of the loss of dry weight.
Fig. 9. Average decrease in fresh weight in starving adult dextral (D) and sinistral (S) forms of the snails *Fruticicola lantzi*. Abscissae: Time in days; Ordinates: Weight in % of the initial value. (From Gause and Smaragdova, 1939.)

6. *On the Relation between Morphological Inversion and Molecular Inversion.* Having considered the physiological differences of the dextral and sinistral forms, we shall now turn to the problem of the possible relation between morphological and molecular inversions. Let us at first note that the heat injury in the dextral strains of *Bacillus mycoides* reminds one of the heat injury observed when different lower organisms, such as yeast, were cultured on unnatural isomers of amino acids (Gause and Smaragdova, 1938). When the yeast *Torula utilis* was grown on the natural isomer of leucine, which enters into the composition of all living organisms, the velocity of growth was that always observed in typical growth-temperature curves, but when it was cultured on the unnatural isomer of leucine, the increase in the velocity of growth became always less and less with the rise of temperature.
These experiments were repeated with the optical isomers of the following amino acids: leucine, histidine, phenyl-alanine and valine (Gause, 1939). The results obtained are given in Figure 10. Typical heat injury at temperatures extending from 18° to 28° may be observed in the growth of *Torula utilis* on the unnatural isomers of leucine and of histidine but not on those of valine and phenyl-alanine.

![Figure 10](image_url)

Fig. 10. Growth of the yeast *Torula utilis* on optical isomers of various amino acids, at different temperatures. Abscissae: Temperature in degrees C; Ordinates: Increase in the number of cells in 40 hours. (From Gause, 1939.)
Similar data were obtained also in the study of the growth of the mould *Aspergillus niger* on the optical isomers of leucine and valine.

The same relation between temperature and rate of growth is thus observed in the unusual strain of *Bacillus mycoides* grown on natural substrates and in the yeast or fungi grown on unnatural substrates. In both cases, there must be an inhibitive factor of growth. It may be conjectured that in the case of yeast or fungi the unnatural isomer of the amino acid dissolved in the culture medium surrounding the cells caused a retardation of growth because its spatial configuration did not coincide with the spatial configuration of the basic constituents of protoplasm. In the case of *Bacillus mycoides* the retardation of growth in the unusual form would be caused by the presence inside of the cells of the unnatural optical isomer of some organic substance which participated in the determination of the morphology of the cell.

7. Morphological Inversion and the Theory of Spiral Growth. The investigations on various physiological properties of dextral and of sinistral strains in *Bacillus mycoides* and in *Fruticicola lantzi* have brought out the two following points: (1) In the optical properties of their protoplasm these strains are alike. This follows from their behavior towards optically isomeric nutritive substances. It has, furthermore, been confirmed by direct observations made by Kiesel, Efimovkhina and Rall (1939), who isolated the same natural amino acids from both dextral and sinistral strains of the snail *Fruticicola lantzi*. (2) The inverted individuals of both bacteria and snails are physiologically weaker than the typical ones. These observations suggest that while in the typical individuals the organic substances which participate in the determination of the twist might well have the same laevo-rotatory configuration as the other constituents of protoplasm, in the inverted individuals some enzymatic disturbance might have occurred.
Castle (1936) has recently undertaken the study of the mechanism of spiral growth in *Phycomyces*. He claims that the spiral structure of the growing cell wall is not strictly predetermined but depends on the interaction of forces which exert their action in the growth region. The twist of the growing elastic elements of the wall may be the result of their resistance to turgor. Castle constructed a model to illustrate this process. If the elastic elements of the wall are distributed symmetrically, dextral spirals will be obtained in 50 per cent of the cases and sinistral spirals in the other 50 per cent. As the left direction of the spirals is typical for *Phycomyces*, one must assume a dissymmetric distribution of the elastic elements of the cell wall, which later, under the action of turgor, lead to the formation of sinistral spirals.

As Castle himself points out, the mechanism of spiral growth can be different in different organisms, and one cannot directly transfer his explanation of the mechanism of twisting to the bacteria, the more so since the cell wall of the latter is thought to consist of some specific protein material closely related by its nature and origin to cellular protoplasm (John-Brooks, 1930).

But one can assume that in the spiral growth of the cell wall of a bacterium, as in that of *Phycomyces*, two factors are involved: (1) Some pre-existing asymmetric system (distribution of the elastic elements of the wall in *Phycomyces*; optically active secondary protoplasmic constituents in bacteria); (2) a system of forces directly inducing the spiral twist (turgor in Castle's experiments). The interaction of these two factors would bring about the dextrality or sinistrality of the spiral growth according to the following scheme:

Secondary substance of metabolism → Asymmetric structure of the cell wall → Spiral growth

↑ Forces directly inducing the spiral twist
One may conjecture that the inversion of the direction of the spiral growth in *Bacillus mycoides* is related to an optical inversion of some secondary substance in metabolism. The latter would bring about the inversion of some structures in the cell wall and, from the interaction of these with the forces inducing the spiral twist, there would result an inversion in the direction of the spiral growth of the cells.

But to what extent can one assume that the secondary substances of metabolism which participate in the structure of the wall of the bacterial cell can undergo an optical inversion? Some recent data on the chemical structure of bacterial capsules obtained by Bruckner and Ivanovics (1937) in the laboratory of Professor Szent-Gyorgyi, are of interest in this connection. As is known, the cell wall in bacteria consists of two layers: (1) A very thin internal cuticle, and (2) An external gelatinous layer which is sometimes developed into an envelope called a capsule. (John-Brooks, 1930, remarks that the bacteriologists have come to look upon capsule formation as a general feature which is common to all bacteria, but which reaches the proportions that we know, only in certain species.) Bruckner and Ivanovics (1937) who studied the chemical properties of the capsule of *Bacillus anthracis* and of some other species of bacteria, all of which are aerobic spore formers, standing near *Bacillus mycoides* in the classification, found that the capsule of these bacteria consists of a polypeptide substance, the hydrolysis of which yields d(-)glutamic acid. The laevorotatory isomer of this amino acid is unnatural, and it has not been found previously anywhere in the organic nature. So, the presence of the unnatural glutamic acid in the structure of the envelope of the anthrax and of some other bacilli has already been recorded in the literature. Further investigations in that direction may reveal significant data on the present problem.
SUMMARY

1. In organisms which possess a spiral structure, as in some bacteria, in snails, etc., one observes a larger number of "typical" individuals, that is, of individuals twisted in one direction, while the "inverse" specimens are rarer.

2. The properties of protoplasm related to optical activity are alike in dextral and sinistral forms. The same natural amino acids have been isolated from either the dextral or the sinistral snails (Fruticicola lantzi). Both dextral and sinistral bacteria (B. mycoides) grow better on the natural than on the unnatural isomers of amino acids.

3. The "inverse" forms are physiologically weaker than the "typical." When the culture temperatures are varied from 20° to 36°, the "inverse" bacteria present a decreasing growth rate not observed in "typical" bacteria; furthermore, the "inverse" forms show some enzymatic deficiencies. In the "inverse" snails the velocity of catabolic loss and the mortality rate, on starvation, exceed those of the "typical" individuals.

4. It is suggested that some secondary substances which may determine the morphological inversion are optically inverted, or that some subsidiary process in metabolic activities is changed in the mutant snails and bacteria, whereas the basic protoplasmic constituents are not. This would explain the disturbance in the enzymatic coordination and the physiological weakness observed in the inverted specimens.

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CHAPTER V

ANALYSIS OF VARIOUS BIOLOGICAL PROCESSES
BY THE STUDY OF THE DIFFERENTIAL
ACTION OF OPTICAL ISOMERS

Asymmetric Analysis. When one analyses the action upon protoplasmic functions of dextrorotatory and of laevorotatory isomers of various organic substances, one often notices a difference in the effectiveness of the two isomers. The existence or the absence of such a difference, as also its quantitative value, are evidently related to the physical structure and the chemical composition of protoplasm. One can, therefore, study the mechanism of various biological processes by examining how they are influenced by optical isomers of various substances. It is thought that this method of analysis, which we call "Asymmetric Analysis," could contribute to the clarification of several important problems of comparative physiology.

With the idea of elaborating some systematic methods of "asymmetric analysis", Gause and his associates have undertaken the following investigations: 1. An analysis was made of the mechanism of toxic action of optically isomeric nicotine upon lower and higher animals. 2. The mechanism of toxic action of optically isomeric organic acids upon lower and higher animals was similarly studied. These two investigations will be reviewed and discussed in the first section of this chapter. The study of the effect of nicotine isomers in various animals led to important observations on the evolution of the nervous system. These observations will be discussed in the second section. 3. A study was made of the action of optically isomeric cinchonines upon various functions of the cell. This study will be summarized in the third section.

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SECTION I

ANALYSIS OF THE MECHANISM OF TOXIC ACTION

A. TOXIC ACTION OF THE OPTICAL ISOMERS OF NICOTINE

A Case of Identical Mechanism of Action in the Two Optical Isomers. Pictet and Rotschy, in 1904, prepared pure dextrorotatory (unnatural) nicotine, tested its toxicity on rabbits and guinea pigs, and ascertained that it was less toxic than the natural L-nicotine. They expressed the view, which subsequently was adopted by a number of authors, of a different mechanism of toxic action by the dextro and by the laevo isomers, the symptoms of poisoning having been found different.

Macht (1929) studied the pharmacological synergism of stereoisomeric nicotines. He found that the toxic action of a mixture of the L- and dl- forms was stronger than the additive action of these isomers. The conclusion that he reached then was that "an individual cell may possess receptor groups of a laevo and dextro type, and a mixture of two stereoisomers would thus have a double point of attack in place of a single one, in case only one of the optic isomers was used." The three following points in the work of Macht are open to criticism. First, his important final conclusion is based on a very small number of experiments. Then, the author did not attempt to obtain a concentration-toxicity curve which would allow one to make some quantitative calculations. Moreover, dl-nicotine is not the most suitable for such experiments, as it usually contains some hydronicotine which influences the physiological effect (Gause, 1936); only the purest dextro nicotine obtained by repeated crystallizations with laevotartaric acid should be used.

In the experiments of Gause and Smaragdova (1939) the dextro isomer, in accord with the data of Pictet and Rotschy, was found less poisonous than the laevo form,
but the relation of the increase in toxicity with the concentration was the same in the two isomers and a complete identity of the temperature characteristics of toxic action of the dextro and laevo forms was also observed (in cold-blooded animals: fishes and tadpoles). The identity of the relation of toxicity to concentration and the identity of temperature characteristics are taken as an indication of the identity of the mechanism of toxic action of the two isomers. Both of them seem to act on the same link in the system of physiological processes, though with different speed.

To illustrate these conclusions, let us consider in some detail the results of recent experiments made with a brood of the fish, *Leuciscus idus var. orfus* (Gause and Smaragdova, 1939). The animals were placed in neutralized solutions of nicotine of different concentrations, prepared with redistilled water, and the killing time in seconds was recorded. Figure 11 (upper part) represents the relation of killing time to the concentration of nicotine.

It is to be pointed out that in the calculation of the relative toxicity of L- and D-nicotine one cannot use indiscriminately results taken at various arbitrarily chosen concentrations. The relative efficacy of the isomers changes with change in absolute concentration. For comparison of the physiological effect of the optical isomers one has to employ such characteristics of corresponding curves of toxicity which are determined not by any values of the absolute concentration of the poison but by some physiological action. The most convenient is to take the minimal lethal concentration of the poison (the constant *n*, cf. formula below). In L-nicotine *n*<sub>L</sub> = 0.0022%; in D-nicotine *n*<sub>D</sub> = 0.0064%. The coefficient of relative toxic action (α) = *n*<sub>D</sub>/*n*<sub>L</sub>, which indicates how much the L-isomer is more powerful than the D-isomer, is 2.91. (This coefficient will be called, hereafter, the "stereo-coefficient.")
Fig. 11. Killing action of the optical isomers of nicotine on the fish _Lenciscus idus_. The lower graph represents the toxicity curves in logarithmic coordinates. (From Gause and Smaragdova, 1939.)

According to the principles of quantitative toxicology, concentration-toxicity curves can usually be expressed by the empirical equation of Ostwald:

\[ y = \frac{k}{(x-n)^m} \]

where \( y \) is the killing time, \( x \) the concentration of the poison, \( n \) its minimal lethal concentration, and \( k \) and \( m \) are constants. The constant \( m \) shows how rapidly the toxicity increases with the concentration; it thus characterizes the dynamics of the killing process. If one plots \( \log y \) on ordinates and \( \log (x-n) \) on abscissae, the relation between these variables will be represented by a straight line. The slope of this straight line is measured by the constant \( m \).
The lower part of Figure 11 represents the data on the toxicity of dextrorotatory and laevorotatory nicotines for Leuciscus, plotted in the manner just indicated. It is evident that the slopes of the straight lines, characterizing the dynamics of the increase of toxicity with concentration, are identical. It is therefore reasonable to conclude that in these experiments the mechanism of killing action in the two optical isomers of nicotine is identical in the sense defined. The unnatural dextro nicotine is weaker only in the sense that a higher dose is required to attain killing. Similar results were obtained also in experiments with birds (Acanthis flammea), lizards (Lacerta viridis) and tadpoles (Rana temporaria). No difference in the symptoms of poisoning by the two optical isomers was observed.

Investigations were then carried out to determine the temperature coefficients of toxicity of dextrorotatory and laevorotatory nicotine for various animals. It is known that if one plots the logarithms of the killing rate against reciprocals of absolute temperature, one usually obtains a linear relation. The slope of this straight line is generally represented by $\mu$, which is known as the temperature characteristic (cf. Crozier, 1924). This characteristic shows how the killing process is speeded up by the rise of temperature. Physiological processes of different nature, i.e., in which different mechanisms are at play, usually possess different temperature characteristics.

The temperature characteristics of toxicity for tadpoles and for the fish Leuciscus were determined according to the following procedure. Two solutions of dextro and laevo nicotines were placed in a constant temperature bath. After the temperature equilibrium was attained, a number of fish or of tadpoles were immersed in the vessels and the killing time was recorded. Figure 12 shows the killing rate (a value inverse to the killing time in seconds) in the fish Leuciscus, due to the action of optic isomers of nicotine at different temperatures. Approxi-
Fig. 12. Effect of temperature on the killing rate of the fish *Lenciscicus idus* by the optical isomers of nicotine. The lower graph represents the killing rate plotted logarithmically. (From Gause and Smaragdova, 1939.)

Matter isotoxic concentrations of the isomers, i.e., 0.007% for L-nicotine and 0.014% for D-nicotine, were used. The temperature characteristics of the two isomers are practically identical; in the D-form \( \mu = 37,500 \), and in the L-form \( \mu = 36,800 \). Such an identity of temperature characteristics was also observed in experiments with tadpoles; in the D-nicotine \( \mu = 14,400 \), and in the L-nicotine \( \mu = 14,600 \). Hence the relation of toxic action to temperature strongly supports the view that the mechanism of toxic action is identical in the two optically isomeric nicotines.

The same relations, that is, (1) a high toxicity of the natural isomers, (2) the same relation between the in-
crease in toxicity and the concentration, and (3) the same temperature characteristics for the two isomers, have been observed by Gause and Smaragdova (1938, 1939) with nicotine on vertebrates (natural = laevorotatory), with tartaric acid on fishes (natural = dextrorotatory), and with cinchonine on paramecia (natural = laevorotatory. Figure 13 shows that the dynamics of toxic action are identical for dextrorotatory and for laevorotatory tartaric acids on the brood of the fish Lebistes reticulatus. The temperature characteristics of toxic action for dextrorotatory tartaric acid was found to be 10,200 (cf.

![Graph](image)

Fig. 13. Killing action of the optical isomers of tartaric acid on the fish Lebistes reticulatus. The lower graph represents the toxicity curves in logarithmic coordinates. (From Gause and Smaragdova, 1938.)
Fig. 14) and for laevorotatory tartaric acid 9,700, in other words, they were of the same order of magnitude. Similar data were obtained also in experiments with the brood of another species of fish, *Platypoecilus maculatus* (Gause and Smaragdova, 1938).

Fig. 14. Effect of temperature on the killing rate of the fish *Lebistes reticulatus* by the optical isomers of tartaric acid. The lower graph represents the killing rate plotted logarithmically. (From Gause and Smaragdova, 1938.)
Further, in experiments on the toxic action of optically isomeric cinchonines upon paramecia, it was found that the laevorotatory isomer inhibits the mechanism of ciliary movement more rapidly than does the dextro form (Gause, Smaragdova and Alpatov, 1938). The dynamics of toxic action in dextro and laevo cinchonines were found to be identical (cf. Fig. 15). A study of the temperature characteristics of toxicity has also shown that these are practically identical, 14,200 in the laevorotatory and 14,000 in the dextrorotatory isomer (cf. Fig. 16).

**Fig. 15.** Killing action of the optical isomers of cinchonine on *Paramecium caudatum*. The lower graph represents the toxicity curves in logarithmic coordinates. (From Gause, Smaragdova and Alpatov, 1938).
B. TOXIC ACTION OF THE OPTICAL ISOMERS OF ORGANIC ACIDS

1. A Case of Different Mechanism of Action of the Two Optical Isomers. In the cases reported so far the natural isomer was more powerful in its physiological action than the unnatural, the relation of increasing toxicity to con-

Fig. 16. Effect of temperature on the killing rate of Paramecium caudatum by the optical isomers of cinchonine. The lower graph represents the killing rate plotted logarithmically. (From Gause, Smaragdova and Alpatov, 1938.)
centration and the temperature characteristics of toxic action were identical for the two isomers. There are cases in which it seems that none of these relations hold. Gause and Smaragdova (1938) reported this situation in the action of malic acid on the brood of two species of viviparous fish, *Lebistes reticulatus* and *Platypoecilus maculatus*.

In these experiments they compared the natural laevorotatory with the racemic malic acid (the significance of the use of a racemate will be indicated below). It was found that the natural laevorotatory malic acid is less toxic than the racemic. The toxic action of weak (0.05%) solutions of the laevorotatory and racemic malic acids on *Lebistes reticulatus* at different temperatures (16°, 18°, 21°, 26°, and 31°) is recorded in Figure 17. It is quite apparent that the temperature characteristics of

![Figure 17](image)

**Fig. 17.** Effect of temperature on the killing time of the fish *Lebistes reticulatus* by the optical isomers of malic acid. The lower graph represents the killing rate plotted logarithmically. (From Gause and Smaragdova, 1939.)
tomic action are different in the racemate and in the laevo-
rotatory isomer. At temperatures from 16° to 26°C the
racemate is more toxic than the laevo-rotatory isomer,
whereas at the temperature of 31°C the latter is rela-
tively more toxic than the racemate. The temperature
characteristics of toxic action are also quite different. In
the laevo-rotatory isomer $\mu = 12,300$, and in the racemic
form $\mu = 9,340$.

In similar experiments with the fry of Platypoecilus
maculatus results of the same kind as those obtained with
Lebistes were recorded (cf. Fig. 18). The temperature
characteristic of toxic action of the left isomer of malic
acid is 16,930 and that of the racemic form 12,880.

The results obtained with fish were duplicated in a
study of the action of optically isomeric malic acids on
tadpoles of Rana temporaria (Gause and Smaragdova,
1939).

Some experiments on the action of $l(-)$ and of $d(+)$
leucine on the yeast Torula utilis (Gause and Smarag-
dova, 1938) also bring confirmatory evidence that the
unnatural form $d(+)\) exerts a stronger action than the
natural and that their effect is of different nature.

Concerning the experiments in which racemic malic
acid was used, it should be mentioned that, in dilute
aqueous solutions, the racemic acid is completely disso-
ciated into dextrorotatory and laevo-rotatory constituents
(Ostwald, 1889). Therefore the greater biological activ-
ity of the racemate must probably be attributed to a
higher toxicity of the unnatural dextrorotatory compo-
nent.\footnote{It is to be remembered that the natural
dextrorotatory tartaric acid and the natural laevo-rotatory malic acid belong to the same steric
series.}

If our last assumptions are correct there would be a
series of cases in which, contrary to what has been de-
scribed above, the unnatural optic isomers are physio-
logically more effective and in which the mechanism of
Fig. 18. Effect of temperature on the killing time of the fish *Platypoecilus maculatus* by the optical isomers of malic acid. The lower graph represents the killing rate plotted logarithmically.
action of the two optic isomers is different. One could not, then, speak of a single receptive protoplasmic substance which would simply react to a different degree to the two isomers, as is probably the case when the mechanism of action of the two isomers is the same.

2. *Dual Activity of Organic Acids.* The mechanism of toxic action of the optical isomers of organic acids can be also investigated from another point of view. Heilbrunn (1928), among others, called attention to the dual nature of the action of organic acids upon living systems: (1) Organic acids produce an electro-chemical effect upon the surface of the cells, primarily due either to a destruction of the negative charge of the cell surface by positively charged hydrogen ions or to other physico-chemical *surface phenomena*; (2) Owing to their relatively weak electrolytic dissociation, the solutions of organic acids contain a considerable proportion of non-dissociated molecules which penetrate into the interior of the cells where they produce transformations of a *chemical nature.* Koltzoff's experiments (1915) on the action of different acids on the feeding activity of fresh-water vorticellids furnish an example of the first type of action. There the biological effect of the organic acid depends only on the pH and the mechanism of this action consists in electro-chemical changes upon surfaces directly accessible to hydrogen ions. The simultaneous occurrence of the first and second type of effects is illustrated in the experiments of Stiles and Rees (1935) who showed that the killing action of monobasic organic acids of the aliphatic series first diminishes with the elongation of the chain of carbon atoms in the molecule, then reaches a minimum with valeric acid and finally again increases with the further elongation of the chain. This phenomenon was explained on the idea that the degree of electrolytic dissociation diminishes with the increase in the weight of the molecule, while the killing action of the non-dissociated molecules increases with the increase of molecular weight.
ASYMMETRIC ANALYSIS

The observed lethal action, which is the resultant of the partial lethal action of hydrogen ions and of that of non-dissociated molecules, would then decrease first and increase afterwards as we indicated. Other investigations on the mode of action of organic acids have been summarized by Lepeschkin (1937).

Since optical isomers have all their physical and chemical properties identical, except those which are directly related to their structural configuration, one will observe that, if the common properties only are involved in the killing mechanism, the two isomers should produce the same effect, while, if the properties which are different in the two isomers are involved in the killing action, the two isomers will produce a different effect. It is furthermore assumed that the properties which are specific to each isomer will be involved in the interaction of these isomers with the protoplasm itself, within the cell, in optically active medium, while the properties common to the two isomers, such as the electric charge, the electric conductivity (observed by Ostwald, 1889, to be the same in the isomers of tartaric acid), the osmotic pressure, etc., will be involved in such processes as conduction toward the protoplasmic matter itself. Consequently, if our assumptions are correct, when solutions of dextrorotatory and of laevorotatory acids are equally toxic for a given animal, one may infer that the killing results from physico-chemical injuries concerned with conduction or the like. If, on the other hand, the two optical isomers are not equally toxic, it is natural to think that the surface effects just described could not induce death, so that non-dissociated molecules have time to penetrate inside the cells and there carry out their stereo-specific destructive actions.

With these ideas in mind, Gause and Smaragdova (1938) determined the coefficient of relative toxicity of the optical isomers of tartaric acid on various fresh water animals. Some of their results are given in Table 18.
The figures represent the mean value ($M$) of the data for all the animals of a given phylum. The probable error (P.E.) from the mean is also given.

The coefficient of relative toxicity in Protozoa is close to unity, which means that the dextrorotatory and laevo-rotatory tartaric acids are equally effective. On the contrary, in fishes, the optical isomers of tartaric acid differ strongly in their killing power, the stereo coefficient being 1.305. The other groups of invertebrates investigated occupy an intermediate position between the protozoa and the fishes in their differential sensitivity to the two isomers, the coefficient of relative toxicity reaching 1.048 in the worms and 1.064 in the crustacea.

Similar results were obtained also with the optical isomers of malic acid.

According to the assumptions made, these data would show that, in the killing of lower animals by tartaric and malic acids, there predominates some electro-chemical surface injury, while in higher animals internal chemical injuries caused by non-dissociated molecules would occur.

Cushny (1903, 1926) called into question the observations on the differences in the killing power of the dextrorotatory and the laevo-rotatory isomers of tartaric acid in vertebrates, because the weak specific action of tartaric acids might, according to his opinion, be totally concealed by the more powerful effect of the hydrogen ion.
concentration which is known to be identical in the solutions of both optic isomers. But the objections of Cushny must be considered in the light of the following recent observations: 1. It is at present doubtful that the effect of hydrogen ions is always dominant over the specific action of non-dissociated molecules of organic acids (cf. Gause, 1936). 2. Furthermore, Sizer (1937), in a work on the stimulative effect of organic acids on various animals has shown that Balanus balanoides is more susceptible to the action of hydrogen ions, while in Fundulus heteroclitus the effect of these ions does not predominate over the specific action of nondissociated molecules.

There is another essential point in the investigations of Gause and Smaragdova. If the animals studied are arranged in the order of increasing difference in the toxic power of the two optical isomers: Protozoa < Worms < Crustacea < Pisces, one obtains the phylogenetic series of gradually increasing differentiation. This is not surprising if one considers the fact of the progressively diminishing relative vital importance of the physico-chemical injury of integuments when one ascends the animal series. The nature of susceptible integuments, the injury of which, according to our assumptions, brings about death in lower animals, is not known. It is possible that the respiratory surfaces are among the most susceptible. In Protozoa the whole surface of the cell is the respiratory surface. When one ascends the animal series, respiratory surfaces become more localized and more differentiated and the physico-chemical injury of these surfaces progressively diminishes in magnitude as a cause of death. The results of further investigations along this line have recently been published by Gause and Smaragdova (1939).
SECTION II

ANALYSIS OF THE EVOLUTION OF THE NERVOUS SYSTEM

1. Stereo-coefficients of Action of the Optical Isomers of Nicotine in the Phylogenetic Series. Since the two optical isomers of nicotine exert their killing action by the same mechanism but with a different strength one can, by measuring this difference of potency in various animals, study the properties of the specific receptive substance in different species. In higher animals, as has been already recorded above, there is some specific sensitive substance which is affected to different degrees by the toxic action of the dextro and the laevo isomers of nicotine. Protozoa do not possess, as some observations have shown, such a sensitive substance, and the dextro and laevo isomers of nicotine are for them equally toxic. The question arises of the nature of this specific substance and of the stage of evolution at which it first appears.

Greenwood, as early as 1890, carried out an extensive comparative investigation on the action of common laevo-rotatory nicotine on invertebrates, attempting to establish a parallelism between the toxic effect of this alkaloid which affects, as is known, the nervous system of animals, and the evolution of the nervous system. On the basis of purely qualitative observations he reached the conclusion that "the toxic effect of nicotine on any organism is determined mainly by the degree of development of the nervous system. Thus for Amoeba the substance cannot be regarded as exciting or paralysing; it is rather inimical to continued healthy life. As soon as any structural complexity is reached, the action of nicotine is discriminating in such a fashion that the nervous actions which are the expression of automatism, that is, which imply coordination of impulses, are stopped first. This is seen dimly in Hydra, and it is more pronounced among the medusae. When structural development goes
farther, the selective action of nicotine is traced readily, as for example in *Palaemon*. Greenwood writes further that: "Animals which have enough in common to stand near each other in classification, may yet react differently to nicotine, each according to what I may perhaps call its own balance of organisation."

Gause and Smaragdova (1939) made quantitative determinations of the toxic action of the two optically isomeric nicotines. The advantage of the use of the two isomers will appear in the discussion of the results.

Experiments on vertebrates showed that the stereo-coefficients of toxic action of optical isomers of nicotine (α) are of the same order of magnitude in all animals studied:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Species</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bird</td>
<td><em>Acanthis flavicula</em></td>
<td>3.1</td>
</tr>
<tr>
<td>Lizard</td>
<td><em>Lacerta viridis</em></td>
<td>2.4</td>
</tr>
<tr>
<td>Tadpoles</td>
<td><em>Rana temporaria</em></td>
<td>3.0</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Leuciscus idus</em></td>
<td>2.9</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Lebistes reticulatus</em></td>
<td>2.4</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>2.8</td>
</tr>
</tbody>
</table>

Inasmuch as the mechanism of toxic action is identical in optically isomeric nicotines, one can, by the difference of their effects, judge of the difference in spatial properties of the specific receptive substance assumed. As the difference of effects remains constant, one can conclude that the chemical nature of the receptive substance in the vertebrates also remain essentially constant.

Since the procedure for the introduction of nicotine was not the same for all the animals used—the poison was introduced in the muscle of *Lacerta* while *Lebistes* were immersed in the solutions of nicotine—the identity of the stereo-coefficient is an experimental proof that the conditions of the penetration of nicotine do not affect significantly either the mechanism of toxic action or the reaction of the specific receptive substance.

Furthermore, the absolute sensitiveness to nicotine in *Acanthis* is considerably higher than in *Lacerta* (0.8
mg. per 100 gr. of weight as compared to 5.6 mg. per 100 gr.), but, practically, this difference in sensitiveness does not influence the stereo-coefficient. The constancy of the latter, despite a different sensitiveness, is also significant in the study of the properties of the specific receptive substance.

The results of investigations on the toxic action of the two optical isomers of nicotine on fresh water and marine invertebrates are given in Table 19. One sees that all the invertebrates examined by Gause and Smaragdova can be divided into two groups. The first includes the animals for which the dextro and laevo isomers are equally toxic, and for which the toxicity curves of the two isomers fully coincide. The second includes the organ-

<table>
<thead>
<tr>
<th>Animal</th>
<th>Comp. Toxicity</th>
<th>Animal</th>
<th>Comp. Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td></td>
<td>Annelida</td>
<td></td>
</tr>
<tr>
<td>1. Paramecium caudatum</td>
<td>=</td>
<td>15. Saccocirrus papilloscerus</td>
<td>$\alpha = 2.3$</td>
</tr>
<tr>
<td>2. Euplotes patella</td>
<td>=</td>
<td>16. Perinecreis cultrifera</td>
<td>$\alpha = 1.9$</td>
</tr>
<tr>
<td>3. Stentor coeruleus</td>
<td>=</td>
<td>17. Arenicola grubii</td>
<td>$\alpha &gt; 1$</td>
</tr>
<tr>
<td>4. Spirostomum ambiguum</td>
<td>=</td>
<td>18. Pristina longiseta</td>
<td>$\alpha = 2.09$</td>
</tr>
<tr>
<td>Coelenterata</td>
<td>=</td>
<td>19. Limnodrilus hoffmeisteri</td>
<td>=</td>
</tr>
<tr>
<td>5. Hydra fusca</td>
<td>=</td>
<td>20. Helobdella stagnalis</td>
<td>$\alpha = 3.45$</td>
</tr>
<tr>
<td>6. Cladonema radiatum</td>
<td>=</td>
<td>21. Nais communis</td>
<td>$\alpha = 4.0$</td>
</tr>
<tr>
<td>Platyhelminthes</td>
<td>=</td>
<td>22. Chactogaster longiseta</td>
<td>$\alpha = 2.41$</td>
</tr>
<tr>
<td>Turbellaria</td>
<td>=</td>
<td>23. Styliaria lucustris</td>
<td>$\alpha = 3.13$</td>
</tr>
<tr>
<td>7. Polycelis nigra</td>
<td>=</td>
<td>24. Aelosoma variegatum</td>
<td>$\alpha &gt; 1$</td>
</tr>
<tr>
<td>8. Phacocora sp.</td>
<td>=</td>
<td>25. Aelosoma hembriichi</td>
<td>$\alpha = 1.76$</td>
</tr>
<tr>
<td>9. Dalycellia brevimana</td>
<td>=</td>
<td>Chaetognathia</td>
<td>$\alpha = 1.84$</td>
</tr>
<tr>
<td>10. Procerodes lobata</td>
<td>=</td>
<td>26. Sagitta setosa</td>
<td>$\alpha = 2.7$</td>
</tr>
<tr>
<td>11. Leptoplana tremelaris</td>
<td>=</td>
<td>Arthropoda</td>
<td>=</td>
</tr>
<tr>
<td>Rotatoria</td>
<td>=</td>
<td>27. Daphnia magna</td>
<td>=</td>
</tr>
<tr>
<td>12. Euchlanis triqueta</td>
<td>=</td>
<td>28. Cyclops serrulatus</td>
<td>=</td>
</tr>
<tr>
<td>13. Rotifer vulgaris</td>
<td>=</td>
<td>29. Gammarus marinus</td>
<td>=</td>
</tr>
<tr>
<td>Nemertinea</td>
<td>=</td>
<td>30. Drosophila melanogaster</td>
<td>=</td>
</tr>
<tr>
<td>14. Linens lacteus</td>
<td>=</td>
<td>(2-days old larvae were immersed in nicotine solutions.)</td>
<td>=</td>
</tr>
</tbody>
</table>
isms in which the laevo isomer of nicotine is more toxic than the dextro-isomer. The two groups correspond to large divisions of the animal kingdom, and within each division, there are hardly any exceptions.

All the representatives of Protozoa, Coelenterata, Turbellaria, Rotatoria and Nemertinea studied belong to the first group. They are devoid of spatially specific receptive substances in the process of poisoning by nicotine.

It should be mentioned that the stereo-coefficients in invertebrates are not affected by the differences in the absolute sensitiveness to nicotine, exhibited by various species, as it has been noticed in vertebrates. Thus, for example, Leptoplana is considerably more sensitive to nicotine than Procerodes, but both these turbellarians are characterized by an equal effect of the dextro and laevo isomers. There are many other examples of the independence of these characters.

The lowest groups in the phylogenetic series, in which a stronger effect of the laevo isomer of nicotine is observed, are the annelids, and particularly the Archannelids (Saccocirrus), the Polychaeta and the Oligochaeta and the primitive representatives of Deuterostomia (Sagitta setosa). In Arthropoda (Crustacea and Insecta) this effect is absent, an equal toxicity of the dextro and the laevo isomers is again observed.

Let us now compare the stereo-coefficients in vertebrates and in those invertebrates which show a higher sensitivity to the laevorotatory nicotine. The following values were recorded in invertebrates:

\[
\begin{align*}
Saccocirrus\ papillocercus & \quad \alpha = 2.3 \\
Perinereis\ cultrifera & \quad \alpha = 1.9 \\
Pristina\ longiseta & \quad \alpha = 2.1 \\
Limnodrilus\ bottmeisteri & \quad \alpha = 3.4 \\
Helobdella\ stagnalis & \quad \alpha = 4.0 \\
Nais\ communis & \quad \alpha = 2.4 \\
Chactogaster\ langi & \quad \alpha = 3.1 \\
Aelosoma\ variegatum & \quad \alpha = 1.8 \\
Aelosoma\ hemprichi & \quad \alpha = 1.8 \\
Sagitta\ setosa & \quad \alpha = 2.7 \\
\text{Mean } \alpha & = 2.6
\end{align*}
\]
The limits of error in measuring the coefficient $a$ may extend over a rather wide range. Thus the following values were obtained in two independent measurements: in *Nais* 2.57 and 2.25; in *Limnodrilus* 2.9 and 4.0; in *Acelosoma variegatum* 1.79 and 1.74; in *Acelosoma hempritchi* 1.50 and 2.18. Nevertheless the order of magnitude of the average is significant. We find nearly the same value in invertebrates (2.6) and in vertebrates (2.8).

The data above mean that the Protozoa, Coelenterata, Turbellaria, Rotatoria and Nemertinea are deprived of the spatially specific receptive substance which responds differentially to the left isomer of nicotine. Annelides, Chaetognatha and Vertebrates possess this receptor, while in Arthropoda it is absent again.

2. The Acetylcholine System and the Differential Effect of the Optical Isomers of Nicotine. Considering that it is the nervous system in animals which is affected by nicotine and that there is an identity of stereo-coefficient in invertebrates and in vertebrates, in spite of essential differences in the morphology of their nervous system, we come to the conclusion that there is some uniform receptive substance distributed in the various nervous systems of these animals. However, this chemical constituent is not an obligatory component of every nervous system; even some quite differentiated nervous systems of lower invertebrates (Turbellaria and Nemertinea) are deprived of it.

A study of the present views on the mechanism of nicotine toxic action will furnish more information on the nature of the receptive substance. Thomas and Franke (1924, 1928, 1933) have shown that it is the paralysis of the peripheral neuro-muscular junctions of the respiratory muscles which is the cause of death of higher animals in acute nicotine poisoning. This view was confirmed by Gold and Brown (1935). We are thus led to the old classical observations of Langley (1904) that in the "neuro-muscular junction" there is a certain sensi-
tive "receptive substance" which is the first to be affected by nicotine.

On the other hand, since the classical works of Loewi, it is known that, in the transmission of impulses from nerves to effectors the various steps are as follows (1) nerve impulse → (2) chemical mediator → (3) receptive substance → (4) specific response (for literature see Cannon and Rosenbluth, 1937). There are some indications that the chemical mediator in the voluntary muscles of higher animals is acetylcholine. Its action on the receptive substance in this case reminds one of that of nicotine: in small doses it excites, and in larger doses it paralyses, and according to the current views, nicotine, in case of an acute poisoning, affects in some irreversible way the receptive substance, upon which acetylcholine mediation is no more effective. In other words, nicotine (at least in experiments of our type) acts upon neuro-effector synapses of voluntary muscles. In its action it reminds one of acetylcholine, the substance which transmits the excitation in these synapses. Consequently the receptive substance in nicotine poisoning has some close relation to the receptive substance for chemical mediation.

The experiments just described permit one to divide the animals into two groups according to the nature of the receptive substance affected by nicotine. It might be that animals possessing a receptive substance differentially affected and those possessing a receptive substance identically affected by optically isomeric nicotines differ also in their receptivity to the normal chemical mediator, and consequently in peculiarities of the transmission of nerve impulses.

An examination of the data on the distribution of acetylcholine in different groups of invertebrates will throw a new light on this problem. Despite the often questionable findings concerning the presence of this substance which is ascertained by the action of extracts
on different organs while not a single of the ordinarily used organs is strictly specific, as Cannon and Rosenblueth (1937) pointed out, the results may be regarded as sufficiently reliable if they are repeatedly observed with several different procedures. The most extensive and elaborate investigations were carried out by Baeq (1935) at the Biological Station of Naples. He did not find acetylcholine nor the enzyme which destroys it, choline-esterase, in the tissues of different Coelenterates. The muscles of Annelids and of lower Deuterostomia (Holothuria) contained acetylcholine and choline-esterase. In the muscles of Crustacea he found so little acetylcholine that he concluded that the transmission of impulses from the motor nerve to the muscle in these animals is not accomplished by means of this mediator. He insisted on this point at the conference devoted to this problem held in Cambridge in 1937. On the other hand, there are some preliminary communications by Nachmanson (1937), according to which there is some choline-esterase in the ganglions of Crustacea. What is certain, however, is that neuro-effector synapses of the muscles of Crustacea are not typical acetylcholine systems, if only for the reason that they are extremely insensitive to the action of externally applied acetylcholine.

In the accompanying table we compare the observations of Baeq with those of Gause and Smaragdova. In six animal groups the two series of independently obtained results coincide. If our suggestions are correct, the differential killing action of optical isomers of nicotine could be employed to detect the presence of the specific receptor characteristic for the acetylcholine system in the neuro-effector synapse of voluntary muscles (Gause and Smaragdova 1939). But further investigations are necessary for a final conclusion on this problem.
TABLE 20


<table>
<thead>
<tr>
<th>Animals</th>
<th>Stereo-Differential Action of Nicotine (Gause and Smaragdova, 1939)</th>
<th>Acetylcholine Mediation (Baeq, 1935)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Coelenterata</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>2. Annelida</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>3. Lower Deuterostomia</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>(Holothuria for acetylcholine and Chaetognatha for nicotine)</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>4. Crustacea</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>5. Insecta</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>6. Vertebrata</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

SECTION III

ANALYSIS OF THE MECHANISM OF VARIOUS PHYSIOLOGICAL FUNCTIONS IN PROTOZOA

The following attempt at an "Asymmetric Analysis" of physiological functions in protozoa is based on the fundamental principle of dissociability of physiological processes. The action of the optical isomers of some organic substance will be studied on some particular function and the stereo-coefficient of action determined for that function. From the similarity or dissimilarity of the coefficient various conclusions can be drawn on the nature or mechanism of the function.

It should be noticed that a similar method is followed in the temperature analysis of biological processes (cf. the recent discussion of this subject by Hoagland, 1935). When two separate processes reveal different temperature relations it is believed that they are not directly controlled by some common "master reaction". (Concerning the caution with which the notion of "master reaction" should be used, cf. Burton, 1936 and Hoagland, 1937.)
The experiments of Gause, Smaragdova and Alpatov (1938) to be reported here, consisted in the analysis of the action of the optical isomers of cinchonine on the rate of the following functions of the infusorian Paramecium caudatum: (1) The feeding rate, as measured by the number of food vacuoles formed in water suspensions of india ink; (2) The velocity of expulsion of gastric vacuoles; (3) The division rate; (4) The velocity of locomotion in thin glass tubes, according to the method of Glaser (1924); (5) The death rate, death being diagnosed by the complete cessation of all motion.

It was found that the stereo-coefficient of action of cinchonine ($\alpha$) was of the same order of magnitude (about 1.3) in the case of the inhibition of the following three functions: the formation of the gastric vacuoles, the expulsion of these vacuoles, and the division rate. In other words cinchonine inhibits some susceptible system which controls these three functions. The gastric vacuoles in paramecia separate from the gullet, being, so to say, pulled away by the active protoplasm (cf. Metalnikoff, 1910 and Bozler, 1924). Their formation and their expulsion are evidently connected with the degree of activity of the protoplasm. These two functions, as well as the rate of division, are, therefore, expected to be controlled by the rate of metabolism in the endoplasm of the paramecium. In all probability optically isomeric cinchonines, inhibiting one of the phases of metabolism in the endoplasm, depress all the three functions together.

On the other hand, the cessation of the motion in paramecia by cinchonine is evidently connected with the poisoning of the system of locomotory cilia. This system is localized in the ectoplasm (cf. Kalmus, 1931). The stereo-coefficient of action of the optical isomers of cinchonine is significantly different from that previously recorded, it reaches 1.98 (the left isomer being, as before, more powerful than the right). These data are presented in Table 21.
TABLE 21
Stereo-Coefficients of Action of the Optical Isomers of Cinchonine on Various Functions in Paramecium caudatum,
(From Gause, Smaragdova and Alpatov, 1938.)

<table>
<thead>
<tr>
<th>Function</th>
<th>Stereo-coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of</td>
<td></td>
</tr>
<tr>
<td>endoplasm</td>
<td>$\alpha = 1.36$</td>
</tr>
<tr>
<td>1. Formation of</td>
<td></td>
</tr>
<tr>
<td>gastric vacuoles</td>
<td>$\alpha = 1.36$</td>
</tr>
<tr>
<td>2. Release of</td>
<td></td>
</tr>
<tr>
<td>gastric vacuoles</td>
<td>$\alpha = 1.24$</td>
</tr>
<tr>
<td>3. Division rate</td>
<td></td>
</tr>
<tr>
<td>Inhibition of</td>
<td>$\alpha = 1.98$</td>
</tr>
<tr>
<td>ectoplasm</td>
<td></td>
</tr>
<tr>
<td>1. Mechanism of</td>
<td></td>
</tr>
<tr>
<td>ciliary motion</td>
<td></td>
</tr>
</tbody>
</table>

The differences observed in the value of the stereo-coefficient suggest that, in the ectoplasm, cinchonine inhibits a receptive substance different from that of the endoplasm. A physiological differentiation of the cells into ectoplasm and endoplasm would then be brought into evidence in Paramecium caudatum by our method of "asymmetric analysis".

However, in another species of paramecia (Paramecium bursaria), the stereo-coefficients of the action of optically isomeric cinchonines on various functions did not disclose such a difference; the same stereo-coefficient of inhibition was observed for the endoplasmic and ectoplasmic function.

For the study of the effects of the optical isomers of cinchonine on the rate of movement of paramecia the procedure was, in general, as follows. To 2 cc. of cinchonine solution of a given concentration 5 drops of a culture of Paramecium caudatum were added. A little quantity of the cinchonine solution with infusoria was then transferred with a pipette into a thin glass tube and the latter was placed on a graduated glass plate on which the velocity of motion was measured with the aid of a stop-watch. The determinations were made every ten minutes for eighty minutes.

While, for a time, no significant difference in velocity could be observed in the control and in the right isomer solution, the paramecia in the left isomer of the same
strength presented a considerable increase in the rate of movement. The left isomer of cinchonine definitely called forth at first a strong stimulation of movement; subsequently the motion slowed down and finally the paramecia died. With the right cinchonine the stimulation phase was entirely absent under all concentrations employed, only the inhibition phase could be observed.

So only the laevorotatory isomer has the specific power of stimulating the ciliary movement. One can suppose that the left isomer, because of peculiarities of its spatial configuration, interacts with the system of reactions which control the ciliary motion, while this system remains as if "closed" for the dextrorotatory isomer. In distinction from this stimulating effect, the less specific process of toxic destruction of the locomotory force of the cilia is carried out qualitatively in the same way by both optic isomers of cinchonine, the rate of the reaction only is different. This situation has its parallel in the following observation of Krebs (1936). He has recently pointed out that in the metabolism of amino acids some specific transformations such as the splitting of the imidazole ring in histidine (Edlbacher and Neber, 1934), or the oxidation of the ring in tyrosine (Bernheim, 1935), are open only to the natural amino acids of the left series and are closed for the right forms. On the contrary, in other less specific reactions, such as deamination, both optic isomers of amino acids can participate.

Further data on the action of optical isomers of cinchonine upon various protozoa are given in the original paper by Gause, Smaragdova and Alpatov (1938).

SUMMARY

1. The study of the mechanism of various biological processes by examining how they are influenced by optical isomers of various substances is presented as a method of investigation called "Asymmetric analysis." This method is applied here in the study of (1) the
mechanism of toxic action, (2) the evolution of the nervous system, (3) the mechanism of various physiological functions in protozoa.

2. The two optical isomers of a toxic substance may exhibit different degrees of toxicity (the natural isomer being more toxic) but possess the same mechanism of toxic action, as judged by the identity of the relation of increasing toxicity to concentration and by the identity of the temperature characteristics. Such conditions have been observed, in particular, in nicotine. There are cases in which none of the two relations just mentioned hold. The last series of cases cannot be accounted for by the assumption of a receptive substance diversely affected by the two isomers.

3. The coefficient of relative toxicity of the two isomers of tartaric acid increases from 1 to 1.305 when one passes from the protozoa to the fishes through the worms and the crustacea. The killing action, in the lower forms, seems, then, to be due to factors which are common to the two isomers, while, in the higher forms, it is due to factors which differ in the two isomers. It is suggested that the factors of the first type are those which act mostly on the surface of organisms, and the factors of the second type, those which act internally. The problem of the mode of action of toxic substances is then linked to that of the evolution of the integuments in fresh water animals.

4. The study of the toxic action of nicotine in animals of variously developed nervous systems points to the absence of a spatially specific receptive substance in Protozoa, Coelenterata, Turbellaria, Rotatoria and Nemertinea, and to the presence of such a substance in Annelids, Chaetognatha and Vertebrates. In Arthropoda it is absent again. A comparison of its distribution with that of acetylcholine in different groups of animals leads to significant data on the evolution of the nervous system. The receptive substance in nicotine poisoning shows some close relation to the receptive substance for chemical mediation in the transmission of the nerve impulse.
The results of the toxic action of the optical isomers of cinchonine on *Paramecium caudatum* bring into evidence a difference in the physiological functions controlled by the ectoplasm and those controlled by the endoplasm. Of the two isomers of cinchonine only the laevorotatory showed the specific power of stimulating ciliary movement.

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APPENDIX

ASYMMETRY OF PROTOPLASM AND THE STRUCTURE OF THE CANCER CELL

When, in 1923, Otto Warburg reported that the oxidative metabolism of cancer cells is somewhat defective and that growth and multiplication in cancerous tissue is provided mainly by fermentative processes accompanied by the formation of large quantities of lactic acid, it was thought that the solution of the old mystery about the nature of cancer had been found. Numerous attempts of cancer therapy based on this principle, and consisting mostly in inhibiting anaerobic processes in malignant cells, were made. But all these attempts failed. It was then learned that anaerobic metabolism is not a characteristic feature of malignancy, it is observed in many embryonic tissues.

In view of such failures, James Ewing, director of the Memorial Hospital, New York, remarked before the National Academy of Science (April, 1938), that the fundamental nature of malignant growth is probably an insolvable problem and that investigations on this problem have consumed much time and money without providing knowledge of practical value. There is, according to him, urgent need for greater support of clinical investigations which yield some practical results.

In spite of that skeptical attitude toward the prospects of fundamental cancer research, the year 1939 brought forth an important discovery on the structure of the cancer cell. Two distinguished Dutch chemists, Fritz Kögl and Hanni Erxleben isolated from proteins of malignant cells the unusual optical isomer of glutamic acid (of the right steric series), which never occurs in proteins of healthy cells. This important finding was rapidly followed by significant practical applications. Waldschmidt-Leitz (1939) discovered in the serum of cancer patients proteolytic enzymes with unusual stereo-
chemical behaviour. These enzymes are absent from the serum of healthy persons.

Kög1 and Erxleben also isolated several other amino acids from proteins of normal and malignant tissues and measured their optical rotatory power. Serine and proline, which undergo easily a partial racemization in hydrolysis, were obtained as partially racemic products in healthy tissues. Proline instead of specific rotation $\alpha_1 = -84.9^\circ$ gave a value of $\alpha = -82.4^\circ$, and serine, instead of $\alpha = +14.45^\circ$, gave $\alpha = +8.38^\circ$. But such amino acids as valine, leucine and glutamic acid were practically optically pure when isolated from healthy tissues, while partially racemic preparations were isolated from malignant tissues (Table 1).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Expected specific rotation</th>
<th>Observed specific rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>$+15.4^\circ$</td>
<td>$+13.2^\circ$</td>
</tr>
<tr>
<td>Lysine</td>
<td>$+14.6^\circ$</td>
<td>$+13.5^\circ$</td>
</tr>
<tr>
<td>Valine</td>
<td>$+28.8^\circ$</td>
<td>$+26.9^\circ$</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>$+31.7^\circ$</td>
<td>$+ 4.6^\circ$</td>
</tr>
</tbody>
</table>

In the case of glutamic acid, racemization is most evident, since as far as 42.7% consists of the unusual d(−) form. Such observations have led Kög1 and Erxleben to conclude that the unusual optical isomers of some amino acids participate in the composition of cancer cells. The latter would, then, be characterized by some particular spatial molecular configuration, on account of which the growth-controlling enzymes would be disturbed.

According to Kög1 and Erxleben, partial racemization of glutamic acid in cancer tissues is most evident and it could be checked easily. This observation has been severely criticized by Chibnall (1939) and also by Graff (1939) who reported to have isolated only optically pure
1(+) glutamic acid from malignant cells. Kögl and Erxleben (1939) immediately pointed out that their opponents did not pay sufficient attention to the different solubilities of the optical isomers. Racemic dl-glutamic acid, in the form of both chlorhydrate and barium salt, is two times more soluble than l-glutamic acid. The pure natural isomer consequently crystallizes first and, if the crystallization is not complete, the racemic isomer will be left in the mother liquid.

Lipmann and his collaborators (1940) also opposed their findings to Kögl’s and Erxleben’s data. On account of some difficulties in the ordinary isolation procedures, Lipmann attempted to determine the total d-amino acid content of the human tumors and of normal tissues by means of d-amino acid oxidase with the aid of the Krebs enzyme. He found 1.85% of d-amino acids in hydrolyzates of normal tissues and 1.84% of d-isomers in those of cancer tissues, that is, practically the same value in the two cases. However, Lipmann himself admits that the accuracy of his method is not great. Moreover, since each hydrolysis inevitably leads to a partial racemization of such labile amino acids as serine and proline, the determination of total d-amino acid content loses some of its significance.

On the other hand Kögl’s data have been confirmed by Arnow and Opsahl (1939). The glutamic acid which they isolated from normal tissues had an optical rotation of \( \alpha = +31.0^\circ \), and that isolated from malignant tissue had an optical rotation of \( \alpha = +5.5^\circ \).

If one assumes that Kögl’s data are correct, it is, however, not clear whether the unusual optical isomer of glutamic acid pre-exists in the cancer cell, or whether the partial racemization observed is of a factitious nature. If glutamic acid, in the protein molecule of cancer cells, enters into some special labile compound different from that in which it exists in usual protein molecules, it is conceivable that hydrolysis could lead to a partial racemization in the cancer cell but not in the normal cell. This
possibility can be checked. Lipmann reports that most of his hydrolyses were carried out in HCl containing heavy water. Subsequent determinations of the content of deuterium attached to the alpha carbon atom of glutamic acid will show, according to the suggestion of du Vigneaud whether or not the partial racemization is due to the process of hydrolysis.

If the recent data of Waldschmidt-Leitz and Mayer (1939) are confirmed, they will undoubtedly lead to new developments in the study of the problem of cancer from the viewpoint of protoplasmic asymmetry. According to Waldschmidt-Leitz, if unusual optical isomers of some amino acids really enter into the composition of malignant cells, there should be specific proteolytic enzymes to catalyze the spatially unusual metabolic processes. It is known that peptidases of healthy animal tissues do not split up such polypeptides which consist of unusual optic isomers of amino acids. Waldschmidt-Leitz showed this to be true also of the aminopolypeptidase and the dipeptidase from the serum of healthy persons. But the properties of peptidases from the serum of cancer patients are radically different, they can split up polypeptides consisting of unusual optic isomers. This feature was used by Waldschmidt-Leitz in his diagnosis method.

One can hope that more light be shed on this important problem in the near future.

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Corrigenda

P. 20, 18th line from the bottom, read 1939 instead of 1938.
P. 29, line 13, read Brockmann instead of Blockmann.
P. 59, 8th line from the bottom, read dextrorotatory instead of laevorotatory.