Some applications of organic chemistry to biology and medicine / by George Barger.

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THE GEORGE FISHER BAKER
NON-RESIDENT LECTURESHIP IN CHEMISTRY
AT CORNELL UNIVERSITY

VOLUME 5

Some Applications of Organic Chemistry to Biology and Medicine

by

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PREFACE

This book is based on a course of lectures delivered during the Second Semester of 1927–1928 under the Non-Resident Lectureship in Chemistry endowed by Mr. George Fisher Baker.

The composite nature of my audience, consisting of biologists and of chemists, induced me, when lecturing, to aim at a general survey and the exposition of first principles. In the subsequent writing of several chapters I have either confined myself to recent work or entered into greater detail, as in Chapter V, where I hope that every significant observation has been noted.

I shall always gratefully remember the kindness of Professor Dennis, my perfect host, and of his colleagues in the Baker Laboratory, that monument of careful planning and organization, worthy of a great university.

INTRODUCTORY LECTURE

International Relations in Science

In this my introductory lecture, I take pleasure, at the very outset, in expressing my gratitude to the president of your university, to Professor Dennis and to the other authorities responsible for having addressed to me an invitation to come to you as lecturer on the Baker Foundation. I regard this invitation as a great honor, altogether beyond what I have deserved, and it will be my earnest endeavor, during my stay in your midst, to discharge the duties of this lectureship to the utmost of my ability.

The first of these duties in point of time is to deliver an introductory lecture on a non-technical subject. The choice of such a subject for the present occasion has caused me not a little difficulty. At the outset it seemed to me that since the purpose of the Baker Foundation is to bring over lecturers from other lands and thus facilitate intercourse between workers of different nationalities, I might perhaps suitably discuss the nature and extent of the intercourse between scientific men in different countries, both in the past and in the present. When I mentioned my plan to a friend who had lectured in a western university, he told me that the relations between European scientists would not be of any particular interest here, where a single nation stretches right across a continent. He further suggested that Americans are not so well informed about the national peculiarities of Europeans as I imagined, that they have no sympathy for our difficulties and are indeed somewhat impatient of European squabbles and jealousies. I was advised to discuss instead some more concrete chapter in scientific research and to treat it in a popular fashion. This suggestion, that America stands aloof from European affairs, did not, however, agree with my own impressions, previously gathered from American colleagues visiting Europe. The very fact that your university makes a prac-

tice of inviting foreign lecturers and the assurances of two of my predecessors in the Baker lectureship, convinced me that here in the east, at any rate, you are not only well acquainted with conditions in Europe, but also understand our difficulties, so that I still hope I may bring before you certain general considerations affecting scientific progress, and thus discharge my obligation, without discussing in detail any particular line of research.

Apart from its effect on the progress of science, the intercourse which I propose to discuss may contribute largely to the mutual understanding of nations. If the consent of scientific men, instead of that of parliaments, were required for the making of wars, the peace of the world would not indeed be assured, but I venture to think —I certainly hope—that it would be less precarious than it is at present. Scientific research is one of the most international forms of human endeavor. Perhaps it might be considered second to music in this respect, since music is independent of human speech; we can enjoy the compositions of foreign composers without knowing their mother tongue. Yet for this very reason music does not greatly help us to understand other nations and the music of the east may even be unintelligible to the west. Athletic contests and games like chess bring about the meeting of competitors from distant lands, but these international competitions affect only a handful of champions. The Olympic games no longer bring together the nations of the modern world as they united the communities of ancient Greece. But scientific phenomena are universal; they are the same all the world over. How often does the specialist worker remain isolated in his own country and find that his particular field is only cultivated by workers abroad! My further lectures will furnish many examples of this, as indeed all scientific lectures are apt to do. Let me, for the purposes of the present occasion, anticipate. The intense physiological action of extracts of the adrenal gland was discovered by two Englishmen and practically at the same time by two Poles. The isolation of epinephrine was then attempted by an Irishman and by an Austrian; it was accomplished in the United States by one of your country-

men and by a Japanese. The substance was next investigated by a Frenchman, an Englishman and by several Germans, one of whom synthesized it; it was also synthesized by an Englishman. Allusion to this chain of researches, extending over less than a decade, has already involved me in the mention of eight nationalities. Hence it is clear that international relations play a considerable part in scientific research.

Western science originated with the Greeks and in the Hellenistic period became concentrated at Alexandria; other civilizations, such as that of the Chinese, remained isolated, and facts known to them were rediscovered later in the west. In ancient times there was a good deal of intercourse among philosophers all around the eastern half of the Mediterranean. Already the Ionian Greeks of the fifth century B. C. were characterized by a love of travel for the sake of the "wonders" to be seen in strange lands. Thales (624–547 B. C.), the founder of Greek geometry and of Greek astronomy, traveled in Egypt, and Pythagoras also undertook extensive journeys. Mathematical discoveries, whether made in Asia Minor in the east or in Magna Graecia in the west, became widely known by an intercourse facilitated by a common language and apparently not hampered greatly by political differences or even by wars; yet the harmful effect of war on scientific progress was early illustrated by the killing of Archimedes by a Roman soldier at the sack of Syracuse in 212 B. C.

The decay of the Roman empire was accompanied by that of Greek science, which passed at a later period to the Moors. Not until the Renaissance did the pursuit of science spread to the nations of the west, and then, for a time, it would seem to have been more international than other forms of human activity. In the school of medicine at Salerno and the earliest universities of Bologna, Padua and Paris, the universal use of Latin established a freemasonry among the learned, where accidents of nationality did not count and difficulties of communication were overcome. Vesalius, a Belgian, taught anatomy at Padua, Paracelsus traveled widely in troublous times, and science

appeared wholly dissociated from politics. Although Spain was the chief nation concerned in the discovery of America, Columbus was an Italian, and the name of your continent is likewise of Italian origin.

The number of foreign students in medieval universities was great. A document of the year 1228, exactly seven hundred years ago, records the presence at Padua of French, English, Norman, Provençal, Spanish and Catalan students. This was only six years after the foundation of that university. Later the number of foreigners increased still further. They came "non ex propinquis tantum regionibus, non ex ultima solum Italia, sed . . . ex toto prope terrarum orbe." Ultimately twenty-two "nations" were represented, ten from beyond the Alps, twelve from various regions of Italy. Each "nation" elected one or more councilors who assisted the rector in the government of the university. Traces of this divisional arrangement of the students survive in certain Scottish universities. In the fifteenth century there were about a hundred French students at Padua, nearly as many English and Scottish, and over three hundred German. Even now the crests of students from many nations (that of Harvey among the number) may be seen in the old loggia and aula of Padua University and afford interesting testimony to the international character of medieval learning. Professorships were not infrequently held by foreigners; it is early recorded that the highest office of the university, the rectorship, was held by a Pole in 1271.

At first the study of science was the work of a few devotees who communicated their discoveries by personal intercourse or in the form of books. To these men science was a passion or an obsession, in any case their main interest in life. In course of time the amateur also made himself felt. Otto von Guericke, burgomaster of Magdeburg, was presumably as much occupied with civic affairs as with his air pump; although King Charles II of England founded the Royal Society and sometimes attended its meetings, his main interest can not be said to have been scientific. The diaries of Pepys and of Evelyn give an interesting sidelight on the attitude towards science of the

amateur of that period. One of your earliest statesmen, Benjamin Franklin, was distinguished for his important contributions to natural knowledge. Priestley, one of the discoverers of oxygen, was in later life much more interested in theology than in the constituents of the atmosphere. The importance of the work of amateurs, or at least of men not holding official positions, seems to me to have been specially characteristic of British science; I need only mention the names of Boyle and of Cavendish, both scions of noble houses, and of Joule, a brewer.

The growing interest in science led, in the second half of the seventeenth century, to the foundation of societies and academies, who published short communications in their proceedings. The Royal Society of London received its charter in 1662 and arose out of informal earlier meetings at Oxford. Its "Philosophical Transactions" were first published in 1665. About the same time were founded the Accademia del Cimento of Florence (1657), the Academy of Vienna (1652) and the Académie Royale of Paris (1666); the memoirs of the latter began in 1699; in 1700 the Berlin Academy was founded. At first the publications of these various societies preserved the appearance of private intercourse, for they frequently were in the form of letters addressed to the secretary. As an example I may refer to the important microscopic discoveries of Anthoni van Leeuwenhoek, who during the latter years of the seventeenth century wrote several hundred letters from his sleepy little town of Delft to the secretary of the newly founded Royal Society of London; a portion of these letters, published in Dutch, occupies four large volumes. Leeuwenhoek, employed as janitor at the town hall, became, in his spare time, an expert in the grinding of lenses and made his own very powerful simple microscopes, tiny instruments compared with the compound microscopes of a later date. His equipment was indeed in strange contrast to that of the chemical laboratory of this university, which, I understand, has a special section devoted to the application of the microscope to chemistry. Yet Leeuwenhoek's discoveries were of a fundamental kind; thus he first saw and figured infusoria and spermatozoa, and investigated the

process of reproduction in various animals. Another famous microscopist of that time, Malpighi, an Italian, also communicated his discoveries to the Royal Society; his original letters, with those of Leeuwenhoek, form an interesting part of the archives of the society.

One of the effects of the foundation of national academies was an increased use of the native tongue in scientific communications, and instead of, or in addition to Latin, it has now become necessary for the man of science to know several modern languages. The abandonment of Latin as the universal language proved an obstacle to scientific intercourse. When lectures at the universities were no longer given in Latin, it became more difficult to obtain teachers from abroad. The change made itself felt in the beginning of the eighteenth century. In the middle of the previous one it was still possible to call to Leiden a Hanoverian physician, Franciscus Sylvius, to teach chemistry and medicine; he indeed founded there the first university chemical laboratory, a humble precursor of the magnificent building in which we are now assembled. When later, early in the eighteenth century, the school of medicine, to which I myself belong, was developed at Edinburgh; the use of spoken Latin, which, as we have seen, had done so much for the medieval universities, had declined; the teachers were all Scotsmen, who had indeed been influenced by the great Boerhaave, of Leiden, but did not use Latin to any large extent in their own lectures. As a written language Latin survived to a much later date, particularly in academic publications, such as doctoral theses which at Edinburgh, for instance, continued to be in Latin until about one hundred years ago; in Germany Latin was still employed for this purpose until about the middle of the last century. Today the use of Latin in scientific publications is rare and almost restricted to a few botanical and zoological works of reference chiefly of interest to the systematist. Its use as a spoken language is extremely rare; apart from ceremonial occasions at the older English universities, I have myself heard it only twice at international gatherings; on both occasions it was used by Swedes.

The use of the vernacular instead of Latin caused at least a relative setback in the intercourse between the scientific men of various nations. The growth of nationalism in the nineteenth century acted in the same direction and it was not until travel had been facilitated through the spread of railways that the abandonment of Latin as a universal language was compensated for by the greater ease of communication.

In giving facilities to advanced students from abroad, for some time Paris and later on the German universities took a leading part and thus contributed greatly to the furtherance of international relations, not the least by spreading a knowledge of French and German among scientific men. Thus the laboratory of Wurtz attracted many foreign chemists to Paris, as did that of Liebig to Giessen. The Pasteur Institute later drew bacteriologists to Paris and towards the end of the century it became comparatively common, particularly for American and English scientific men, to spend a year or so in research at a foreign university. They thus acquired a knowledge of the spoken language, which sometimes proved useful in strange circumstances. When, as the result of the Armistice, the Allied chemical experts inspected certain German chemical factories which had been used for the production of munitions of war, there was at least one occasion when an English and a French chemist met a German expert and the victors had to speak the language of the vanquished, for German was the only language known to all. Personally, I remember a chemical congress held a few years after the war, at Cambridge. England and France were largely represented; there were no Germans; there was a distinguished chemist from Japan who had studied in Germany and spoke its language fluently, but did not speak French. I took pleasure in introducing him in German to his French colleagues who too spoke that language fluently, and later admitted to me privately that it was a very useful one.

Among the advantages of foreign study may therefore be counted the acquisition of a thorough knowledge of a foreign language and some insight into the character of

another nation. For various reasons residence abroad has however of late become less frequent, at least relatively so. The great development of your own universities has diminished the inducement to your students to spend some years in Europe, when they find at home an extensive choice of distinguished teachers and of excellent laboratories. The late war has had a great effect in the same direction, particularly on the younger workers in my own country. Formerly it was common for British students to spend a year or two at a German university, in order to obtain the degree of doctor of philosophy, but as a result of the late war practically all British universities have copied Germany in instituting such a degree; the effect has certainly been good in stimulating research among British students at home, but it has also tended to make the younger generation more insular and less acquainted with foreign life and thought. To some extent this is compensated for by the increased number of traveling fellowships, mostly founded by Americans; to these I shall refer later.

We have seen that the disuse of Latin as a vehicle of instruction made the occupation of teaching posts by foreigners more difficult, but the practice has never died out. Thus in 1845 Prince Albert, the Consort of Queen Victoria, and a German who did much to stimulate scientific research in his adopted country, secured the migration to London of A. W. Hofmann. The nineteen years which Hofmann spent in England not only saw the production on a commercial scale of mauve, the first aniline dye, by his pupil, Perkin, but Hofmann's stay in England did also much to further Anglo-German chemical relations. A number of German chemists settled in England, and Hofmann, after he had returned to his native country to occupy the chair of chemistry at Berlin, brought about the foundation of the German Chemical Society on the model of the English society, with which he became familiar during his years in London. While Hofmann was in England, another German organic chemist, Kekulé, was professor in Belgium, at the University of Ghent, and there worked out his famous benzene formula; he soon afterwards returned to Germany, but yet another German,

Körner, migrated permanently to Italy, where he had many pupils and died only a few years ago. Such examples of migration are most frequent in the smaller European countries whose size restricts their choice of native candidates. Moreover, in a country such as Holland every university student knows English, French and German, so that there is no difficulty about a foreigner lecturing in one of these languages until he has learned the vernacular. There are always a few Germans among the professoriate of the Dutch universities. About thirty years ago an Englishman was appointed to a theological professorship at Leiden, and when he migrated to the United States, he was succeeded by a Norwegian; in this way the Dutch government attempted to avoid the *odium theologicum* which would have resulted from the appointment of a native. Dutchmen have from time to time occupied chairs abroad; thus van't Hoff left Amsterdam for Berlin, and within recent years Holland has supplied a professor of physics to Scotland, one to Germany and a professor of medicine to Vienna. Sweden has a German professor of chemistry, and an English professor of pharmacology, who came there after holding a chair in Switzerland. This latter country is, of all, the most ready to appoint foreigners; indeed, at one time a Swiss chair was frequently a stepping-stone to a more important one in Germany. Besides quite a number of Germans I can think of one or two Frenchmen, several Poles and Russians, two Americans, an Englishman, a Dutchman and an Austrian, who have in recent times held Swiss professorships. Such a lively interchange would however offend the nationalism of the larger countries, where there is moreover a larger choice of native candidates and where the wars of 1870 and 1914 have produced a serious setback in international exchanges.

After 1870 politics entered into science as never before. French science became national, almost insular. Germans no longer studied in Paris, and for many years no French workers came to German laboratories; by slow degrees formal relations were ultimately resumed, more readily perhaps by the victors than by the vanquished. Franco-German susceptibilities became the chief stumbling-block

in any international organization, as they did in European politics. Among the most noteworthy of these organizations are various congresses at which devotees of the same branch of science meet periodically for communication and discussion of their researches. One of the oldest and most successful of these is the congress of physiologists, started in 1889 on the initiative of Michael Foster and, except during the late war, held at intervals of three years. In a gathering of this kind the very choice of a meeting place is already influenced by politics. Just as the International Postal Union and the League of Nations meet in Switzerland and the International Court of Justice in Holland, so the congress of physiology began by meeting in small countries to avoid the jealousies of the larger ones. The first six meetings were held in Switzerland, in Belgium, again in Switzerland, in England, in Italy and again in Belgium. Although Germany has important physiological laboratories, it took eighteen years for the congress to come to that country (Heidelberg, 1907). In Paris, in 1920, no Germans were present, and in 1923 at Edinburgh, the great problem was to bring the late belligerents together again. The organizer of the latter congress received strong expressions of opinion from American and English physiologists that they would welcome the presence of German and Austrian colleagues, and invitations were accordingly sent to them, but this very fact kept away many Frenchmen and Belgians. Those who were present realized, however, how the restoration to the congress of its truly international character increased its scientific value, and three years later at Stockholm, it was generally agreed that the Franco-German difficulty was at end among the physiologists. The next meeting is to be held in 1929 at Boston, and this decision illustrates yet another problem, not political, but geographic and financial, for it will have taken the congress exactly forty years to come to America.

This is far from satisfactory. American physiologists have attended previous meetings in large numbers, they have enhanced the scientific value of the congress by their communications, yet many of them could ill afford the expenses of a journey to Europe. Of course many Euro-

pean university teachers are even less able to defray the cost of transatlantic travel. There is here a difficulty inherent in the spread of science over two continents. Yet it is to be hoped that, in spite of this difficulty, a numerous contingent from Europe may find it possible to accept the warm invitation of their American colleagues. Thus the visitors will be able to learn at first hand about divisions of their subject which have been developed by American pioneer work and have as yet hardly been studied on the continent of Europe.

The political difficulties in other departments of knowledge have varied. It would seem that after the war international relations were most readily resumed in those sciences which are most remote from practical considerations. Where, as in chemistry, industrial or military aplications interfere, progress has been less rapid.

Thus the late war had very little effect on astronomers, but industrial rivalry and chemical warfare have delayed a *rapprochement* among the chemists. Yet here also progress may be recorded. Thus, Professor Richard Willstätter, a leader of German chemistry, who, you may recall, visited this university less than a year ago, was invited to give the Faraday lecture to the Chemical Society of London, and generously allowed himself to be reëlected an honorary fellow of that society. The celebration of the Berthelot Centenary in Paris last October, the most distinguished chemical gathering in which it has been my privilege to take part, was attended by nine German and by two Austrian delegates.

Mention should also be made of the International Research Council formed as a result of meetings in London and Paris in 1918 and at Brussels in 1919. It is practically a union of academies formed for the purpose of facilitating international coöperation in scientific work, and promoting the formation of international unions in different branches of science. The statutes of the Research Council were so framed that the Central Powers were excluded; their inclusion immediately after the Great War would indeed have been surprising. Seven years later, however, at Brussels, in 1926, the Royal Society of London, at the

instigation of Holland and Denmark, proposed that the five German academies should be invited to join the International Research Council, an invitation which has not yet been accepted. Its non-acceptance must be a disappointment to the Dutch and Danish academies, and to all who wish to see science dissociated from politics. The accession of the German academies might not be very important in itself, but it would bring with it membership of the various unions. One of these is the "Union internationale de la chimie pure et appliquée." I purposely quote its French title, for since its inception it has been largely under French influence, and at its first four annual meetings there was no question of admitting German chemists. Any one acquainted with the magnitude of the contribution which Germany has made to chemical science will realize that the union thereby greatly handicapped itself. At the sixth meeting at Bucharest in 1925, a motion was finally carried expressing the wish that the International Research Council should modify its statutes, so as to permit the entry into the affiliated unions of all countries who are members of the League of Nations. Apart from the furtherance of individual scientific intercourse, which may be secured in other ways, this entry would bring about the coöperation of the Germans in the attempt to secure a uniform chemical nomenclature, which without them is a somewhat sterile labor, since the chief exhaustive chemical dictionaries and cyclopedias have been published as the result of German enterprise and diligence. For the advancement of science in general, and of chemistry in particular, it is very much to be hoped that the German academicians will accept the invitation to join the International Research Council and thereby facilitate coöperation among the younger men.

On the whole the setback in scientific intercourse produced by the late war seems to me not so great as the magnitude of the struggle might lead one to fear; the cleavage between France and Germany is no greater than it was after 1870. Moreover, we can record the beneficent effect of certain agencies which have only come into being during recent years. Thus the League of Nations, in its

public health work, has incidentally brought medical men together, and from the outset German delegates have taken part. For instance, international standards have been adopted for the strength of certain drugs, and the biological methods used in testing them have formed a subject of research by pharmacologists of various nations. While this country of yours is so remote from the turmoil of European affairs that it has remained outside the League of Nations, I need hardly say that American delegates have heartily coöperated in the health work of the league, as in some other of its activities. The attitude of your government has not prevented private individuals and foundations from exercising a powerful influence in favor of the resumption of international intercourse and the furtherance of scientific coöperation. It is peculiarly appropriate that in addressing you I should record here, in the first place, the work of the distinguished president of this great university, who was the first chairman of the League of Red Cross Societies, at Geneva and Paris. Then I would mention the work of the Rockefeller Foundation, particularly in regard to medical education. I well remember the impression produced by a large gift to the medical school of University College, London, a few years after the war. The idealism, shown by giving so large a sum to a foreign institution, aroused feelings of enthusiasm and admiration among British men of science, and since then medical education has benefited in other countries, regardless of politics. I take pleasure in recording that the medical school with which I am myself associated has received several benefactions from the Rockefeller Foundation. Moreover, by giving traveling fellowships regardless of nationality, the Foundation has done much to further scientific intercourse, particularly by enabling the younger men to visit foreign laboratories. Thus the first visitor from Central Europe to work in my laboratory after the war was enabled to do so by a Rockefeller Traveling Fellowship, and several of my pupils owe experience gained in American laboratories to the same endowment. The annual review of the work of the Foundation gives an idea of its world-wide activities. Thus in 1925, in addition

to taking measures for the combating of hookworm disease, yellow fever and malaria, the Foundation contributed to the progress of medical education in many countries, maintained a modern medical school in Peking, provided, directly or indirectly, fellowships for 842 men and women from forty-four different countries and financed the travel of fifty other persons, officials and professors. Such activities are indeed a powerful and beneficent factor in international scientific intercourse. The International Education Board, established in 1923 by Mr. John D. Rockefeller, Jr., is an agency working in the same direction. In theory it may include the United States in its field of work. In practice, however, its interests lie mainly in other countries, since the General Education Board, founded by Mr. John D. Rockefeller, Sr., in 1902, is limited by its charter to the advancement of education in the United States. During the year 1925–1926 the International Education Board made ninety-seven first awards of fellowships and twenty-nine renewals; the holders came from twenty-five different countries. The voluntary migration of three hundred or more young scientists under the auspices of the Board since its foundation provides interesting indications where, in the opinion of the European and American sponsors, the more favorable conditions for research may be found at the moment. Thus in mathematics there is a marked migration toward France, Germany and Italy, in physics the trend is definitely toward England, the United States, Denmark and Germany. The primary object of the Rockefeller Foundation is the improvement of health and of education; a valuable secondary result of their activities is the promotion of international amity. This latter object is the primary one in the case of certain other benefactions, such as that of the thirty-two scholarships for American students, founded a generation ago by an Englishman, Cecil Rhodes, in his own University of Oxford. In this, as in other matters, Rhodes was a pioneer. His foundation has now a counterpart in the Commonwealth Fund, supported by gifts from the late Mrs. Stephen V. Harkness, which Fund has established a number of fellowships for British graduates, tenable at American univer-

sities. I may perhaps quote from the official memorandum: "In creating these Commonwealth Fund Fellowships the Directors of the Fund have been impelled by a belief in the value of international opportunities for education and travel to young men and women of character and ability, and by a conviction that such opportunities offered to British students will promote the mutual amity and understanding of Great Britain and the United States." The John Simon Guggenheim Memorial Foundation indirectly furthers the same object by giving fellowships to American graduates for study abroad. All these factors are bound to have a favorable effect on the outlook of the younger generation of scientific workers; half a century ago they did not exist; in the main we owe them to your country.

National characteristics have an interest, comparable to that which the student of natural history takes in the various species of animals and plants. National psychology may be as interesting as the nesting habits of birds. Each nation has its own particular genius, without which the world would be the poorer. It is interesting to inquire which nations show the greatest aptitude for scientific research, and why they do so. I feel convinced, as a result of a statistical inquiry, into which I can not enter here, that the small nations are preëminent in this respect. Per million of population, Holland, Switzerland and the Scandinavian countries at present seem to contribute more to the progress of science than any of the larger nations. Why this is so it is difficult to say. It is also interesting to speculate on the reasons which make pure mathematics flourish in Italy and Sweden, music and organic chemistry in Germany, biochemistry and psychology in the United States, physiology in Britain. While we need not agree wholly with the opening words of Wurtz's dictionary of chemistry, which claims this science as French and Lavoisier as its founder, we must recognize that we owe bacteriology to Pasteur and to France. The various nations have each their peculiar aptitude which by itself constitutes a reason for furthering international relations in science; my main reason for having brought this sub-

ject before you is, however, a desire to promote, in the words of the Commonwealth Fund Memorandum, "mutual amity and understanding." This object has already appealed to a number of your citizens; with the westward trend of civilization it is all the more desirable that the difficulties of an enfeebled Europe should be understood by America, which has become the economic mistress of the modern world, just as Rome in the third century B.C. became the political mistress of the Mediterranean. Europe, like Greece, has suffered from internal strife, yet the influence of Greece was not extinguished by the loss of political independence; the Academy survived for seven centuries, and the migration of Greek scholars began the Renaissance. Similarly, the influence of Europe will survive her economic adversity; America will doubtless become even more interested in European affairs, just as Rome looked more and more to Greek civilization.

I hope I have not wearied you with the dissensions of European men of science. In discussing them I have had in mind the words which mark so impressively the tomb of your great countryman, Grant, on the east bank of the Hudson River. These words, used after a great crisis in your own political history, I would apply to scientific affairs of today: "Let us have peace."

CHAPTER I

The Chemistry of the Hormones

Introduction

The object of this section is to outline the earlier chemical work on hormones and to deal more fully with recent developments; a few elementary physiological explanations may help students of chemistry. In the first place we must explain the term "hormone" and see how the concept originated.

In 1902 Bayliss and Starling[1] were following up the experiments of Pavlov and Popielski on the factors which bring about secretion of the pancreatic juice after the ingestion of food. The secretion does not begin until it is wanted, that is, until the food passes from the stomach into the duodenum. In analogy to what had been observed with other glands, such as the salivary gland, they sought for a nervous control of pancreatic secretion, but found that the pancreas still secreted after all the nerves supplying it had been cut, and after the spinal cord had been destroyed. They were thus led to infer an action of the acid stomach contents on the duodenal mucous membrane, resulting in a substance which should somehow cause the pancreas to secrete. They therefore prepared an acid extract of this mucous membrane, neutralized it, and injected it into the blood stream; the extract at once produced a powerful secretion of pancreatic juice. Bayliss and Starling gave the name "secretin" to the substance responsible for this effect. They imagined that normally it is set free by the acid of the gastric juice, acting on the duodenal mucous membrane, and is then absorbed into the blood; traveling round in the circulation it reaches the pancreas and excites this organ to secrete. Having searched for a nervous control and having found a material substance, Bayliss and Starling were im-

[1] J. Physiol., 28, 325 (1902).

pressed by the contrast, and recognized that secretin was a "chemical messenger."

The existence of other chemical messengers could be inferred at the time when secretin was discovered. Eight years previously Oliver and Schafer[1] first observed the pressor action of extracts of the suprarenal gland and its hormone had actually beeen obtained in a crystalline form a year before Bayliss and Starling's experiments. The fact that the effects of thyroid deficiency can be made good by oral administration of the gland, was still older knowledge; and the effects of castration, known to the ancients, might also be attributed to the absence of a chemical messenger, normally supplied by the testis or ovary.

A few years later Starling suggested the term *hormone* for substances of this class, derived from ὁρμάω, I stir up or excite, and this name has been commonly adopted. Starling included among the hormones non-specific substances, such as carbonic acid, which, accumulating in the blood during asphyxia, stimulates the respiratory center. Usually the term has been restricted to specific secretions of certain glands, but we shall see later that it is not quite easy to draw the line of demarcation.

The contrast between a nervous impulse and a hormonal stimulus may be illustrated as follows: A person at a distance may be induced to act in a certain manner by receiving an electrical stimulus, a cable message, or he may be reached by a material agent, for instance, an advertising circular. The latter would correspond to a hormone, and just as many circulars are wasted, much of the hormone is presumably wasted. Again, a hawk hovering over the valley is stimulated to swoop down on the field mouse by an optical stimulus, but hounds give tongue because of the transference to their noses of a chemical substance from the fox. The material method of stimulation is doubtless the more ancient, and the only one in unicellular organisms without nerves. Hence the importance of chemotaxis, which may be illustrated by

[1] J. Physiol., 16, i-iv (1894), Proc. Physiol. Soc.

a recently discovered example. Harington[1] found that the free-swimming larva of a wood-boring mollusk, the shipworm (*Teredo*) is attracted to wood by a soluble constituent of the latter which diffuses through the surrounding sea water. Chemotaxis in the antherozoids of ferns and mosses, in bacteria, etc., has long been known to vegetable physiologists, but since animal physiologists have occupied themselves mostly with the higher animals, the transmission of stimuli through nerves first engaged their attention, to the exclusion of the material method. The latter is by no means uncommon. There are quite a number of glands which send chemical messengers into the blood stream; such glands may be called hormone glands. Since these glands secrete their products inwards, in contrast for instance to the salivary or to the sweat glands which secrete outwards, the hormone glands are often called endocrine organs or glands with internal secretion. The Germans often refer to hormones as *increta* (in contradistinction to the products of external secretion, *secreta*), but this term is not generally used in English.

The discovery of a hormonal action naturally suggests attempts to isolate the active principle in a state of purity. This is usually much more difficult than the isolation of the active principle of a vegetable drug, partly because the supply of the endocrine organ may be very limited. The isolation of hormones has, nevertheless, attracted many biochemists, because these substances prepared by the body for its own use, are drugs of quite specific activity and extraordinary potency. So far the isolation has been achieved in the case of hormones from the adrenal (or suprarenal) gland, and from the thyroid gland; perhaps the hormone of the pancreas has also been obtained in a state of purity. Epinephrine (= adrenaline) and thyroxine have moreover been synthesized; it should be borne in mind that, strictly speaking, thyroxine does not occur as such in the gland, and is really only an active fragment of a much more complex protein-like substance. One of the main difficulties in isolating hormones is due to the

[1] Biochem. J., **17**, 736 (1921).

minuteness of the quantity present in the glands. Thus the improved method of isolating thyroxine, due to Harington, yields only 0.027 per cent. Most hormones are exceedingly unstable, being hydrolyzed very readily by acids, alkalies and proteolytic enzymes; some are readily oxidized. They present no special chemical or physical properties, such as enable alkaloids or essential oils to be separated from a large quantity of vegetable matter. Some hormones are adsorbed with great readiness on precipitates formed in their solutions, but this very property, through the use of more or less specific adsorbents, may be turned to account to bring about a preliminary concentration, as is done with vitamins and enzymes. Like diamonds and gold, the hormones interest the chemist on account of their rarity, not on account of their chemical properties. Most of all they are, however, interesting on account of their intense physiological activity.

ADRENALINE (EPINEPHRINE)

The usual sequence of events in the study of a hormone may be illustrated by reference to the adrenal gland. In 1849 Addison called attention to the importance of this tiny organ on clinical grounds; he showed that a rare disease, now called after him, is associated with lesions of the gland. Brown-Séquard then showed in 1856 that extirpation of both suprarenals in animals is rapidly fatal. In the same year Vulpian found that the adrenal medulla contains a peculiar chromogen, a substance giving a green coloration with ferric chloride and a red coloration with iodine. This histological curiosity led to a number of investigations during the next four decades, without any real progress being made. Then in 1894 interest in the adrenal glands was suddenly enormously stimulated by the discovery, by Oliver and Schafer in London, of the extraordinary pharmacological activity of suprarenal extracts, which shows itself most prominently by a rise of blood pressure on intravenous injection. It is interesting to observe that this discovery was made independently and almost simultaneously by Szymonowicz[1] in Poland

[1] Bull. intern. acad. Cracovie, Classe d. Sc. math. et nat., 56 (1895).

(1895). Attempts were now made in various laboratories to isolate the active principle. Abel at Baltimore attempted this by eliminating the protein from an extract of the gland and then separating the active substance, which he termed "epinephrine", by means of its insoluble benzoyl derivative. The subsequent removal of all three benzoyl groups proved impossible, however, and Abel at first described as epinephrine the *N*-benzoyl compound which perhaps owed its activity to adherent traces of the active substance. The green coloration with ferric chloride suggested the presence of a catechol compound which von Fürth[1] at Strassburg precipitated as lead, zinc and iron salts, without obtaining what he termed "suprarenin" in a crystalline state. This was done simultaneously in 1901 by Takamine[2] after a visit to Abel's laboratory, and by Aldrich[3] in the laboratory of Parke, Davis and Company. The method consists essentially in extracting the minced glands with hot acidulated water, heating the extract to coagulate proteins, concentrating the solution *in vacuo* and precipitating with 2 or 3 volumes of alcohol; after evaporation of the alcohol, ammonia is added to the residual aqueous solution when adrenaline separates, on standing, in sphero-crystals. Throughout, oxidation must be guarded against by using a vacuum or a carbon dioxide atmosphere; thus the final precipitation may be carried out under a layer of light petroleum.

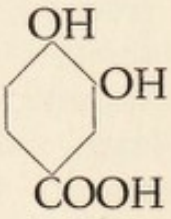
Constitution of Adrenaline. Once the active principle had been obtained in a crystalline state, it did not take organic chemists more than two or three years to determine its constitution. The surprising thing about the substance was its small molecular weight. Previously discovered active substances of the animal body (ferments, antitoxins) had been found to have a very high molecular weight. The molecular formula of adrenaline was found by Aldrich to be $C_9H_{13}O_3N$. Two of the oxygen atoms and six of the carbon atoms could at once be as-

[1] Z. physiol. Chem., **24**, 142 (1898); **26**, 15 (1898); **29**, 105 (1900); Beitr. chem. Physiol. Path., **1**, 243 (1901); Monatsh., **24**, 261 (1903).

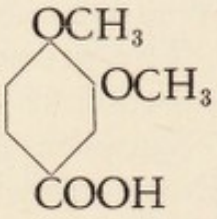
[2] Am. J. Pharm., **73**, 523 (1901).

[3] Am. J. Physiol., **5**, 457 (1901).

signed to an *ortho*-dihydroxybenzene or catechol nucleus, because of the intense green color reaction produced by ferric chloride, and because of the ease with which adrenaline is oxidized. Like catechol it may indeed be used as a photographic developer (to reduce silver salts attacked by light). (See Fig. 1) The presence of this nucleus was demonstrated by Takamine who obtained protocatechuic acid (*I*) by fusion



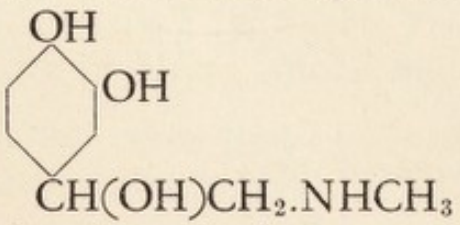
I

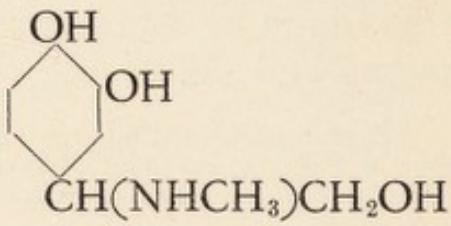


II

with potash, and by Jowett[1] who oxidized completely methylated epinephrine with permanganate and obtained veratric acid (*II*) and trimethylamine. The methylation protects the hydroxyl groups of the catechol nucleus, which would otherwise not survive the action of the oxidizing agent. These experiments showed also the position of the side chain relative to the two phenolic groups. In the case of three substituents in the benzene ring it is most convenient to resort to numbers. If we assign position 1 to the carboxyl group of protocatechuic acid (representing the side chain), the hydroxyl groups are in positions 3 and 4, an arrangement met with in a large number of vegetable substances. Protocatechuic acid is therefore 3,4-dihydroxybenzoic acid and veratric acid is its dimethyl ether.

Von Fürth established the presence of an alcoholic hydroxyl and of a methylamino group, both in the side chain, and since adrenaline is optically active, there must also be an asymmetric carbon atom in the side chain. This leads to the two following formulas:





[1] J. Chem. Soc., 85, 192 (1904).

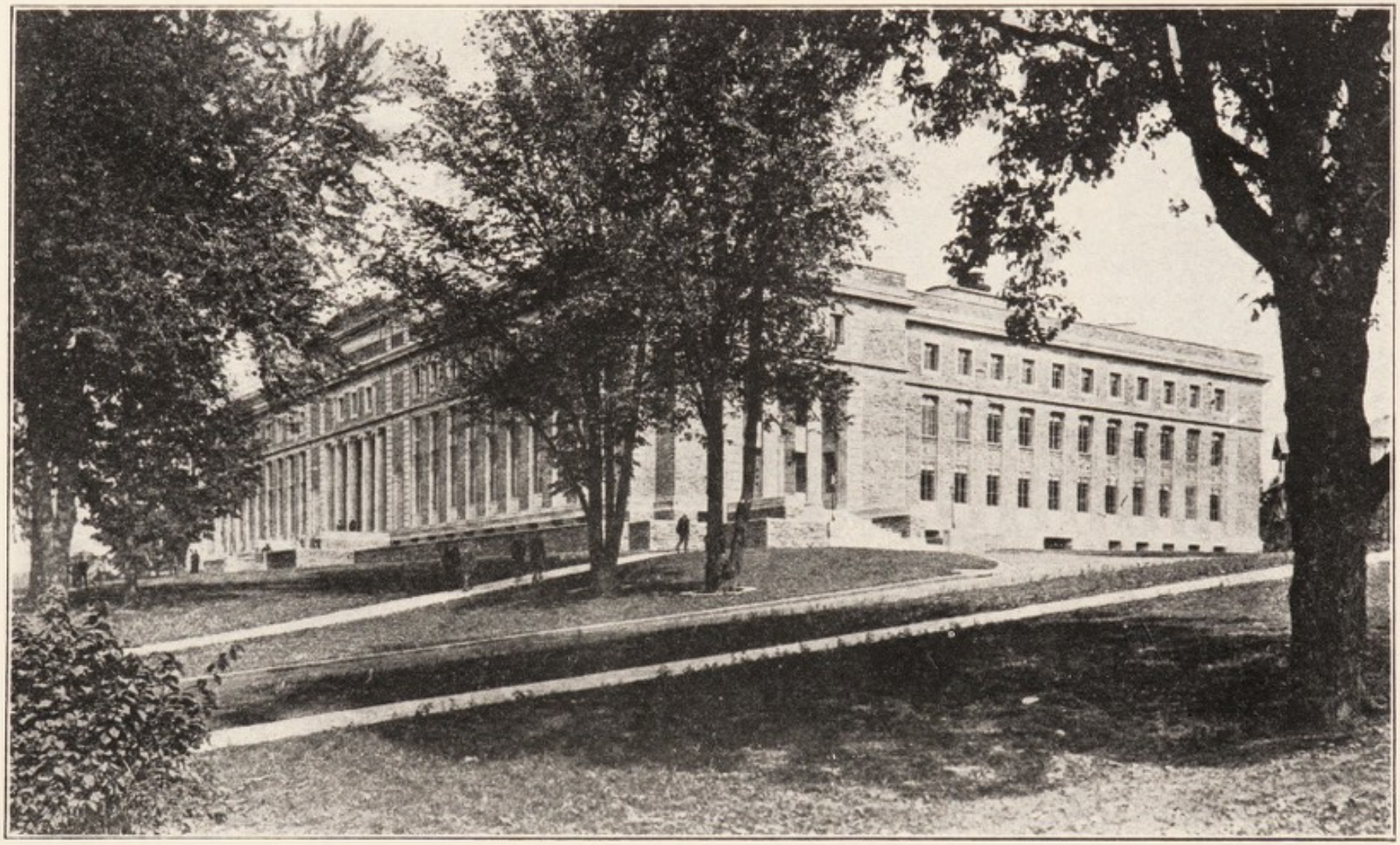
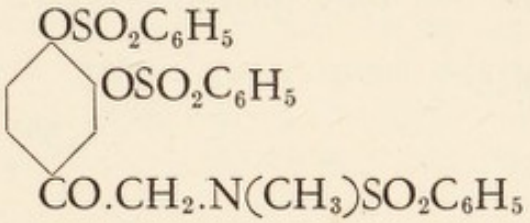


FIG. 1

View of Baker Laboratory of Chemistry (From a slide developed with adrenaline)

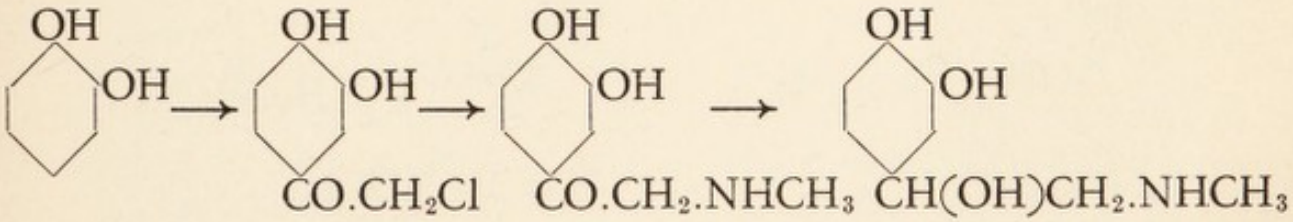
The former of these was shown to be correct by synthesis. Thus adrenaline is a secondary alcohol, in the other case it would be a primary one. Friedmann[1] later protected the catechol nucleus by introducing two benzenesulphonyl groups; a third is attached to the nitrogen atom. Now the resulting tribenzenesulphonyl compound undergoes oxidation to a ketone and not to an aldehyde or acid; this ketone therefore has the constitution shown below,



and epinephrine is a secondary alcohol. In order to give the substance a systematic name we may best regard it as ethane with three substituents, a methylamino group on one carbon atom and a 3,4-dihydroxyphenyl group plus a hydroxyl group on the other. It is therefore α-methylamino-β-hydroxy-β-(3,4-dihydroxyphenyl)ethane.

Synthesis of Adrenaline. The synthesis of adrenaline was carried out by Stolz[2] in the laboratory of the German dye works of Meister, Lucius and Brüning, and almost at the same time by Dakin[3] in London.

The starting point was catechol, which under the influence of phosphorus oxychloride condenses with chloroacetic acid on heating



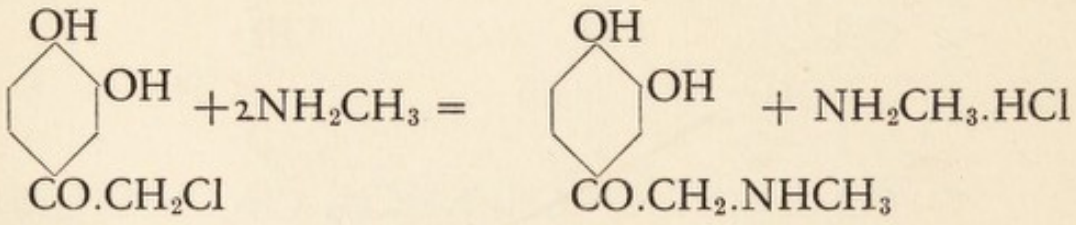
to form chloroacetocatechol. This ketone is an acetophenone derivative and its synthesis takes place somewhat like that of acetophenone from benzene and acetyl chloride, by the well-known Friedel and Crafts reaction. The yield

[1] Beitr. chem. Physiol. Path., 8, 95 (1906).
[2] Ber., 37, 4149 (1904).
[3] Proc. Roy. Soc., B76, 491, 498 (1905).

is bad; much tar is formed and even the improved directions of Mannich and Hahn[1] result in only 35–50 per cent of the theory. Ott[2] therefore recently investigated the mechanism of this reaction. He found that equimolecular proportions of pure chloroacetyl chloride and catechol in boiling benzene solution form the chloroacetate, without resinification. By warming the latter substance with 5–10 per cent of phosphorus oxychloride it is isomerized to chloroacetocatechol. We may indeed imagine that $POCl_3$

first transforms the chloroacetic acid into chloroacetyl chloride, and the condensation takes place with the evolution of hydrogen chloride.

Chloroacetocatechol had been discovered some ten years previously by a Russian chemist, Dzergowsky, who had already attempted to replace the chlorine atom by methylamine, quite in ignorance of its relationship to adrenaline, which then had not been isolated. The reaction can be brought about by using a large excess of concentrated methylamine solution in the cold.



The resulting methylamino ketone already shows the pharmacological properties of adrenaline, although it is much less active. Its reduction to crystalline adrenaline caused some difficulty. It was originally carried out electrolytically, or by means of aluminum amalgam;

[1] Ber., **44**, 1548 (1911).
[2] Ber., **59B**, 1068 (1926).

more recently Hoshino[1] has described a catalytic reduction with hydrogen and palladium. The synthetic adrenaline ("suprarenin") thus obtained was at first thought to have the same pharmacological activity as the natural product, but here a disappointment awaited the manufacturers. On careful comparison of the two substances Cushny[2] found that in all its actions the natural product was nearly twice as active as the synthetic. The reason for this is that the latter is a racemic mixture of *d*- and *l*-adrenaline, while the former contains only one variety (the *levo*), which is much more active than its mirror image. The constitutional formula shows us that one carbon atom of the side chain has four different groups attached to it, viz., the catechol nucleus, a hydrogen atom, the alcoholic hydroxyl group and the group CH_2NHCH_3.

This asymmetric carbon atom confers a lopsidedness on the whole molecule which can therefore exist in two mirror images, left handed and right handed. One of these so-called enantiomorphs turns the plane of polarized light to the left and alone occurs in nature; the other stereoisomer can only be produced artificially. On reduction of the ketone to the secondary alcohol in the laboratory, the chances for the production of the two forms are of course equal, and so a racemic mixture results, which has no effect on polarized light. Now the nerve endings, on which adrenaline acts, are adapted to the naturally occurring *l*-variety, but much less so to the artificial *d*-variety which is foreign to the body. We must therefore imagine that the ultimate structure affected by the hormone is itself asymmetric. We are reminded of the action of the enzymes maltase and emulsin which hydrolyze respectively α- and β-glucosides, which led Emil Fischer to his famous simile of the lock and key, or better, of the hand and glove. An asymmetric substrate requires the appropriate enzyme just as a glove fits only the appropriate hand. The analogy is not quite strict, however, because the two glucosides are not mirror images. A closer analogy is perhaps provided by the fact

[1] Japanese Patent 42531, *see* J. Chem Soc., 126, i-284 (1924).

[2] J. Physiol., 37, 130 (1908); 38, 259 (1909).

that (a presumably asymmetric) lipase hydrolyzes one variety of a racemic optically-active ester more rapidly than the other.

In the case of the adrenalines it was ultimately found by direct comparison of the *l*-variety with the pure *d*-variety (not at first available) that the former is about 12–16 times as active as the latter. Hence it is not surprising that racemic adrenaline is, weight for weight, little more than half as active as natural *l*-adrenaline, since 50 per cent of it is the feebly active *d*-variety.

The German manufacturers might perhaps have issued synthetic "suprarenin" (as they called their product) in 1:500 solution to compete with the natural product at 1:1000, but this they did not choose to do. Instead they resolved the racemic substance by means of its bitartrate, a discovery due to Flächer.[1] When the *dl*-adrenaline *d*-tartrate is heated with methyl alcohol the *d*-adrenaline *d*-tartrate dissolves, and the *l*-adrenaline *d*-tartrate remains behind. This is obtained optically pure by recrystallization from 95 per-cent methyl alcohol. In order to obtain quite pure *d*-adrenaline the crude soluble *d*-adrenaline *d*-tartrate is converted into *d*-adrenaline *l*-tartrate which is now less soluble than the accompanying *l*-adrenaline *l*-tartrate and is thus also obtained pure. Pure *d*-adrenaline is, however, of scientific interest only. In manufacture it is not wasted, but racemized by heating with hydrochloric acid. On resolution a further quantity of the *l*-variety is obtained and by repeating this process, in the end, nearly all the material is obtained in the desirable *l*-form. This is the first and as far as I know, the only case of a resolution of a racemic compound on the industrial scale. It enables the manufacturers to claim complete identity, both chemical and physiological, of their product with the natural drug.

Adrenaline is a case in which the natural and the synthetic product have both been in use for a number of years. The small yield obtainable from the gland is amply outweighed by the difficulties of the synthesis, so that the natural product has not been driven off the market. A

[1] Z. physiol. Chem., 58, 581 (1908).

synthetic product can only obtain the monopoly under favorable conditions. This was so with alizarin; its synthesis from anthracene is a simple one and the natural product soon became extinct or nearly so. The same applies to vanillin. The technical production of artificial indigo was much more difficult and it was some decades after the substance was first synthesized in the laboratory before indigo planters began to feel the competition of the dye works; in the end the dye works triumphed. On the other hand the attempts at the industrial preparation of rubber, seriously undertaken in Germany during the war, have since been abandoned; here the natural product holds the field. The synthesis of glucose, possible in the laboratory, is very far from being attempted in chemical works.

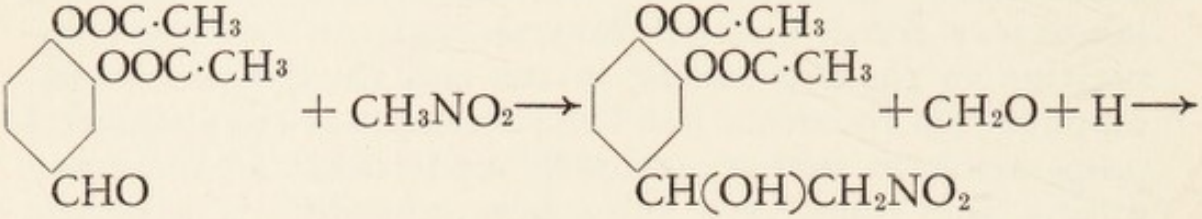
It would seem that at present *l*-adrenaline can be produced more cheaply from the products of the highly organized Chicago packing houses, than by synthesis in chemical works. The process of extraction is a simple one and the preparation of the hormone is a student's exercise in several American medical schools. Since its discovery several tons of adrenaline have been produced in the United States. The yield is about 0.21 (even up to 0.24) per cent of the weight of the fresh beeves glands employed, or nearly 1.5 per cent of their dry weight, which according to Reid Hunt[1] is the amount actually present as determined by blood pressure experiments. Hence the yield in manufacture approximates to the theoretical. A pair of fresh beeves glands weighs 20–25 grams, so that a ton of adrenaline is the product from something like 20 million animals. Several millions of glands are used annually in manufacture. Besides the original synthesis due to Stolz, a number of other processes have figured in the patent literature.[2] A more recent one is due to Nagai.[3] Diacetylprotocatechuic aldehyde is condensed with nitromethane and the crystalline condensation product is reduced with zinc dust and acetic acid in the presence of

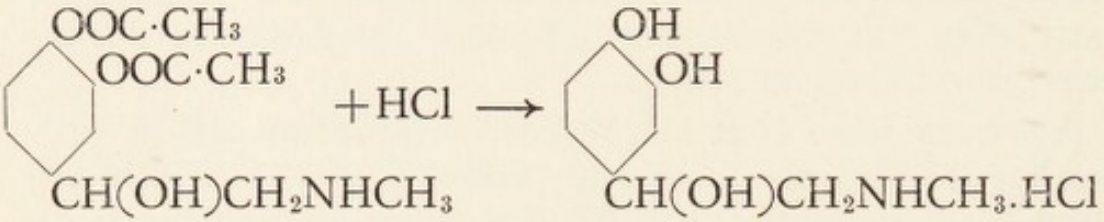
[1] J. Am. Med. Assoc., 47, 790 (1906).

[2] Barger, *The Simpler Natural Bases*, p. 86 (London, 1914).

[3] Japanese Pat. Nos. 32440 and 32441. J. Chem. Soc., 118, i-43 (1920).

an equivalent amount of 35-per-cent formaldehyde. After removal of the zinc, enough hydrochloric acid is added to hydrolyze the acetyl groups and form a salt of the base, and the solution is evaporated *in vacuo* at a low temperature, when adrenaline hydrochloride is said to crystallize out.





Of late years, probably owing to the expiration of the original German patent of 1903, a number of synthetic adrenaline preparations have appeared on the market, which are either racemic or incompletely resolved and therefore have a low activity. The necessity of their control by physiological means (or, in the case of chemically pure preparations, by polarimetry) has been pointed out by Tiffeneau.[1] The physiological assay, universally applicable, is best carried out by the blood pressure method[2] and the error need not exceed 5 per cent.

Colorimetry of Adrenaline. A large number of (more rapid) colorimetric assay methods have been described, some of which may be employed with advantage in certain cases. They are of three types:

A. The green coloration with ferric chloride, due to the catechol nucleus. It is greatly influenced by the hydrogen ion concentration and is, according to Elliott, quite unsuitable. Nevertheless Schmallfuss, Spitzer and Brandes[3] have recently shown that equimolecular amounts of vari-

[1] J. pharm. chim., **23**, 313, 366 (1921).
[2] Elliott, J. Physiol., **46**, xv, (1913), Proc. Physiol. Soc.
[3] Biochem. Z., **189**, 226 (1927).

ous catechol derivatives give the same intensity of (purple) coloration in slightly alkaline solution, in the presence of excess of ferric chloride.

B. The red coloration produced from adrenaline by the action of a variety of oxidizing agents, such as iodine, iodic acid, mercuric chloride, persulphates, potassium ferricyanide, brown oxides of manganese, sodium nitroprusside and ammonia, bleaching powder, chlorine, bromine, ammoniacal silver solutions, osmic acid.[1] Most of these reagents have only been used by a single worker, or only for qualitative purposes. The one most frequently employed is iodic acid, suggested by Fränkel and Allers[2] and by Krauss[3] and recommended for quantitative use by Hale and Seidell.[4] It has also been used more recently by Scoville,[5] for the colorimetric estimation of adrenaline. The reaction is quite delicate; for instance, the absolute amount of adrenaline in Scoville's method is only about 0.5 mg.

More recently mercuric chloride has been employed by Bailly,[6] and Johannessohn[7] has employed a modification of the iodic acid reaction, due to Bayer,[8] which consists in adding sulphanilic acid, and increases the limit of sensitiveness from about 1:300,000 to 1:5,000,000 making the reaction however less specific.

C. A third type of color reaction depends on the reduction of tungstic acid to lower, deep blue, oxides of tungsten. In contradistinction to type B the color here arises from the reagent, not from oxidation products of adrenaline. The phosphotungstic reagent was first introduced by Folin and Denis for the estimation of uric acid, and then found by Folin, Cannon and Denis[9] to give a more delicate reaction with polyphenols generally and adrenaline in particular, the limit for the latter substance

[1] See Barger, *op. cit.*, p. 89-91.
[2] Biochem. Z., **18**, 40 (1909).
[3] Biochem. Z., **22**, 131 (1909).
[4] Am. J. Pharm., **83**, 551 (1911).
[5] J. Ind. Eng. Chem., **12**, 769 (1920).
[6] J. pharm. chim., **30**, 404 (1924).
[7] Biochem. Z., **76**, 377 (1916).
[8] Biochem. Z., **20**, 178 (1909).
[9] J. Biol. Chem., **13**, 477 (1913).

being 1:3,000,000; about 0.003 mg. adrenaline can be detected. Since its introduction it has become the favorite reagent for chemical assay, partly because it was shown at the outset to agree substantially with the blood pressure method of assay carried out by Cannon; the differences between the two methods was never greater than 7 per cent. Elliott[1] confirmed this agreement and Johannessohn[2] found the colorimetric method to agree with that of Läwen-Trendelenburg, depending on vasoconstriction. The first criticism came from Lewis[3] who found much more adrenaline in human foetal adrenals than the biological method revealed; he considered this due to uric acid which, as has been pointed out, also reduces the reagent, and he warned against the use of the method in gout. Maiweg[4] decomposed adrenaline solutions partially, by passing a current of air through them, and found a considerable discrepancy. Maiweg's figures may be quoted, because they also show the instability of adrenaline at pH 7.35 and 38°.

Average values after	0	1	2	3	4	hours
Folin, Cannon and Denis	100	75	55	33.5	25	per cent
Blood pressure	100	55	27.5	12.5	6	per cent

Thus, at body temperature, with a current of air passing through the neutral solution, the half-life period of adrenaline is about 1 hour, but the adrenaline so inactivated continues to reduce Folin, Cannon and Denis' reagent to some extent. Evidently partially decomposed solutions cannot be estimated colorimetrically. Something of the kind was indeed already observed by Moore and Vincent[5] when they found that adrenal glands kept for 7–10 days in alcohol, still gave the reaction with ferric chloride, but contained no pressor substance. Maiweg points out, as was first done by Borberg,[6] that *l*- and *d*-

[1] J. Physiol., 46, xv (1913), Proc. Physiol. Soc.
[2] Biochem. Z., 76, 377 (1916).
[3] J. Biol. Chem., 24, 477 (1916).
[4] Biochem. Z., 134, 292 (1922).
[5] Proc. Roy. Soc. London, B62, 280 (1898).
[6] Skand. Arch. Physiol., 27, 341 (1912).

adrenaline are colorimetrically identical, which one would indeed expect. Hence colorimetry cannot be applied to pure synthetic preparations (unless checked by polarimetry).

Another source of error may be the presence in the gland of a precursor of adrenaline; there is some indication that such a precursor may be present since adrenaline, poured out into the circulation after (electrical or chemical) stimulation, is soon replaced by a futher supply. Such a precursor might well be a catechol derivative, and thus also reduce the reagent. It is to this that Frowein[1] attributes the high values he found by the colorimetric method, as shown in the following table, giving the content of one adrenal gland in milligrams, as found by the two methods:

		Folin, Cannon and Denis	Blood Pressure
Cattle	1	105	83
"	2	42	22
"	3	120	57
Hog		118	89
Rabbit	1	7–8.5	4.6
"	2	20–25	6.4

Frowein concludes: "Es kann hiernach kein Zweifel sein dass das Folin'sche Verfahren vollkommen unzuverlässige Ergebnisse liefert." This criticism is altogether too sweeping; Frowein's values moreover show great variation for the same species. Beznák[2] in a paper not directly concerned with assay, obtained incidentally a much better agreement between the method of Folin, Cannon and Denis and that of Läwen-Trendelenburg. It would, however, appear that the colorimetric method is only applicable to pure solutions of natural adrenaline, and with a rather larger error to fresh glands or to glands dried when fresh. The ratio of the color intensity with uric acid to that with adrenaline was given by Folin, Cannon and Denis as 1:2.9–2.95, by Johannessohn[3] as 1:2.98, by Auten-

[1] Biochem. Z., **134**, 559 (1922).
[2] Biochem. Z., **141**, 8 (1923).
[3] Biochem. Z., **76**, 377 (1916).

rieth and Quantmeyer[1] as 1:3.3. A knowledge of this ratio is only required when pure uric acid, instead of pure adrenaline is used as a standard. The blue color is not permanent, so that Autenrieth and Quantmeyer, as well as Kodama[2] have suggested permanent color standards. That of the latter author seems preferable and consists of copper sulphate with some water blue and nigrosine. Copper sulphate alone does not give an exact match. Details for preparing the uric acid and reagent have been suggested by Benedict and Hitchcock.[3]

Johannessohn[4] finds the Folin method inapplicable to adrenaline solutions containing cocaine or procaine and uses the Fränkel-Allers-Bayer method in such cases. He examined the stability of commercial preparations on keeping. After $3\frac{1}{2}$ years originally good preparations had lost half their activity, after $4\frac{3}{4}$ years only one third of the activity remained. The rapid decay due to a current of air, investigated by Maiweg, has already been referred to; this was at pH 7.35. Yet at pH 4 air can be passed through adrenaline solutions for hours without appreciable loss. Hence the importance of a small amount of excess acid in adrenaline solutions to prevent oxidation. Sulphurous acid and sulphites have a similar protective effect, but light and ultra-violet rays hasten decomposition (Vacek[5]), as do traces of iron.

Synthetic Derivatives. The number of crystalline derivatives of adrenaline is extraordinarily small, particularly of the optically active varieties, where the chief salt is a crystalline bitartrate. Racemic adrenaline yields a crystalline hydrochloride and oxalate. Heated with 1–4 equivalents of hydrogen chloride in methyl or ethyl alcohol, racemic adrenaline, according to Funk and Freedmann,[6] yields the crystalline hydrochlorides of its methyl and ethyl ether, *e.g.* $(OH)_2 \cdot C_6H_3 \cdot CH(OC_2H_5) \cdot CH_2 \cdot NHCH_3$ and also an anhydride formed by the loss of a molecule

[1] Münch. med. Wochenschr., **68**, 1007 (1920).
[2] J. Biochem. Japan, **1**, 280 (1922).
[3] J. Biol. Chem., **20**, 619 (1915).
[4] Biochem. Z., **76**, 377 (1916).
[5] Biochem. J., **21**, 457 (1927).
[6] J. Am. Chem. Soc., **45**, 1792 (1923).

of water from the alcoholic hydroxyl groups of two molecules (diadrenaline ether hydrochloride).

The Precursor of Adrenaline. Since the amount of the active principle in the glands is so small (50 mg. in a pair of beeves glands, not much more than 10 mg. in the two human suprarenals) it will probably be difficult to isolate a precursor. Possibly this might be attempted more successfully in *Bufo agua*, a Central American toad, where Abel and Macht[1] found as much as 5 per cent of the dried venom secreted by the parotid gland to consist of adrenaline, identical in every respect with that in the adrenal gland of mammals. The biological significance of this large adrenaline content is quite obscure; *Bufo agua* is not immune to its own poison, and reacts like a frog to minute doses of injected adrenaline.

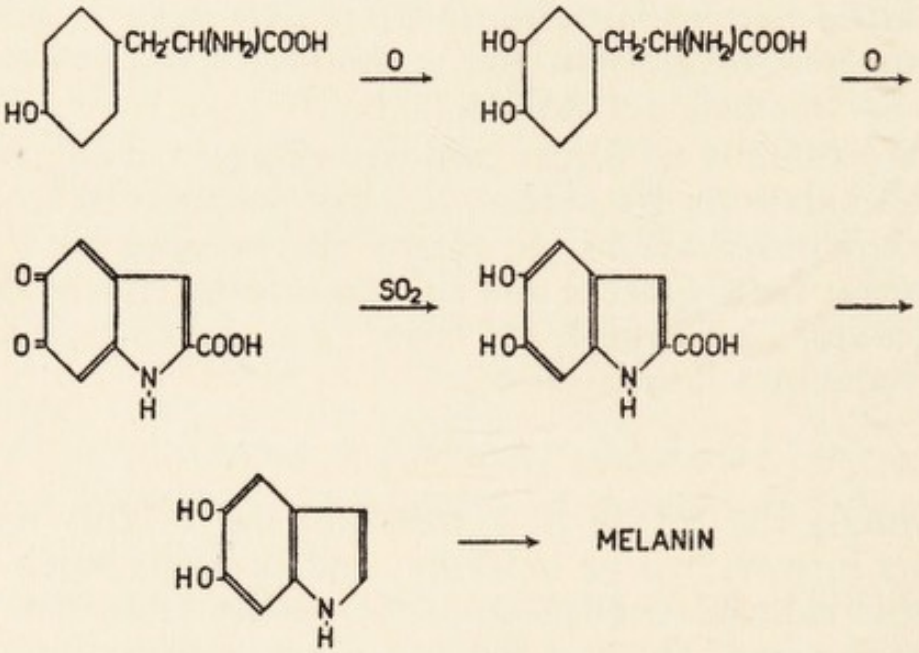
Since there is no direct evidence of a precursor, we can only speculate on its nature, and are encouraged to do so by recent observations. In plants the benzene ring probably arises from glucose and this transformation would be particularly intelligible in the case of the abundant phloroglucinol derivatives:

$$C_6H_{12}O_6 = C_6H_6O_3 + 3\ H_2O.$$

In animals the origin of a benzene ring in this manner appears however to be unlikely, and it seems much more probable that adrenaline somehow arises from the tyrosine of proteins. The first step in the transformation would almost certainly be the introduction into tyrosine of a second phenolic hydroxyl. For this there is considerable indirect evidence. In the first place the substance resulting from such a transformation, 3,4-dihydroxyphenylalanine has been isolated from bean pods (*Vicia faba*) by Guggenheim.[2] It is this substance which, when oxidized, makes the pods black. Later this amino acid was found in the cocoon of a moth (*Samia cecropia*) by Przibram and Schmallfuss[3] and in the elytra (hard anterior wings) of the June bug (*Melolontha vulgaris*) by Schmallfuss and

[1] J. Pharmacol., 3, 319 (1912).
[2] Z. physiol. Chem., 88, 276 (1913).
[3] Biochem Z., 187, 467 (1927).

Mueller.[1] It is genetically connected with tyrosine, for Raper[2] actually isolated it in the pure condition as an intermediate product in the conversion of tyrosine to melanin by the oxidase of the meal worm (*Tenebrio molitor*). The 3,4-dihydroxyphenylalanine undergoes further oxidation to a red substance, possibly the *o*-quinone of indolecarboxylic acid, from which, by reduction with sulphur dioxide, Raper obtained dihydroxyindolecarboxylic acid (isolated as the dimethyl ether). This acid is decarboxylated and the resulting dihydroxyindole then condenses to a black pigment, melanin.



Dihydroxyphenylalanine has not been isolated from the higher animals, but there is reason to assume that it is present in small quantities, for Bloch[3] has demonstrated an enzyme in the skin ("dopase") which converts 3,4-dihydroxyphenylalanine ("dopa") into melanin. This enzyme is extraordinarily specific and does not act on tyrosine, adrenaline, tryptophane, hydroquinone, homogentisic acid, pyrogallol, caffeic acid, 3,4-dihydroxyphenylglycine, 3-methoxy-4-hydroxyphenylglycine, 3-methoxy-

[1] Biochem. Z., **183**, 362 (1927).

[2] Biochem. J., **20**, 735 (1926); **21**, 89 (1927).

[3] Z. physiol. Chem., **98**, 226 (1916-17); Bloch and Schaaf, Biochem. Z., **162**, 181 (1925).

4-hydroxyphenylalanine, 3,4,5-trihydroxyphenylalanine and glycyl-3,4-dihydroxyphenylalanine. The effect of the enzyme is apparently restricted to "dopa" and small quantities of this amino acid can be estimated in the presence of tyrosine by melanin-colorimetry according to Schmallfuss and Lindemann.[1] Bloch and his collaborators[2] have suggested a relationship between the enzyme dopase and the pigmentation in Addison's disease; they suppose that a precursor of adrenaline, no longer used up by the diseased adrenal gland, now becomes oxidized to a pigment by the ferment of the skin. Whether this parent substance of adrenaline is dihydroxyphenylalanine itself, or a related substance, is not clear; but an interesting additional argument for a genetic relationship between these two substances was supplied by Chikano[3] and by Hirai and Gondo,[4] according to whom 3,4-dihydroxyphenylalanine when injected, raises the sugar of the blood (from the normal of about 0.10 to as high as 0.34 per cent), while the isomeric 2,4- and 3,5-derivatives do not do this. It is well known that adrenaline on injection produces such a rise of blood sugar (hyperglycaemia) and it may be that the injected dihydroxyphenylalanine is converted into adrenaline.

A very different and complicated series of reactions, involving the formation of 3,4-dihydroxyphenylserine as an intermediate, has been suggested by Rosenmund and Dornsaft[5] as the path of adrenaline synthesis in the body. Knoop[6] has vigorously criticized these speculations, as being wholly opposed to what we know about intermediate metabolism in the animal body.

THYROXINE

The thyroid is a much more obvious organ than the adrenal gland. In 1882 Kocher recognized that the operative removal of the thyroid in goiter produced symptoms similar to those of myxoedema which was attributed to thyroid deficiency. This suggested the trans-

[1] Biochem. Z., **184**, 10 (1927).
[2] Deut. Arch. klin. Med., **121**, 262 (1917); Biochem. Z., **162**, 181 (1925).
[3] Mitt. med. Ges. Osaka, **25**, 6 (1922) (quoted according to Hirai and Gondo).
[4] Biochem. Z., **92**, 189 (1927).
[5] Ber., **52**, 1734 (1919); **53**, 317 (1920).
[6] Ber., **52**, 2266 (1919); **53**, 716 (1920).

plantation of normal thyroid tissue into the myxoedematous patient; success in these experiments led Murray[1] to inject an extract and soon afterwards he found oral administration of the gland to be quite as effective in myxoedema and also in cretinism, a permanent infantile condition due to defective development of the thyroid. Thus the first great triumph of organotherapy was achieved, and it became evident that the thyroid gland contains an active principle. The favorable effect of iodine therapy in thyroid disease led Kocher to suspect that this active principle was associated with iodine, and when Baumann[2] showed that iodine is a constituent of the normal thyroid gland, a stimulus to chemical research was given. By boiling the gland with 10 per cent sulphuric acid, Baumann obtained an amorphous brown substance, containing from 4 to 10 per cent of iodine and possessing the physiological properties of the thyroid gland to a limited extent. We may now regard it as a very impure and partly decomposed thyroxine. Oswald[3] later separated from the gland a protein, thyreoglobulin, of small variable iodine content (0.5–1.0%) and possessing the physiological activity of the thyroid gland. This substance can hardly be regarded as pure and since its physiological effect is not abolished by the digestive enzymes of the alimentary canal, the search for an active fission product was indicated. Tryptic digestion of thyroglobulin *in vitro* splits off a large part of the iodine in the ionic condition. We do not know whether this also happens in the alimentary canal or if not, why not. Peptic digestion precipitates a substance with something like 5 per cent of iodine, by means of which Hutchison[4] unsuccessfully attempted to obtain a pure active principle. Success was first achieved by E. C. Kendall at the Mayo Clinic by substituting a moderate alkaline hydrolysis of the gland for the boiling acid employed by Baumann. Kendall conducted the hydrolysis in steps, first with 5 per cent sodium hydroxide, then with baryta; he utilized the solubility of thyroxine in alkaline

[1] Brit. Med. J., 1891-ii, 796.
[2] Z. physiol. Chem., 21, 319 (1895-1896).
[3] Z. physiol. Chem., 27, 14 (1899); 32, 121 (1901).
[4] J. Physiol., 20, 474 (1896); 23, 178 (1898-1899).

solution and its insolubility in acids. He was guided by iodine estimations on the crude products at various stages, and had the advantage of a satisfactory analytical method which he had previously worked out.[1] In this way he obtained a pure crystalline substance, containing 65 per cent of iodine which he named "thyroxine"[2] (contracted from thyroid oxindole; he erroneously considered it to be an oxindole derivative). It was found to have a very great physiological activity of the same kind as thyroid gland substance. Thus in adult patients suffering from thyroid deficiency (myxoedema) it raised the basal metabolic rate by 2–3 per cent per milligram injected. This rate indicates the intensity of chemical action (combustion) in a patient whose organs are doing as little work as possible, *i.e.* in a patient lying in bed. It is ascertained by measuring the output of carbon dioxide from the lungs. Thyroxine stimulates the combustion processes in the body; hence it may be used in obesity to bring about the combustion of superfluous fat. Kendall's thyroxine also stimulates development to an extraordinary degree. Thus, in minute concentration it hastens the metamorphosis of tadpoles, and 20–30 milligrams stimulate the growth and development of children with deficient thyroid (cretins) to a remarkable extent. There is no doubt that, at least when given intravenously, thyroxine can replace thyroid gland.

The yield of thyroxine in Kendall's experiments was minute. He obtained 33 grams from 3 tons of fresh gland, representing about 600 kilos of desiccated gland. The iodine content of this would hardly be less than 0.2 per cent so that the 3 tons of fresh gland contained probably something like 1200 grams of iodine. As 33 grams of thyroxine contain about 22 grams of iodine, Kendall obtained less than 2 per cent of the iodine in the form of thyroxine. The substance was placed on the market by E. R. Squibb and Sons of New Brunswick, N. J., but at a price which precluded its use in ordinary medical practice, and made its purchase for chemical investigation quite prohibitive.

[1] J. Biol. Chem., **19**, 251 (1914).

[2] J. Biol. Chem., **20**, 501 (1915); **39**, 125 (1919).

Kendall proceeded to investigate the chemical constitution of his product. He assigned to it the composition $C_{11}H_{10}O_3NI_3$ and also a constitutional formula. In 1919 he wrote: "The empirical and structural formulas were determined during the summer of 1917. In December 1917 Mr. Osterberg succeeded in synthesizing a small amount of thyroxine. The synthesis was repeated and the structural formula confirmed in April 1919." No proof of the constitution was ever given (except the above claim of synthesis, which was subsequently withdrawn) and to organic chemists Kendall's constitutional formula appeared inherently improbable, nor was it clear how the position assigned to the iodine atoms could be determined by existing methods. The interest in the problem and the impossibility of obtaining sufficient natural material nevertheless stimulated various synthetic experiments. Harington[1] prepared 3,4,5-triiodophenylpyrrolidonecarboxylic acid, with two hydrogen atoms less than Kendall's supposed formula; it was inactive. Kalb, Schweizer, Zellner and Berthold[2] attempted the synthesis of a compound similar to that assumed by Kendall, and at least one German chemical works was likewise led to futile synthetic experiments. Hicks[3] determined the absorption spectrum of thyroxine and concluded that it was consistent with the presence of an indole nucleus, as required by Kendall's formula. Abderhalden and Haas[4] used the same method and came to the opposite conclusion. The futility of all this work became clear when it was found that thyroxine has a quite different molecular formula with four carbon atoms, one hydrogen atom, one oxygen atom and one iodine atom more than Kendall supposed; that it is not a tryptophane or indole, but a tyrosine derivative; and that Kendall's constitutional formula and his alleged synthesis were entirely erroneous.

The only way to solve the problem at a moderate cost seemed to be to improve Kendall's method of preparing natural thyroxine so that this might become sufficiently

[1] J. Biol. Chem., **64**, 29 (1925).
[2] Ber., **59B**, 1860 (1926).
[3] J. Chem. Soc., **127**, 771 (1925).
[4] Z. physiol. Chem., **166**, 82 (1927).

cheap. This Harington undertook,[1] after his synthetic "shot in the dark" mentioned above. He had the advantage of a supply of dried gland containing as much as 0.55 per cent of iodine. This can now be obtained from the Liebig's Extract of Meat Company, London, who presumably get it from their South American estates. Harington had further the advantage of financial resources adequate to his comparatively slight requirements. It is a pleasure to record here that these were of American origin, for the laboratory at University College Hospital Medical School, London, in which he worked, had benefited by an endowment from the Rockefeller Foundation. Harington turned his advantages to good account; he greatly improved Kendall's method of isolation, and thus obtained enough material to determine the true constitution of thyroxine, which in its turn enabled him to synthesize it.

Harington obtained from dried thyroid a yield of 0.12–0.13 which represents about 0.027 per cent of the fresh gland, as compared with 0.0011 per cent obtained by Kendall, *i.e.* about 25 times as much. Yet Harington's yield corresponds to only 14 per cent of the iodine present. How much of the total iodine, not isolated by Harington as thyroxine, is originally present as such and is split off during the isolation process, is uncertain. From Kendall's and Harington's work it seems not improbable that iodine-containing substances, other than thyroxine, may be present in the gland. Without going into details it may be said that the essence of Harington's method is the use of baryta for hydrolysis with glands, first in 10 per-cent solution, later in 40-per-cent solution. It was by baryta hydrolysis that Drechsel had already long before obtained iodogorgonic acid from corals; this acid was later identified as 3,5-diiodotyrosine and is related to thyroxine, as it ultimately turned out. With glands sufficiently rich in iodine a considerable proportion of thyroxine separates after the first hydrolysis as the crude barium salt. With thyroxine thus made available, Harington proceeded to determine the molecular formula.[2]

[1] Biochem. J., **20**, 293 (1926).

[2] Biochem. J., **20**, 300 (1926).

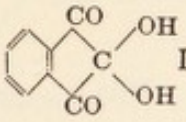
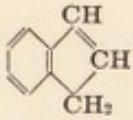
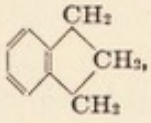
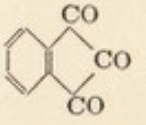
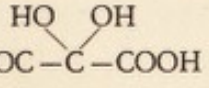
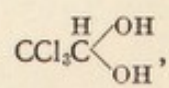
Constitution of Thyroxine. Since 65 per cent of the molecule is iodine, the exact determination of the number of the other atoms, together constituting only 35 per cent, is somewhat difficult. A method was therefore required by which the iodine atoms could be replaced by hydrogen without changing the rest of the molecular structure. By breaking down this iodine-free compound, desiodothyroxine, one might hope to obtain recognizable fragments, which could hardly be expected as long as the iodine was present, since comparatively few organic iodine compounds are known. Harington found an ideal method for this purpose; he shook an alkaline solution of thyroxine with colloidal palladium in a hydrogen atmosphere. This removes the iodine quantitatively as iodide, and by measuring the hydrogen used up, it was found to correspond exactly to the iodine split off, so that no extra addition of hydrogen had taken place. The desiodothyroxine was found to have the composition $C_{15}H_{15}O_4N$. The analysis could be at once interpreted with certainty, which was not the case as long as two-thirds of the molecule consisted of iodine. The solubility was much greater than that of thyroxine, so that the molecular weight could be determined. It followed at once, moreover, that thyroxine has four atoms of iodine, instead of three ascribed to it by Kendall. The formula is actually $C_{15}H_{11}O_4NI_4$ and not $C_{11}H_{10}O_3NI_3$. These two formulas differ chiefly in their nitrogen percentage (1.8 instead of 2.4%) but the difference is not much in excess of experimental error. For desiodothyroxine the difference in the nitrogen content of the two formulas is of course three times as large (1.7%, difference of 5.1% and 6.8%).

$C_{11}H_{10}O_3NI_3$ has C = 22.6 H = 1.7 N = 2.4 I = 65.1%

$C_{15}H_{11}O_4NI_4$ has C = 23.2 H = 1.4 N = 1.8 I = 65.4%

The next step was the determination of the constitution of the substance $C_{15}H_{15}O_4N$. On treatment with nitrous acid, by Van Slyke's method, the whole of the nitrogen was eliminated; it was therefore present as a primary amino group, and the formula may be written $C_{15}H_{13}O_4 \cdot NH_2$. Van Slyke's method consists in shaking

the substance under investigation with nitrous acid which, as is well known, reacts differently with different kinds of amines. In primary amines and amides the amino group NH_2 reacts with nitrous acid to give nitrogen, the volume of which is measured in a special apparatus. Secondary amines and amides react differently without the production of nitrogen. Hence in a protein it is possible to distinguish between the free amino groups NH_2 and the bound imino groups NH which as NH·CO form the link between two amino acids. Thus tryptophane gives up only half of its nitrogen in the Van Slyke apparatus, histidine one-third. Desiodothyroxine gave an intense violet coloration on boiling with triketohydrindene hydrate,[1] the so-called ninhydrin reaction. This is characteristic of α-amino acids, which would suggest a further resolved formula $C_{13}H_{11}O_2 \cdot CH(NH_2) \cdot COOH$. Finally the substance gave the Millon reaction characteristic of phenols like tyrosine. Millon's reagent is a mixture of mercuric and mercurous nitrate, and gives a brick red precipitate on boiling with proteins. It was first used as test for protein, but it is in reality only a test for tyrosine. Many phenols give a similar coloration or precipitate. This leads to resolution into $HO \cdot C_6H_4 \cdot C_7H_6O \cdot CH(NH_2)COOH$. There was at least one benzene nucleus, and considering the composition of the residue (C_7H_6O) a second such nucleus seemed very likely.

[1]Triketohydrindene hydrate has the formula  Indene is a hydrocarbon of coal tar  and may be regarded as indole in which the NH group is replaced by CH_2. Hydrindene is therefore  , triketohydrindene is  . It forms a hydrate in which the carbon atom situated between two (acidic) CO groups is exceptionally capable of holding two hydroxyls. This hydrate formation occurs also in mesoxalic acid  and in chloral hydrate  , where the chlorines are sufficiently acidic. Triketohydrindene hydrate, on boiling with α-amino acids, peptides and proteins, gives an intense violet coloration.

The next step was to break down the amino acid side chain by known stages. For this purpose desiodothyroxine was fully methylated by heating with methyl iodide in alkaline alcoholic solution. A substance was obtained containing 4 more carbon atoms and 8 more hydrogens. This meant that four methyl groups had entered, three would be on the nitrogen and one on the phenolic group, so that no methyl had entered the group C_7H_6O; its oxygen atom therefore could not be phenolic. There resulted a compound $MeO.C_6H_4.C_7H_6O.CHNMe_3$ a so-called betaine.

$$\begin{array}{c} \\ \\ \text{CO}-\text{O} \end{array}$$

The simplest example of this class is obtained by methylating glycine; it is betaine *par excellence*. Betaine is so called because it occurs in the sugar beet, *Beta vulgaris*. It crystallizes with a molecule of water and may then be regarded as a quaternary ammonium hydroxide and at the same time an acid. On heating it loses the molecule of water forming an internal cyclic salt. The nitrogen of the amino acid glycine has become quinquevalent. Now many betaines on heating with concentrated potassium hydroxide lose their nitrogen as trimethylamine with the production of an unsaturated acid. Thus fully methylated alanine, or propiobetaine $CH_3.CH\begin{array}{l}\diagup\text{NMe}_3\\ \\ \diagdown\text{CO}\end{array}\!\!>\!\text{O}$ yields acrylic acid and trimethylamine. The compound from thyroxine behaved in similar fashion: $MeO.C_6H_4.C_7H_6O.CH.N{:}Me_3$ =

$$\begin{array}{c} \\ \\ \text{CO}\cdot\text{O} \end{array}$$

$MeO.C_6H_4.C_7H_5O{:}CH.COOH + NMe_3$. The group C_7H_5O evidently consists of a benzene nucleus plus one other carbon atom, and at first it was thought that this carbon atom was placed between the two benzene nuclei. On oxidation of the above unsaturated acid a substance $MeO.C_6H_4.C_7H_5O_2$ resulted, which was at first considered to be a ketone $MeO.C_6H_4.CO.C_6H_5O$. It was converted

into an oxime, which on heating with phosphorus pentachloride was expected to undergo the Beckmann transformation into an anilide.

The Beckmann transformation may be illustrated by the case of benzophenone $C_6H_5{\cdot}CO{\cdot}C_6H_5$. The oxime of this ketone $\mathrm{C_6H_5\underset{\underset{NOH}{\|}}{C}{\cdot}C_6H_5}$ on heating with phosphorus pentachloride or other reagents undergoes a peculiar molecular re-arrangement to $C_6H_5NH{\cdot}COC_6H_5$, a so-called anilide, in this case the anilide of benzoic acid, which by heating with acid is hydrolyzed to aniline and benzoic acid. In the present case something like the following reaction might have occurred:

$$\mathrm{MeO{\cdot}C_6H_4{\cdot}\underset{\underset{NOH}{\|}}{C}{\cdot}C_6H_4OH \longrightarrow MeO{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}C_6H_4OH}$$

and the resulting substance should have been hydrolyzable to two known substances. Instead, the resulting supposed anilide yielded only a single substance on hydrolysis, an acid still containing all the carbon atoms of the supposed ketone. The explanation was simple: the ketone was in reality an aldehyde; its oxime had merely been dehydrated to the nitrile, and this had been hydrolyzed to the acid $MeO{\cdot}C_6H_4{\cdot}C_6H_4O{\cdot}COOH$.

$$\mathrm{R{\cdot}\overset{H}{C}{:}O \longrightarrow R{\cdot}\overset{H}{C}{:}NOH \longrightarrow R{\cdot}C{\vdots}N \longrightarrow R{\cdot}COOH}$$

This acid was indeed next obtained directly by more energetic oxidation of the unsaturated acid; the latter may therefore be written $MeO{\cdot}C_6H_4{\cdot}(C_6H_4O){\cdot}CH{:}CH{\cdot}COOH$. Thyroxine thus consists of two benzene nuclei with a side chain of three carbon atoms and it became probable that the two benzene nuclei were united by the fourth oxygen atom so that thyroxine would be a derivative of diphenyl ether, $C_6H_5{\cdot}O{\cdot}C_6H_5$. This would explain why this fourth oxygen atom was not methylated. Harington did not immediately come to this conclusion because derivatives of diphenyl ether were not known to occur in

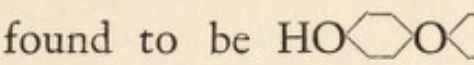
nature, and because he was not at first aware of their extraordinary stability. The conclusion was however forced on him by a different set of experiments. On gentle fusion of desiodothyroxine with potash, at the usual temperature of about 180–200°, Harington at once obtained four substances; three were obtained in small quantity only and were promptly identified as quinol (hydroquinone) $HO{\cdot}C_6H_4{\cdot}OH$, *p*-hydroxybenzoic acid $HO{\cdot}C_6H_4{\cdot}COOH$, and oxalic acid (from the side chain), but the main product was a phenol $C_{13}H_{12}O_2$ giving the Millon reaction. The separation of the four substances is quite simple. The two phenols could be shaken out from sodium carbonate solution by means of ether, the acids remaining in the aqueous layer. The two phenols differed greatly in their solubility in water, hydroquinone being very soluble, the other substance hardly at all; the two acids also differ greatly in solubility. On methylating the more complex phenol only a single methoxy group was introduced; the other oxygen atom was evidently non-phenolic. This other oxygen atom would appear to be in the second hydroxyl of quinol, as well as in the phenolic hydroxyl of *p*-hydroxybenzoic acid. The unknown phenol might thus be $HO{\cdot}C_6H_4O{\cdot}C_6H_4{\cdot}CH_3$. It would be the primary product of the potash fusion, which product only breaks down further after the methyl group has become oxidized to a carboxyl group, making the *p*-diphenyl ether less stable.

$$HO{\cdot}C_6H_4{\cdot}O{\cdot}C_6H_4{\cdot}CH_3 \longrightarrow HO{\cdot}C_6H_4{\cdot}O{\cdot}C_6H_4{\cdot}COOH \longrightarrow HO{\cdot}C_6H_4{\cdot}OH + HO{\cdot}C_6H_4{\cdot}COOH.$$

The phenol $HO{\cdot}C_6H_4O{\cdot}C_6H_4{\cdot}CH_3$ was next synthesized and found to be identical with the product from the potash fusion. It is often thus in the determination of constitution; it may be impossible to break down a natural substance entirely to known degradation products in such a way as to establish its molecular architecture beyond doubt. Generally degradation and synthesis meet at an intermediate point and the ultimate aim then becomes the synthesis of the original substance. In this particular case even the phenol need not have been synthesized, for later, when its constitution was known, it was found that it could be

oxidized to a carboxylic acid and could be split quantitatively by potash fusion at a high temperature (300°). It should be remembered, however, that when as in the present case, the natural substance is too expensive to allow of many degradation experiments whose outcome may be inconclusive, synthetic experiments are cheaper.

Perhaps I may permit myself a slight digression to illustrate this point. Friedländer[1] investigated the royal purple of the ancients. From the glands dissected out of 12,000 whelks (*Murex*) and exposed to light, he extracted enough of the pure coloring matter to analyze it, but no more. The composition and properties corresponded to that of a dibromoindigotin. To fix the position of the two bromine atoms by degradation might have been difficult; in any case the cost of material would have been prohibitive. Friedländer therefore synthesized the various possible dibromoindigotins; with the 6,6-derivative the purple of the ancients was found to be identical.

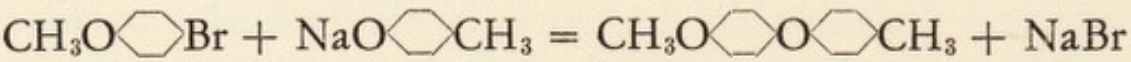
The constitution of the desiodothyroxine was thus found to be  and the next step was to confirm this by synthesis.[2]

Since the substance is an ordinary amino acid, several standard methods were available; the most promising appeared to be that of Erlenmeyer which, in its several modifications, consists essentially in converting an aldehyde group CHO into $CH_2 \cdot CH(NH_2)COOH$, an alanine residue. For instance, the naturally occurring amino acid phenylalanine can be readily made from benzaldehyde by this method; tyrosine is made from *p*-hydroxybenzaldehyde; tryptophane and histidine have also been synthesized from the corresponding aldehydes, illustrating the great utility of aldehydes for synthetic work. It was thus necessary to have $CH_3O \cdot C_6H_4O \cdot C_6H_4 \cdot CHO$. For this

[1] Ber., 42, 765 (1909).

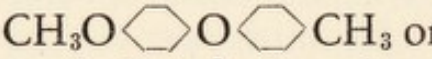
[2] It is a remarkable coincidence that the same conclusions as to the constitution of thyroxine were arrived at quite independently by Dr. H. D. Dakin by analytical reactions. Thus he obtained ordinary tyrosine by heating thyroxine with hydriodic acid. When informed privately of Harington's forthcoming publication in the Biochemical Journal, he withdrew a paper he had himself sent to the Journal of Biological Chemistry, although it would have appeared before Harington's more comprehensive account, an example of scientific generosity pleasantly contrasting with the more common claims for priority.

we must go back to the phenol $HO{\cdot}C_6H_4O{\cdot}C_6H_4{\cdot}CH_3$ already synthesized for purposes of identification. It involves the general reactions for the production of diphenyl ethers. Methyl phenyl ether or anisole, $CH_3OC_6H_5$, is of course readily made by the action of methyl iodide on sodium phenoxide, $CH_3I + NaOC_6H_5$, but such a reaction is not easily applicable to the present case because, as is well known, the halogen attached to a benzene ring is less reactive, less "mobile," than that attached to an alkyl group. However, as was found by Ullmann, the reaction proceeds at a high temperature with moderate yield when it is catalyzed by copper powder (so-called molecular copper or copper bronze). Thus the phenol in question was obtained by heating *p*-bromoanisole with the sodium salt of *p*-cresol and copper powder,



and then hydrolyzing the methoxy group by boiling hydriodic acid. In Ullman's reaction *p*-bromophenol could not be used, for the free hydroxyl group would also form a sodium salt and react in undesirable fashion; hence this group had first to be "protected."

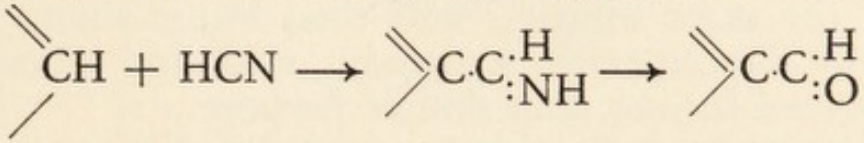
The action of boiling hydriodic acid on the compound



only breaks the ether linkage between methyl and phenyl, not between the two phenyls. Many derivatives of diphenyl ether are very resistant to hydriodic acid, even at 260°, and we have already seen that they also resist potash fusion to a large extent.

Now from the phenol ether $CH_3O{\cdot}C_6H_4{\cdot}O{\cdot}C_6H_4{\cdot}CH_3$ the required aldehyde could be prepared by oxidation, but unless special means are adopted, the oxidation proceeds too far, forming the acid, and the reduction of this to the aldehyde is troublesome. It was therefore found better to introduce the aldehyde group into the lower homologue $CH_3O{\cdot}C_6H_4{\cdot}O{\cdot}C_6H_5$ made in a quite similar manner by Ullmann's reaction. Fortunately there is a good method for introducing an aldehyde group into phenols, the method of Gattermann, and fortunately this group preferentially enters the *para* position, that is the position desired in the present case. The carbon atom of the aldehyde

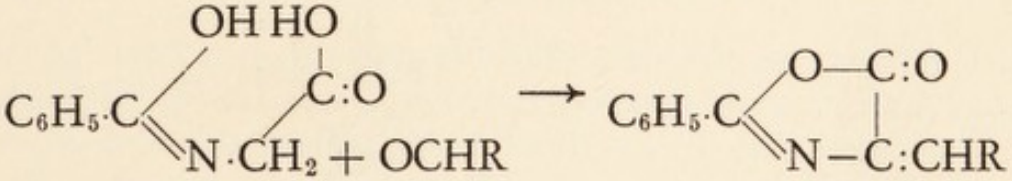
group is supplied as anhydrous hydrocyanic acid which under the influence of hydrogen chloride and a condensing agent such as zinc or aluminum chloride, forms an imino compound, which is readily hydrolyzed to the aldehyde:



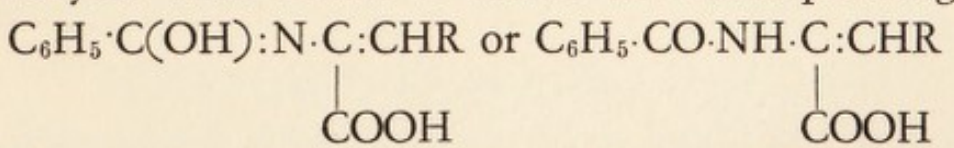
Now having obtained the required aldehyde, Erlenmeyer's synthesis was applied to it. This reaction consists in adding a residue of aminoacetic acid to the aldehyde group and bears a certain resemblance to the well-known Perkin reaction where acetic acid is joined to an aldehyde to form an unsaturated acid. Thus benzaldehyde heated with sodium acetate and acetic anhydride forms cinnamic acid:

$$C_6H_5{\cdot}CHO + H_2CH{\cdot}COOH \longrightarrow C_6H_5{\cdot}CH{:}CH{\cdot}COOH.$$

Aminoacetic acid (glycine) however, will not react thus; the basic amino group must first be protected. Erlenmeyer achieved this by using benzoylglycine or hippuric acid. When this is heated with an aldehyde and the same condensing agents as those used by Perkin, viz., acetic anhydride and sodium acetate, then condensation takes place, with the formation of a so-called azlactone. The hippuric acid may be considered to react in its tautomeric, enolic form:



The resulting compound is a cyclic, internal anhydride of a hydroxy acid, a lactone therefore, and since it also contains nitrogen, it is called an azlactone. On gentle hydrolysis with alkali it forms the corresponding acid

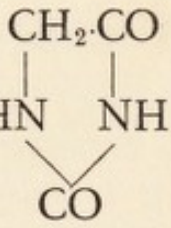


which is next reduced to the saturated acid:

$$C_6H_5.CO.NH.CH(COOH)CH_2R$$

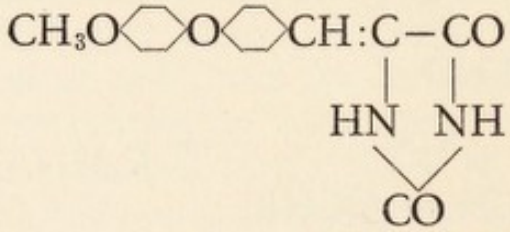
and then only the benzoyl group has to be split off to give the desired compound. Reduction and hydrolysis may be effected in one operation, *e.g.* the reducing agent may be sodium and absolute alcohol, and after adding a little water, the same solution will then bring about hydrolysis; or concentrated hydriodic acid and phosphorus may be used for the same double purpose.

Of recent years Sasaki[1] has introduced some modifications into the Erlenmeyer synthesis, which were used in the present case. Instead of protecting the amino group of glycine by benzoylation, it may be converted into a urea group by heating with potassium cyanate; the reaction is analogous to Wöhler's well-known synthesis of urea. There is then formed ureidoacetic acid or hydantoic acid, $NH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}COOH$, which loses water, forming



the anhydride, hydantoin, HN NH. This compound may

be used instead of hippuric acid in Erlenmeyer's reaction. Harington condensed it with the *p*-methoxyphenyl ether of *p*-hydroxybenzaldehyde, already referred to, and so obtained the compound given below.



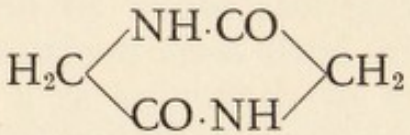
Boiling this with hydriodic acid and red phosphorus achieved a triple object: (a) the double bond is reduced, (b) the urea grouping is hydrolyzed with elimination of CO_2 and NH_3, (c) the methyl ether is hydrolyzed. Thus the amino acid

$$HO{\cdot}C_6H_4{\cdot}O{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$$

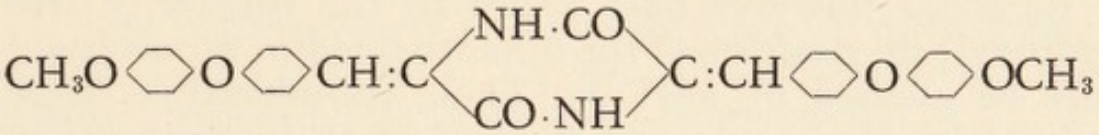
resulted; it was found to be identical with desiodothyroxine. Harington made it in yet another way. Besides hy-

[1] Ber., 54B, 163 (1921).

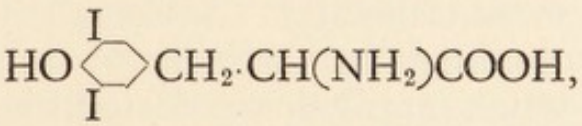
dantoin Sasaki had also used glycine anhydride as a substitute for hippuric acid. Here the amino group of glycine is protected by its own carbonyl group,



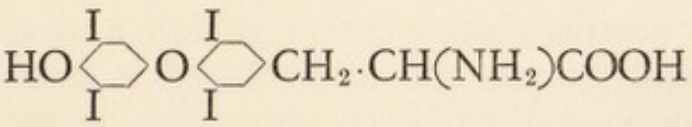
Harington condensed this to the compound



which again, on boiling with hydriodic acid and phosphorus, leads to desiodothyroxine. After the constitution of desiodothyroxine had thus been settled, it was merely necessary to ascertain the positions of the four iodine atoms in thyroxine itself. These were to some extent guessed by analogy. Desiodothyroxine would be tyrosine, $HO \cdot C_6H_4 \cdot CH_2 \cdot CH(NH_2)COOH$, in which the phenolic hydrogen atom is replaced by the group $HO.C_6H_4$, in fact it would be *p*-hydroxyphenyltyrosine. Now one of the very few natural organic iodine compounds of known constitution is 3,5-diiodotyrosine,



found by Drechsel in the skeletal protein of corals, and identified by Henze[1] as 3,5-diiodotyrosine which had been prepared from tyrosine by Wheeler and Jamieson.[2] The same iodine compound was obtained also by Wheeler and Mendel[3] by the hydrolysis of the common bath sponge. It seemed therefore plausible that thyroxine is made up of two molecules of diiodotyrosine, one molecule having lost its side chain:

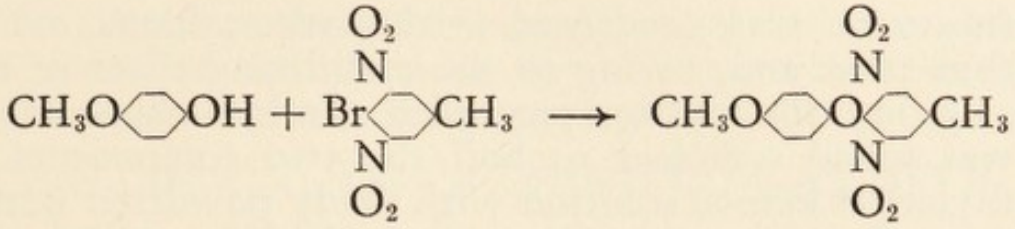


[1] Z. physiol. Chem., **51**, 64 (1907).
[2] Am. Chem. J., **33**, 369 (1905).
[3] J. Biol. Chem., **7**, 1 (1909).

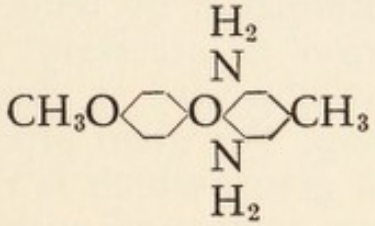
There was indeed some experimental evidence that two of the iodine atoms were both *ortho* to the phenolic hydroxyl. In the first place, a color reaction described by Kendall for thyroxine was found to be characteristic of phenols iodinated in both *ortho* positions; in the second place, potash fusion of thyroxine at a high temperature yielded traces of a substance giving reactions of pyrogallol (1,2,3-trihydroxybenzene). Evidence of this kind, slender as it is, could however only apply to the ring with free hydroxyl; the position of the other two iodine atoms could only be guessed by analogy. These considerations were utilized by Harington and Barger[1] in their synthesis of thyroxine.

Synthesis of Thyroxine. The position of all four iodine atoms was first proved by synthesis of the acid, which was found to be identical with a degradation product obtained from thyroxine in the same way as the corresponding non-iodinated acid from desiodothyroxine. Thyroxine was fully methylated to the betaine, trimethylamine was removed by boiling with alkali, and the unsaturated acid so obtained was oxidized. The synthesis of the resulting product, like that of thyroxine itself, caused some difficulty. Although diiodotyrosine can be readily obtained from tyrosine by direct iodination, this process, when applied to desiodothyroxine, only introduced about two iodine atoms and the resulting product could not be crystallized. It was therefore necessary, before carrying out an Ullmann condensation, to have the iodine atoms of the inner ring already in position, or at least some groups which could be exchanged for them. An already iodinated phenol such as diiodocresol or, what would be very useful, diiodotyrosine, could not be made to condense with *p*-bromoanisole (unlike the unsubstituted cresol which had already been used successfully). On the other hand it was found easy to condense 3,5-dinitro-4-bromotoluene with hydroquinone monomethyl ether, in the presence of pyridine:

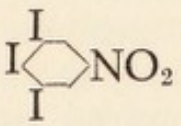
[1] Biochem. J., **21**, 169 (1927).



Here the hydroxyl group and halogen atom have changed rings, as compared with the first use of Ullmann's reaction in this investigation. The bromine has moreover become specially reactive owing to the presence of nitro groups in both ortho positions. The dinitro compound was successfully reduced to the diamino compound

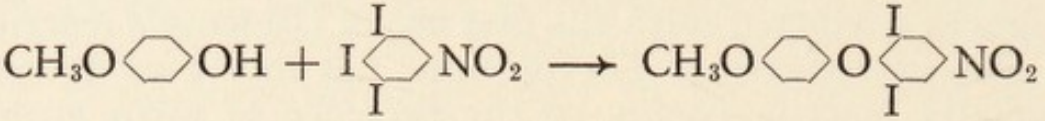


but the diazotization of this substance, previous to the introduction of iodine by the Sandmeyer reaction, caused difficulties which so far have not been overcome. As with other *meta*-diamines the partially diazotized substance condenses very readily with unchanged amine to form highly colored aminoazo dyes. The tendency may, to some extent, be overcome by working in strongly acid solution, but nevertheless this mode of attack was abandoned as unsuitable. We therefore had to fall back on compounds with the "inside" pair of iodine atoms already in position as such. The position between them could not be occupied by a hydroxyl group since this does not react. Hence a halogen atom in this position was indicated.

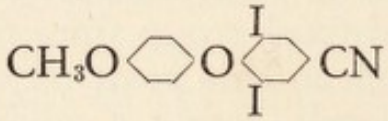
Harington ultimately found a suitable compound in 3,4,5-triiodonitrobenzene: 

Here the nitro group serves a double purpose. In the first place it mobilizes the middle iodine atom in the *para* position without affecting the two *meta*-iodines. In the second place the nitro group provides a starting point for building up the amino acid side chain. This triiodo-

nitrobenzene was condensed with hydroquinone monomethyl ether and, owing to the mobilizing effect of the nitro group, the reaction proceeded at a low temperature. It was found sufficient to boil the two components in methylethyl ketone solution with finely powdered potassium carbonate, to neutralize the hydriodic acid formed:



The nitro group must now be converted into the amino acid side chain, without removal of the iodine atoms, which are easily eliminated by reduction (we have seen that a final reduction is necessary in the Erlenmeyer synthesis). The first objective is the exchange of NO_2 for an aldehyde group. The nitro group is of course first reduced to an amino group which is then diazotized. On boiling the diazonium compound with alcohol the amino group was replaced by hydrogen and it was thought that an aldehyde group could be introduced by Gattermann's reaction with HCN, as had already been done quite easily with the corresponding non-iodinated compound. But here again disappointment awaited us. Gattermann's reaction completely failed in the present case. The difficulty was overcome in another manner. Instead of replacing a diazo group by hydrogen, it can be replaced by a cyanogen group, by means of cuprous cyanide, according to Sandmeyer. This reaction yielded the compound



The cyanide had now to be converted into the aldehyde. This was first accomplished via the corresponding acid, but as is well known, the reduction of an acid to its aldehyde is no easy matter and the yield was very poor. Fortunately a few years earlier Stephen[1] had described a method for preparing aldehydes from cyanides by reduction with anhydrous stannous chloride, and by this method the required aldehyde was obtained in better yield.

[1] J. Chem. Soc., 127, 1874 (1925).

Its condensation with hippuric acid and the partial hydrolysis of the azlactone to the unsaturated benzoylamino acid went normally, giving

$$CH_3O \cdot C_6H_4 \cdot O \cdot C_6H_2I_2 \cdot CH{:}CH(NH \cdot CO \cdot C_6H_5) \cdot COOH.$$

The reduction of this compound was a source of anxiety, since it was feared that the iodine atoms would be eliminated. Boiling concentrated hydriodic acid (with phosphorus) was chosen as reagent since it would also remove the superfluous methyl and benzoyl groups. In the early experiments the yield was only 25 per cent but this was not so much due to elimination of iodine as to the insolubility of the compound. When acetic anhydride was added (which constitutes a very useful addition, for instance, in certain determinations of methoxyl groups by Zeisel's method) the yield was trebled. We thus obtained the compound

$$HO \cdot C_6H_4 \cdot O \cdot C_6H_2I_2 \cdot CH_2 \cdot CH(NH_2)COOH,$$

which required the introduction of two more iodine atoms to convert it into thyroxine. From analogy with tyrosine and other phenols it was assumed that these iodine atoms could be readily introduced by sodium hypoiodite, but with this reagent we failed to obtain a crystalline compound. We, however, found a suitable iodination method by studying a simpler case, which incidentally first definitely fixed the position of the four iodine atoms. We had already obtained the acid $HO \cdot C_6H_4 \cdot O \cdot C_6H_2I_2 \cdot COOH$ by the hydrolysis of its nitrile, as has been indicated, and although we likewise failed to iodinate this acid further to the tetraiodo compound by means of hypoiodite, we obtained an excellent result by dissolving it in concentrated ammonia and dropping in the calculated quantity of potassium triiodide solution, a method already used by Datta and Prosad.[1] The active agent seems to be nitrogen iodide, which is formed as a black cloud on each addition of iodine, and then rapidly disappears on shaking; its persistence marks the end of the reaction. By partially neutralizing the ammonia a sufficient concentration of

[1] J. Am. Chem. Soc., 39, 441 (1917).

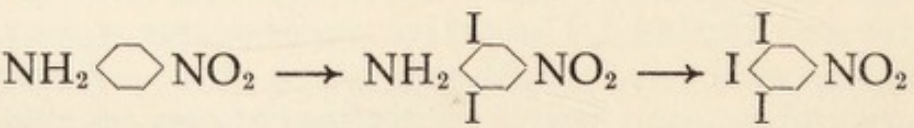
ammonium ions is then set up, which causes the ammonium salt of the desired acid to crystallize out.

The acid was next methylated and was then found to be identical with the degradation product

$$CH_3O \cdot C_6H_2I_2 \cdot O \cdot C_6H_2I_2 \cdot COOH$$

already obtained from thyroxine in the manner described. Thus the position of the iodine atoms was established. Since the melting point of the methoxy acid is very high and really a decomposition point, it was not ideal for the method of mixed melting points. We therefore made from both samples of the acid, natural and synthetic, the methyl and ethyl esters. The latter ester in particular melted low, without decomposition, and a mixture of the esters from both sources showed no lowering of the melting point whatsoever. Having learned how to introduce the two final iodine atoms, we applied the same method to the diiodo amino acid and so readily obtained synthetic thyroxine, at first only in milligrams. Its identity with the natural product was at once obvious. It had the same very slight solubility, the sodium salt showed the same characteristic crystalline form, etc. The identity was confirmed by physiological tests. Professor Murray Lyon[1] found in the Royal Infirmary at Edinburgh, that intravenous injection of the sodium salt raised the basal metabolic rate of myxoedematus patients to the same extent as natural thyroxine (about 2 or 3 per cent per milligram injected).

The synthesis outlined consists of nine successive reactions starting from triiodonitrobenzene which was a known substance. It is, however, not a commercial product and has to be made from *p*-nitroaniline, by iodinating this and then replacing the amino group by iodine according to Sandmeyer



This gives two additional reactions or eleven altogether from the commercial starting point, *p*-nitroaniline. If each

[1] Biochem. J., 21, 181 (1927).

reaction gave a 50 per-cent yield the final yield would be $(\frac{1}{2}^{11}) = 1/2048$ or $1/20$ per cent of the *p*-nitroaniline employed. It is obvious that in a case like this, close attention must be paid to the yield at each stage. As a matter of fact all the reactions involved have been made to yield well over 50 per cent, and the yield of thyroxine is something like 5 per cent of the *p*-nitroaniline used. Harington's isolation process gives a very much better yield than Kendall's and natural thyroxine now costs about $32 per gram, but the synthetic product can be made at about half that price. The process has not been patented—in accordance with medical ethics—and synthetic thyroxine is now being manufactured by several firms in Britain, Germany and Switzerland, so that it can receive an extensive clinical trial. Whether it will replace thyroid gland is very doubtful. It certainly has the advantage of accurate dosage; the activity of the gland varies considerably and does not entirely depend on its iodine content. But thyroid gland is taken by the mouth and thyroxine has so far been mostly given intravenously. Whether thyroxine will be equally effective in oral administration remains to be seen. The sodium salt is little soluble and it should not be forgotten that thyroxine as such does not occur in the thyroid gland but is combined with other amino acids in a protein, thyreoglobulin. This protein is perhaps hydrolyzed in the intestine with liberation of thyroxin and it might be worth while to make some simple peptides of thyroxine for oral administration.

According to Reid Hunt[1] thyroxine has only about two-thirds of the activity of a dose of thyroid gland containing the same amount of iodine and, according to Cameron and Carmichael[2] who used a different method of physiological assay, a given amount of iodine in the thyroid gland is 2–4 times as active as the same amount when present in thyroxine. Possibly therefore the thyroid gland contains a substance still more active than thyroxine, which latter is perhaps a fragment of the real hormone and is converted into it in the body.

[1] Am. J. Physiol., 63, 257 (1923).
[2] J. Biol. Chem., 46, 35 (1921).

The Iodine Content of the Thyroid Gland and Goiter. The iodine content of the thyroid gland varies greatly according to surroundings and food. In general the normal iodine content is 0.1–0.5 per cent of the dried gland, in man and various animals according to Marine.[1] The highest iodine content, 1.16 per cent in the thyroid of the dogfish, was recorded by Cameron,[2] the highest in land animals by Hunter and Simpson[3] in Orkney sheep living on kelp (1.05 per cent). The normal minimum is 0.1 per cent according to Marine and Lenhart.[4] With less than this amount an enlargement of the thyroid occurs which results in goiter. Hence goitrous glands contain little iodine per gram, as already found by Baumann. In some animals there is considerable seasonal variation; Seidell and Fenger[5] found from June to December about 3 times as much iodine as from December to June.

Unconsciously iodine has been used for centuries by various peoples for the treatment of goiter, and it was first so used consciously in 1820 by Coindet, a Geneva physician. J. Inglis,[6] a physician at Harrogate in England, concluded that the inhabitants of this town were free from goiter because the town water supply contained enough iodine, whereas the inhabitants of the surrounding country were subject to the disease on account of an insufficient supply of iodine in their drinking water. In the middle of the last century numerous determinations of the minute quantities of iodine in drinking water, etc., even in Parisian air, were made by Chatin and soon afterwards prophylaxis against goiter by iodized table salt was started in some Alpine districts of France and Savoy. Strangely enough, this prophylaxis was later abandoned and it was not resumed on a firm basis until sixty years later, after the late war. The reasons for this relapse may be various; Chatin's analyses were not trusted and the amount of iodide administered prophylactically was too

[1] Harvey Lectures, 19, 96 (1923-24).
[2] J. Biol. Chem., 18, 335 (1914).
[3] J. Biol. Chem., 20, 119 (1915).
[4] Arch. Internal Med., 4, 440 (1909).
[5] J. Biol. Chem., 13, 517 (1913).
[6] *Treatise on English bronchocoele with a few remarks on the use of iodine and its compounds.* (London, 1838.)

large. Chatin proposed 30 mg. of iodine per annum, but in some departments of France an iodized salt was used containing 0.1–0.5 gram KI per kilo, much too large an amount (von Fellenberg). Saint Lager[1] in a purely literary compilation, without any experimental evidence of his own, recites 42 alleged causes of goiter, each backed by a number of authors, and an additional category to which he reckons the majority of French hygienists of that day, who believed that nothing whatsoever was known about the cause of goiter. Saint Lager's book may have done much to lessen the faith in iodine prophylaxis, for iodine deficiency is only mentioned casually among the 42 alleged causes. He writes: "Dans la choix des eaux à dériver, ne pas se fier aux analyses chimiques, et considérer avant tout l'expérience physiologique des populations. Puisque les chimistes ignorent complètement la nature du principe goîtrigène et les moyens de le reconnaître, il est inutile de leur demander des conseils à ce sujet." Evidently the time was not ripe for a systematic prophylaxis. Physicians had not yet become accustomed to the idea of deficiency diseases, and were more ready to imagine that goiter was caused by the presence of a positive, harmful agent, rather than by the lack of something essential to the normal organism. It was the same in the early study of beriberi; Eykman at first imagined polished rice to be harmful as such, and the effect of the rice-polishings to be one of neutralizing this effect or detoxication. "A similar alternative has arisen in practically every instance, in which the function of a glandular organ has been revealed by its ablation; and I cannot recall any such case in which the later evidence has not decided the issue in favour of an internal secretion, and against a detoxicating action" (Dale). It was not until after the discovery of iodine in the thyroid gland itself that prophylaxis could be placed on a more certain foundation. Even then it was long before the true state of affairs was fully recognized. Thus as late as 1911 Swale Vincent[2] in a résumé on the subject, did not consider the connection

[1] *Études sur les causes du crétinisme et du goître endémique.* (Paris, 1867).

[2] Ergebnisse Physiol., 11, 279 (1911).

between goiter and iodine proved, but Kendall's isolation of a very active substance with 65-per-cent iodine seems to have dispelled all doubt, if any remained at the time. Marine and Lenhart[1] were able to prevent goiter in artificially reared trout by adding small quantities of iodide to the water of the hatchery. Marine and Kimball[2] began prophylaxis with schoolgirls in Akron, Ohio, and since then iodine is systematically added to some of the municipal water supplies in the United States, where iodine prophylaxis has entered commerce in the rearing of hogs and in the advertizing of iodized table salt. Since 1922 such a salt has been publicly supplied in most Swiss cantons; it generally contains 0.005 gm. of potassium iodide per kilo and it is calculated that it is used by half the population of Switzerland. At a daily consumption of 10 gm. this amounts to only 18 mg. of potassium iodide per annum, or 1/20–1/100 of that given sixty years ago. Parts of Bavaria, Austria, Thuringia, northern Italy and Roumania have followed suit. (Compare, for Switzerland, a recent article by Stiner[3]). In New Zealand prophylaxis among school children has been started by Hercus and his collaborators.[4] One of the causes which delayed the general recognition of iodine deficiency as the cause of goiter was the observation that often almost contiguous villages or valleys in mountain districts differed as to the incidence of goiter, but more accurate analysis of the minute amounts of iodine in the soil, in drinking water and in food has shown the existence of considerable irregularities in the distribution of iodine in a given small area, depending on geological conditions. This micro-analysis of iodine has been developed especially by McClendon[5] in the United States and by von Fellenberg[6] in Switzerland. The principle of the method consists in a colorimetric estimation of free iodine dissolved in 0.2–0.5 cu.cm. of chloroform. The manipulative procedure of

[1] J. Exp. Med., **12**, 311 (1910).
[2] J. Lab. Clin. Med., **3**, 40 (1917).
[3] Schweiz. med. Wochenschr., **5**, 391 (1924).
[4] J. Hyg., **24**, 321 (1925); **25**, 49 (1927).
[5] J. Biol. Chem., **60**, 289 (1924); Physiol. Rev., **7**, 189 (1927).
[6] Ergebnisse Physiol., **25**, 176 (1926).

von Fellenberg seems to be the simpler and preferred by Hercus and his co-workers.

McClendon and Williams[1] have published an interesting map of the United States, divided into four areas, representing the incidence of goiter among 2½ millions of recruits examined. The areas with most goiter are those with least iodine, as gauged by the iodine content of representative rivers. There is least iodine and most goiter in the Northwest (Washington, Oregon, Idaho, Montana, Wyoming, Utah) and near the Great Lakes (Wisconsin and Michigan). These areas were not submerged later than the Pleiocene and nearly all their iodine has been washed out. The Southern States along the Gulf of Mexico have most iodine and least goiter because they were covered by the sea at a later geological period. Small amounts of iodine are carried inland by sea spray from the coast, which is shown by the statistics along the Atlantic Coast (the statistical material was not large enough to show the same on the Pacific Coast) but naturally this influence does not extend very far into the interior. A similar correlation between goiter and the iodine content of the soil for various parts of the South Island of New Zealand is expressed by Hercus, Benson and Carter[2] by a curve which resembles a rectangular hyperbola, like the inverse relationship of the pressure and volume of a gas.

Although the amount of iodine in Swiss iodized table salt is so minute that its use would supply only something like 15 mg. of iodine per annum, this appears to be enough to cover dietary deficiency. According to Marine[3] a strictly normal human thyroid gland does not contain more than 25 mg. of iodine. Marine[4] has further shown the coefficient of partition for iodine between the thyroid and the rest of the body to be enormously in favor of the former. Of a single dose of 50 mg. KI (=38 mg. I) 18.5 per cent was secured by this gland although it was only 1/687 of the body weight. By intravenous injection the iodine content of the thyroid may be increased by several

[1] J. Am. Med. Assoc., **30**, 600 (1923).
[2] J. Hyg., **24**, 321 (1925).
[3] Harvey Lectures, **19**, 96 (1923-24).
[4] J. Biol. Chem., **22**, 547 (1915).

hundred per cent within 5 minutes. The iodine so stored is not, however, at once physiologically active; it takes at least 8 hours to obtain signs of increased activity. The synthesis of thyroxine, or whatever the hormone may be, requires time.

Physiological Tests for the Activity of Thyroid Gland and Thyroxine. The test of activity most frequently used is an effect on tadpoles. Their metamorphosis is hastened by minute quantities of thyroid gland or thyroxine, as was first shown by Gudernatsch.[1] During metamorphosis the first effect is a shortening of the tail and by measuring a batch of tadpoles Gaddum[2] was able to show the effect of one part of thyroxine in 10000000 parts of water, and Romeis[3] even observed an effect at one-tenth of this concentration. He regards diiodotyrosine as having a similar effect in solutions 200–1000 times as strong, but Gaddum does not find any specific action of diiodotyrosine. The Mexican axolotl remains permanently larval unless stimulated by thyroid gland or by thyroxine; Zawadovsky[4] and collaborators found 0.03 mg. injected thyroxine enough to bring about the metamorphosis.

A more quantitative idea of the activity of thyroid and thyroxine may be obtained by studying their effect on the basal metabolism, a method introduced long ago by Magnus-Levy; by this means the physiological identity of natural and synthetic thyroxine has been established in myxoedematous patients and the method can also be applied to laboratory animals. A further, peculiar, method for estimating thyroid activity was discovered by Reid Hunt.[5] It consists in observing the increased resistance to acetonitrile poisoning which thyroid feeding confers on mice. As little as 1 mg. of thyroid in 40000 parts of cracker dust can be recognized. The resistance varies greatly with the diet, which must be carefully standardized. Since the resistance of rats and of guinea pigs to acetonitrile is lowered by thyroxine, it is difficult to find

[1] Zentr. Physiol., **26**, 323 (1912); Am. J. Anatomy, **15**, 431 (1914).
[2] J. Physiol., 64, 246 (1928).
[3] Klin. Wochenschr., 1, 1262 (1922).
[4] Pflüger's Arch., **217**, 198 (1927).
[5] J. Biol. Chem., 1, 33 (1905).

a theoretical explanation of the method. Tested by this method diiodotyrosine only shows activity in enormous doses (Wuth[1]). Cameron and Carmichael[2] have attempted to develop a method in which the adverse effect on growth in rats and rabbits is contrasted with a hypertrophy of the organs concerned in metabolism, *e.g.* the heart. Both Hunt and Cameron and Carmichael draw the conclusion that thyroxine is less active than an amount of thyroid containing the same amount of iodine. This may be due to the presence in the gland of a substance more active than thyroxine. It should also be remembered that thyroxine, both synthetic and "natural," is a racemic product, and one of the enantiomorphs may be the more active, as with adrenaline. Harington[3] has prepared the two optically active thyroxines by resolving its immediate precursor in the synthesis, containing only two iodine atoms. (Thyroxine itself, with four iodine atoms was too insoluble). The *N*-formyl derivative of the amino acid, with two iodine atoms, was resolved by means of its salts with active α-phenylethylamine, and the active varieties were then further iodinated. The resulting *l*- and *d*-thyroxines did not show a great difference in rotation nor, according to preliminary tests, a great difference in physiological activity.

A comparison between thyroxine and thyroid gland with equal iodine content does not seem to have been made by means of the basal metabolism; this comparison seems desirable.

[1] Biochem. Z., **116**, 237 (1921).

[2] J. Biol. Chem., **45**, 69 (1920); **46**, 35 (1921).

[3] Biochem. J., **22**, 1429 (1928). This paper, published while these lectures were in the press, gives $[\alpha]_{5461}^{21°}$ for synthetic *l*- and *d*-thyroxine as $-3.2°$ and $+2.97°$, respectively. The former has, according to Gaddum, about three times the physiological activity of the latter. More recently Harington and Salter have obtained *l*-thyroxine by enzymic hydrolysis of the gland, and Harington and Randall obtained from the gland in the same way diiodotyrosine, previously isolated from it by baryta hydrolysis (Private communication).

CHAPTER II

The Chemistry of the Vitamins

So little is known about the chemistry of the vitamins—not a single one has been isolated with absolute certainty—that I have hesitated to include this subject among the applications of organic chemistry. The very extensive contemporary literature on vitamins which takes up much space in journals devoted to biochemistry, contains few chemical facts, and very few that are thoroughly well established. The position in this group is rather worse than in that of the hormones. Both groups comprise extremely active substances present in very small amounts, but whereas the hormones are entirely or almost entirely of animal origin, the vitamins are primarily the products of vegetable activity. Funk[1] was the first to attempt the isolation of an antineuritic substance from rice-polishings and after sucessive precipitation by phosphotungstic acid, by mercuric chloride in alcoholic solution, and by silver nitrate and baryta, he obtained a minute quantity of a crystalline substance which he considered to be a base and called *vitamine*. No evidence has ever been adduced to show that the substance is an amine. Funk was probably influenced in his nomenclature by the discovery some years earlier, by Dale and myself, of the powerful physiological action of histamine, a real amine, which we had isolated by methods similar to those employed by Funk. Later he fractionated his product into two compounds, nicotinic acid $C_6H_5O_2N$ and a substance $C_{26}H_{20}O_9N_4$, considered to be a tetrabasic acid and the real "vitamine".[2] About this time I satisfied myself (by unpublished experiments) that such activity as Funk's preparations possessed was due to adherent traces of a highly potent substance; and I suggested later[3] that all Funk's preparations, both

[1] J. Physiol., 43, 395 (1911).
[2] J. Physiol., 46, 173 (1913).
[3] Barger, *Simpler Natural Bases*, p. 112, (London, 1914).

from rice and from yeast, were more or less impure nicotinic acid. This was confirmed by Drummond and Funk[1] who found choline, betaine, adenine and guanine, in addition to the principle substance nicotinic acid.

It was thus seen that the isolation of the antineuritic principle was far more difficult than had been supposed. Incidentally Drummond and Funk showed that the "oryzanin" of Suzuki, Shimamura and Odake,[2] also contained nicotinic acid and betaine, and the "torulin" of Edie, Evans, Moore, Simpson and Webster[3] from yeast was evidently also an impure substance. During the war Hofmeister,[4] working at Strassburg with Tanaka, obtained a pyridine (?) derivative from rice, isomeric with betaine, of which the crude hydrochloride showed some activity; this was, however, lost on purification. Hofmeister worked under great disadvantages: his Japanese collaborator was interned; his pigeons being in a fortress were confiscated.

These repeated failures, ending in pyridine derivatives, induced Williams[5] to examine synthetic hydroxypyridines, some of which showed slight antineuritic activity, but only in one of two tautomeric forms (the phenol betaine?); Williams and Seidell[6] isolated from yeast a more or less active adenine which became inactive on recrystallization but was reactivated by heating with alcohol to 180°. This remarkable observation has not been confirmed. Seidell[7] subsequently described a picrate of a base $C_6H_{18}O_2N_3$ from yeast, which he considered to be the antineuritic substance.

The recognition that the vitamin is present only in very small amounts, and that it is readily adsorbed on a variety of precipitates, led to a new development. Selective adsorbents were sought in order to effect a preliminary concentration. Such methods have been used with marked success by Willstätter and his pupils in the concentration

[1] Biochem J., 8, 598 (1914).
[2] Biochem. Z., 43, 89 (1912).
[3] Biochem. J., 6, 234 (1912).
[4] Biochem. Z., 103, 218 (1920).
[5] J. Biol. Chem., 25, 437 (1916); 29, 495 (1917).
[6] J. Biol. Chem., 26, 431 (1916).
[7] U. S. Public Health Repts., 39, 294 (1924).

of enzymes. Seidell[1] introduced fuller's earth into vitamin research, a substance previously employed by Lloyd of Cincinnati for isolating alkaloids. From the adsorbate the vitamin was removed by baryta. Levene and van der Hoevens[2] preferred silica gel as the adsorbent, and removed the vitamin from it at pH = 9.0. Kinnersley and Peters[3] used a special charcoal (norit), and removed the vitamin from it by extraction with acid alcohol; they ultimately obtained a highly active concentrate that cured and protected pigeons by a dose of 0.084 mg. per day.

The furthest progress in this direction, by the use of fuller's earth, was made by Jansen and Donath in Java.[4] From 100 kilos of rice-polishings they obtained one quarter of the vitamin as a crystalline hydrochloride, $C_6H_{10}ON_2.HCl$, that was very active. From it they prepared a crystalline gold salt and picrolonate and these, after crystallization from organic solvents and reconversion into the hydrochloride, showed undiminished activity. Since adsorption from organic solvents is much less likely than from water, it may well be that Jansen and Donath have obtained the pure antineuritic principle. Yet it is very desirable that their work should be repeated by others in view of the many earlier and unsustainable claims to have obtained the substance in a pure condition. Jansen and Donath's substance gives an intense coloration with diazobenzenesulphonic acid; they think it may be a glyoxaline, or more likely a pyrimidine derivative. Peters[5] had already shown that the antineuritic vitamin is not destroyed by nitrous acid, so that it does not contain an amino group. The above formula corresponds to a dimethylhydroxypyrimidine and it would be interesting to know how many *N*-methyl groups, if any, are present.

The above statements refer to a substance having a well-defined physiological effect in preventing or curing polyneuritis, best studied in birds, and first recognized by Eijkman in 1897.

[1] Abstracts Bact., 6, Proc. 101 (1922); J. Biol. Chem., 67, 593 (1926).
[2] J. Biol. Chem., 61, 429 (1924); 65, 483 (1925); J. Pharm. exp. Ther., 29, 277 (1926).
[3] Biochem. J., 19, 820 (1925).
[4] Proc. Acad. Sci. Amsterdam, 29, 1390 (1926).
[5] Biochem. J., 18, 858 (1924).

Hopkins[1] showed in 1912 that milk contains a substance which is necessary for the growth of young rats, and McCollum and Davis[2] found it to be fat-soluble; later they discovered that a water-soluble substance is also required for growth and the two substances became known as vitamin A and vitamin B, respectively.[3] The properties and distribution of the latter were similar to the above-discussed antineuritic vitamin, and in 1916 McCollum and Kennedy[4] concluded that the two were identical. This view was widely accepted for about ten years, although of course a rigid proof would require the demonstration that one and the same pure chemical individual has both physiological properties. The work of Goldberger and collaborators, however, has during the last few years led to the recognition of vitamin B as an association of at least two substances.[5] This work was undertaken to ascertain the cause of pellagra, a deficiency disease of people living largely on maize (Carolina, Italy). The maize protein was thought at first to be responsible, but Goldberger and his associates later concluded that the disease is due to the absence from the diet of a special substance, the pellagra preventive (P-P), which accompanies the antineuritic vitamin. Both are necessary for growth.[6] Both can be heated to 100° for one or two hours without appreciable loss, but at 120° the antineuritic vitamin B_1 is destroyed much more rapidly than the growth-factor vitamin B_2 (=the pellagra preventive P-P).

Attempts to isolate the fat-soluble vitamin A received a great impetus when Steenbock and Boutwell showed that it resists hydrolysis and can be concentrated in the unsaponifiable portions of cod-liver oil.[7] The earlier failures of other authors were due to oxidation by atmospheric oxygen, which readily destroys this vitamin in a

[1] J. Physiol., **44**, 425 (1912).
[2] J. Biol. Chem., **15**, 167 (1913).
[3] J. Biol. Chem., **23**, 181, 231 (1915).
[4] J. Biol. Chem., **24**, 491 (1916).
[5] Goldberger and Tanner, U. S. Public Health Repts., **39**, 87 (1924); **40**, 54 (1925); Goldberger, Wheeler, Lillie and Rogers, *ibid.*, **41**, 297 (1926); Goldberger and Lillie, *ibid.*, **41**, 1025 (1926).
[6] Comp. Chick and Roscoe, Biochem. J., **21**, 698 (1927); **22**, 790 (1928).
[7] J. Biol. Chem., **42**, 121 (1920).

hot alkaline solution. By hydrolysis in an inert atmosphere the vitamin from a liter of cod-liver oil can, however, readily be concentrated without appreciable loss in 8 or 9 grams of unsaponifiable matter. Takahashi, Nakamiya, Kawakami and Kitasato[1] claim to have isolated the vitamin A in a state of purity from cod-liver oil by freezing out the cholesterol in alcoholic solution, removing the last traces with digitonin, extracting from solution in 90-per-cent methyl alcohol by petroleum ether, and distilling. At 147°–150°, under a pressure of 0.02–0.03 mm., a substance distilled which satisfied the daily requirements of a rat in a dose of 0.005–0.010 mg. The Japanese authors regarded it as pure and gave it the formula $C_{27}H_{44}O_2$, with one hydroxyl group and one double bond; but since the active material was not crystallized, this claim to purity cannot be upheld, as Drummond, Channon and Coward have pointed out in an investigation on the same subject.[2] These authors showed that about half the unsaponifiable matter of cod-liver oil is cholesterol, and that when the rest is distilled vitamin A passes over at 180°–220° and 2–3 mm. of mercury. The distillate consists of a saturated alcohol, an unsaturated hydrocarbon (spinacene) and one or more alcohols, probably with one hydroxyl group and one double bond, and with a smaller molecular weight than that recorded by the Japanese authors (*e.g.* $C_{20}H_{39}OH$). The cod-liver oil used by the latter was about five times as active as that used by Drummond and his co-workers, whose most active distillate had about one-fifth of the activity of that obtained by Takahashi and his collaborators. Evidently the possibility of isolating vitamin A seems more remote than that of isolating the antineuritic vitamin.

The next vitamin to be recognized by its physiological effects was the antiscorbutic one, vitamin C, found in fresh vegetables, orange juice, and the like. It is particularly unstable and is even decomposed when vegetables are dried. The chance of isolating it seems, at present, quite remote.

[1] Sci. Papers Inst. Phys. Chem. Research, Tokyo, no. 32.
[2] Biochem. J., 19, 1047 (1925).

Lately most interest has centered round the antirachitic vitamin D. Its existence was first recognized by E. Mellanby[1] who showed that rickets is a deficiency disease. Mellanby assumed the antirachitic vitamin to be similar in properties and distribution to the fat-soluble vitamin A, but McCollum, Simmonds, Becker and Shipley[2] showed that the former is much more stable to oxidation than the latter, so that a new name, "vitamin D," was applied to it.

Meanwhile Huldschinsky[3] cured rickets in children by ultra-violet radiation and the importance of light in the etiology of rickets was abundantly confirmed. For a time the two views as to the cause of rickets were hard to reconcile. One school believed in diet, the other in light, as a cure for rickets. Both were shown to be right, as a result of the remarkable observation of Steenbock and Black[4] that irradiation by a mercury-vapor lamp confers growth-promoting and calcifying power on inactive muscle. An indication of the photosynthesis of fat-soluble vitamin had already been obtained by Goldblatt and Soames.[5] Steenbock and Black at once went on to irradiate the whole diet and particularly its fatty constituents, with identical results; and in 1925 no fewer than three groups of investigators[6] discovered independently and simultaneously that cholesterol (or phytosterol) is rendered antirachitic by irradiation.

Rosenheim and Webster[7] next showed that an amorphous substance, which protects rats in doses of 0.01 mg. per day, can be separated from unchanged cholesterol by precipitating the latter with digitonin, a crystalline saponin which forms an insoluble compound with sterols. They also found that a highly unsaturated sterol of fungi, ergosterol, when irradiated becomes "highly protective even in doses of 1 mg." Soon afterwards the same authors[8]

[1] J. Physiol., 52, Proc. xi, liii (1918-19); Lancet, **196**, 407 (1919-i).
[2] J. Biol. Chem., **53**, 293 (1922).
[3] Deut. med. Wochschr., **45**, 712 (1919).
[4] J. Biol. Chem., **61**, 405 (1924).
[5] Biochem J., **17**, 446 (1923).
[6] Steenbock and Black, J. Biol. Chem., **64**, 263 (1925); Hess, Weinstock and Helman, J. Biol. Chem., **63**, 305 (1925); Rosenheim and Webster, Lancet, **208**, 1025 (1925-i).
[7] Biochem. J., **20**, 537 (1926).
[8] J. Soc. Chem. Ind., **45**, 932 (1926).

recognized that ordinary cholesterol contains an impurity which cannot be removed by crystallization, and is destroyed by bromine. When cholesterol is purified via the dibromide, it can no longer be rendered antirachitic by irradiation. This led, finally, to the identification of the impurity in cholesterol. By the joint work of Rosenheim and Webster[1] and of Windaus and Hess[2] this impurity was shown to be ergosterol or some similar sterol. Ergosterol has not been separated from animal fats, such as cod-liver oil, but it is quite certain that minute quantities of irradiated ergosterol (from ergot or yeast) protects rats from rickets. The daily dose required is something like 0.00005 mg. or less. When they first irradiated ergosterol, Rosenheim and Webster obtained complete protection with 0.01 mg., but did not test smaller amounts. That ergosterol is a precursor of vitamin D was also indicated by spectroscopic observations but without the biological tests on rats, such observations can only be presumptive evidence and are not conclusive. Hess and Weinstock[3] showed that when ordinary cholesterol is irradiated, a change in the ultra-violet absorption spectrum occurs; Schultz and Ziegler[4] confirmed and extended these observations and discussed the possibility of the bands being due to an impurity. Heilbron, Kamm and Morton[5] concluded that ordinary purified cholesterol contains another compound in small quantity which can be accumulated in the least soluble fraction; it gives well-defined absorption bands, whereas cholesterol gives only general absorption. On irradiation with ultra-violet light these bands disappear, and since antirachitic properties appear in the same process, these authors concluded that the unknown substance was closely connected with the precursor of vitamin D. This indeed turned out to be the case, but need not have been so, since many cholesterylenes possess almost the same absorption bands as ergosterol, yet cannot

[1] Biochem. J., **21**, 127 (1927); Lancet, **212**, 306 (1927-i).
[2] Nachr. Ges. Wiss. Göttingen, 175 (1926).
[3] J. Biol. Chem., **64**, 193 (1925).
[4] J. Biol. Chem., **69**, 415 (1926).
[5] J. Soc. Chem. Ind., **45**, 932 (1926); Biochem. J., **21**, 78 (1927).

be activated. Pohl[1] found that cholesterol purified by way of the dibromide, at Rosenheim's suggestion, has no absorption band; and then Rosenheim and Webster[2] as well as Pohl[3] showed that ergosterol gives, qualitatively, the same absorption band as unpurified cholesterol but with enormously greater intensity. Pohl also found that on prolonged irradiation the ergosterol bands are replaced by another band in the further ultra-violet, which he assumes to be characteristic of vitamin D. Morton, Heilbron and Kamm[4] calculated that ergosterol, on irradiation, becomes 2000 to 4000 times as active as the purified cholesterol which they had previouly used. They further studied the decomposition of vitamin D by excessive irradiation, and considered that waves shorter than 270 $m\mu$ are responsible for this effect. Since the formation of vitamin D so far has always been accompanied by its decomposition, it has been impossible to get a 100-per-cent transformation of ergosterol into vitamin D, or to obtain the latter substance in a state of purity. Rosenheim and Webster[5] find that the maximum activity may be produced in half an hour, and remains constant for four hours. They imagine that during this time the formation and the destruction of the vitamin proceed at the same rate, until the available ergosterol is exhausted. Since after half an hour's exposure they could recover 90 per cent of the ergosterol unchanged, they estimate that the maximum yield of vitamin obtainable is rather less than 10 per cent of the ergosterol irradiated. The minimum daily requirements of a rat have been given as 0.00005 mg. (= 0.05 γ) but a demonstrable deposition of calcium in the bones of a rachitic rat, the so-called "line" test, may be obtained with as little as 0.02 γ of irradiated ergosterol, according to Coward.[6] On allowing for a 10-per-cent yield, as above indicated, Coward points out that this would correspond to 0.002 γ of vitamin per day.

[1] Nachr. Ges. Wiss. Göttingen, 144 (1927).
[2] Lancet, **212**, 306 (1927-i).
[3] Nachr. Ges. Wiss. Göttingen, 185 (1927).
[4] J. Chem. Soc., **1927**, 2000.
[5] Lancet, **213**, 622 (1927-ii).
[6] Biochem. J., **22**, 1221 (1928).

Now Fosbinder, Daniells and Steenbock[1] had already made an attempt to determine quantitatively the amount of energy necessary to secure a demonstrable deposition of calcium in the bones of a rachitic rat. By using cholesterol and a monochromatic radiation of 265 mμ wavelength, they found that irradiation for 22.5 seconds was necessary for a dose administered over a period of ten days. The energy absorbed was 234 ergs, the number of quanta is calculated at 3.2×10^{13}. According to Einstein this means a synthesis of 3.2×10^{13} molecules of vitamin D, *i.e.* 5×10^{-11} gram-molecules. Assuming the molecular weight of vitamin D to be the same as that of cholesterol (385), this works out at $5\times10^{-11}\times385 = 2\times10^{-8}$ gm. of vitamin given during 10 days, *i.e.* 2×10^{-9} gm. = 0.002 γ of vitamin per day. As Coward points out, the agreement between this calculated value and that determined more directly is indeed remarkable.

It would appear that 3 million million molecules of the vitamin per day can produce a definite change in the calcium metabolism of a rat. In the case of some very powerful drugs a pharmacological effect is obtainable with an even smaller number of molecules.

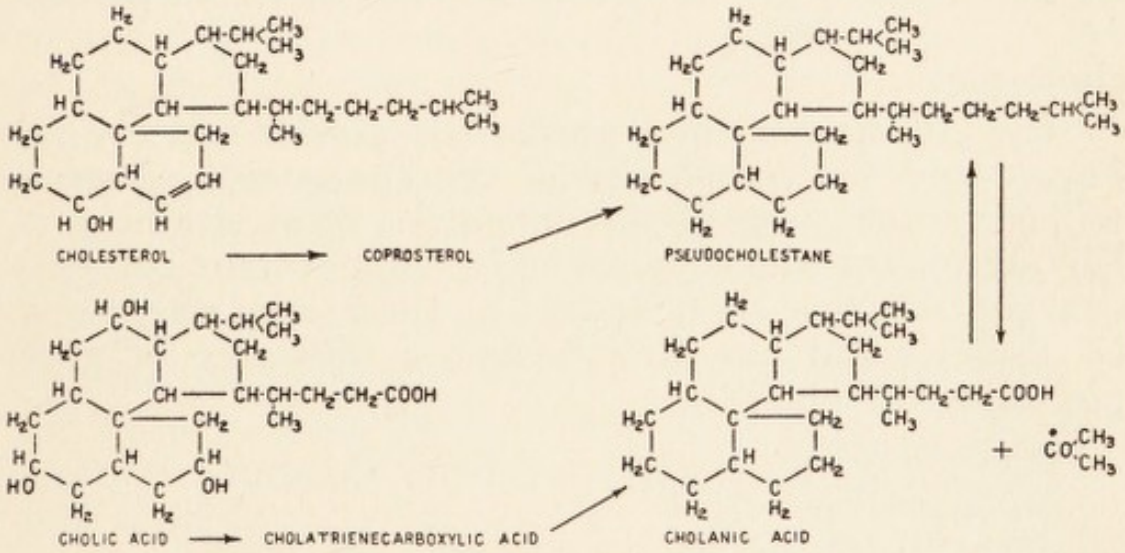
It is not surprising that irradiation of cholesterol, and particularly of ergosterol, is carried out technically. The ergosterol is best obtained from yeast, and crude yeast fat may be employed. The clinical results, chiefly with rickets, have been uniformly successful; doses of 1–5 mg. per day of the (impure) vitamin are given. It is pretty certain that enormous doses of irradiated ergosterol have a harmful effect and may be fatal to rats.

It has been suggested that the actual precursor of vitamin D is not ergosterol, but an impurity present in the latter, necessarily in minute amount. This would resemble the small fleas which have lesser fleas to bite them, "and so *ad infinitum*." It seems, however, that with the recognition of cholesterol as the active constituent of food, and then of ergosterol as the active constituent of cholesterol, the limit has been reached. Recently, Windaus and

[1] J. Am. Chem. Soc., 50, 923 (1928).

Brunken[1] reported that a peroxide of ergosterol is inactive, whether irradiated or no. This peroxide which cannot be activated, may be converted back into ergosterol, which then can be made highly active by irradiation. Hence the vitamin cannot result from an impurity in the ergosterol. The same conclusion is suggested by the concordance between the photometric and the "direct" estimate of the dose necessary to give a "line" test, referred to above.

Although vitamin D has not been obtained chemically pure we know more about it than about other vitamins, for we know its precursor in a photochemical change. Ergosterol has the composition $C_{27}H_{41}OH$ and contains three double bonds. It is obviously related to cholesterol $C_{27}H_{45}OH$ which has but one double bond. Dihydrocholesterol is $C_{27}H_{47}OH$ and still contains 8 hydrogen atoms less than the corresponding saturated aliphatic alcohol $C_{27}H_{55}OH$. Hence cholesterol has four rings. The following formulas, although perhaps not certain in every detail, give a fairly accurate idea of the constitution of cholesterol and its relation to cholic acid of bile.



Interconversion of Cholesterol and Cholic Acid

Cholesterol is excreted in the faeces as a saturated alcohol, coprosterol, $C_{27}H_{47}OH$; in the biochemical reduction the double bond is not only reduced, but a stereochemical change also takes place, so that we pass into a diastereo-

[1] Ann., 460, 225(1928).

meric series, to which cholic acid belongs; apparently the same stereochemical change takes place in the biological oxidation of cholesterol. Replacement of the hydroxyl group of coprosterol by hydrogen yields the saturated hydrocarbon pseudocholestane, which on oxidation with chromic acid breaks up into equimolecular proportions of cholanic acid and acetone, as was shown by Windaus and Neukirchen.[1] Now cholanic acid can be also obtained from cholic acid. Wieland and Weil distilled cholic acid in a vacuum,[2] when it lost three molecules of water, and cholatrienecarboxylic acid (with three double bonds) resulted. When the latter is reduced, cholanic acid is formed. Wieland and Jacobi,[3] more recently, have carried out the reverse transformation from cholanic acid to pseudocholestane, by converting its ethyl ester with isopropylmagnesium iodide into the tertiary alcohol $C_{23}H_{39}.COH\left(CH\begin{matrix}CH_3\\CH_3\end{matrix}\right)_2$ oxidizing off one isopropyl group to the ketone $C_{23}H_{39}.CO.CH\begin{matrix}CH_3\\CH_3\end{matrix}$ and reducing the latter to pseudocholestane.

The constitution of ergosterol is almost entirely unknown and the complexity of the cholesterol structure will give some idea of the difficulties of ascertaining it. Ergosterol, for some years a quite unimportant curiosity of ergot, was first characterized by the French pharmacist C. Tanret,[4] and has now become a substance of great physiological interest.

[1] Ber., **52**, 1915 (1919).
[2] Z. physiol. Chem., **80**, 290 (1912).
[3] Ber., **59**, 2065 (1926).
[4] Compt. rend., **147**, 75 (1908).

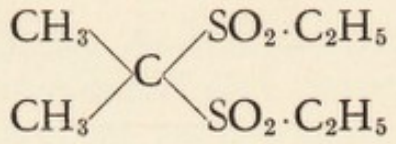
CHAPTER III

Chemical Constitution and Physiological Action

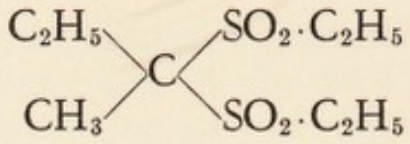
It is nearly 60 years since Crum Brown and Fraser,[1] of Edinburgh, published a series of papers on the changes in the physiological action of certain bases, produced by the addition to their molecule of a methyl group. Ever since, the relationship between chemical constitution and physiological action has been of interest to organic chemists and pharmacologists alike. Crum Brown and Fraser showed that a number of alkaloids (strychnine, brucine, atropine, codeine, morphine, thebaine) on conversion into quaternary salts by the action of methyl iodide, lose most or the whole of their original activity and acquire instead the power of paralyzing motor-nerve endings, a power possessed in an intense degree by the arrow-poison curare which itself owes its activity to quaternary bases. Subsequently the rule was found to apply very widely even to quite simple quaternary iodides. Tetramethylammonium iodide was, of late years, recommended in Germany as a substitute for curare in physiological laboratories. Yet this generalization does not apply universally. A number of quaternary salts of alkaloids and of simpler bases do not show a curare action, and even the change from tetramethyl to tetraethylammonium iodide greatly diminishes this action.

Crum Brown's and Fraser's work was limited almost entirely to natural drugs, for the simple reason that no series of synthetic drugs was as yet known. Such a series first became available as a result of the accidental discovery, by Kast, of the hypnotic action of sulfonal.

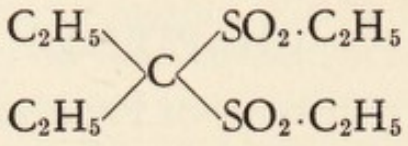
[1] Proc. Roy. Soc. Edinburgh, 6, 228, 461, 556 (1869).



Sulfonal

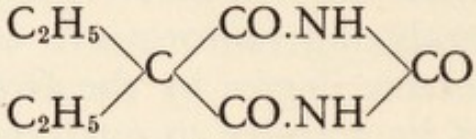


Trional



Tetronal

which Baumann and Kast[1] considered less active than trional with three ethyl groups, and trional again less so than tetronal with four such groups. This supposed order of activity led to the attribution of an hypnotic effect to the ethyl group. The greater activity of tetronal has not been confirmed, and now it has been fully established that ethyl groups are of no special importance in the constitution of a hypnotic. Influenced however by Baumann and Kast's conclusions, E. Fischer and Mering[2] found an important hypnotic in diethylbarbituric acid or veronal,



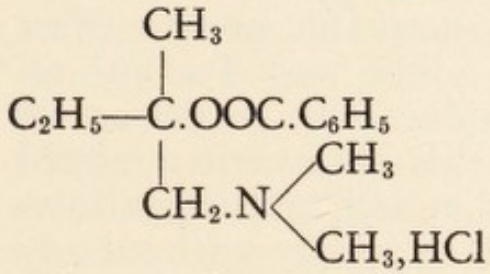
Veronal

but the non-essential character of the ethyl group was later demonstrated in this very class of hypnotics by the successful introduction of other members in which propyl, isopropyl, allyl and phenyl groups take the place of one or both ethyl groups of veronal. In a more general way, however, alkyl groups appear to have some significance.

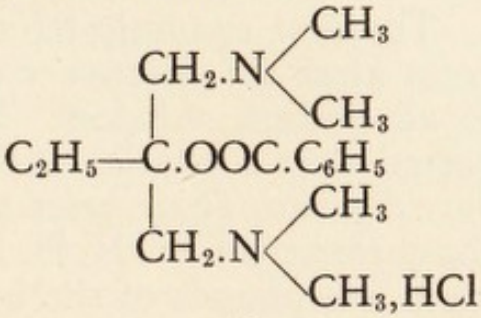
Local anesthetic action is possessed to some extent by a variety of substances (phenol, benzyl derivatives) but all really active substances of this class have two features: they are esters and they are bases. The basic group may be in the alcoholic portion or in the acidic portion or in both. Thus the ethyl esters of *p*-aminobenzoic acid, $NH_2.C_6H_4.COOC_2H_5$, of *p*-amino-*m*-hydroxy-, and of *m*-amino-*p*-hydroxybenzoic acids have all been employed.

[1] Z. physiol. Chem., **14**, 52 (1890).
[2] *Therapie der Gegenwart*, p. 97 (1903).

It is better, however, to have the basic group in the alcoholic part of the molecule which leads to esters of amino alcohols. In order to limit the final acylation (mostly benzoylation) to the alcoholic hydroxyl, and leave the basic group unchanged, a tertiary base is chosen.



Stovaine

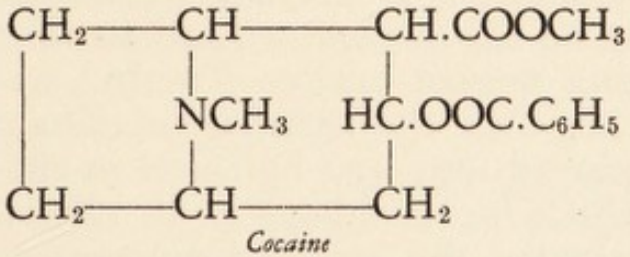


Alypine

For instance ethyl dimethyl carbinol with a hydrogen in one methyl group replaced by $N(CH_3)_2$, gives stovaine on benzoylation, and the same tertiary alcohol with $N(CH_3)_2$ in both methyl groups yields alypine. Procaine has two basic groups, a primary amino group in the benzoic acid portion, and a tertiary one in the alcohol. It is the hydrochloride of the *p*-aminobenzoyl ester of diethylaminoethyl alcohol

$$NH_2.C_6H_4.COOCH_2.CH_2.N(C_2H_5)_2,HCl$$

The amino group in the benzene nucleus is a primary one, because it is formed by reduction of a nitrobenzoyl ester. The various synthetic substances mentioned were suggested, directly or indirectly, by the powerful natural local anesthetic cocaine,



Cocaine

itself the benzoyl ester of an amino alcohol with a tertiary amino group. (The group $COOCH_3$ has no particular significance for the action.)

The group of local anesthetics which has thus been illustrated by a few examples, evidently shows a closer connection between chemical constitution and physiologi-

cal action than that of the hypnotics previously referred to. We will next discuss two other well-marked examples of such a connection, relating for the most part to substances which have only a theoretical interest and have not met with practical application.

The first example refers to some simple amines. Some years after the discovery of the remarkable pressor effect of adrenaline, Abelous, Ribaut, Soulié and Toujan[1] reported that a similar effect is produced by an extract of putrid meat. Their brief note on this observation excited the curiosity of Dr. H. H. Dale and myself, and after allowing a few pounds of the best steak to putrify without any bacteriological precautions, we were able to confirm the observation of the French authors. A large and transitory rise of blood pressure was rapidly produced in the decerebrate cat by intravenous injection of an extract of of the putrid meat. We at once began an attempt to isolate the active principle. Walpole and I allowed a large quantity of minced horse meat to putrify at 37°;[2] the protein was then coagulated by boiling water, and filtered off. These operations were conducted in the open air on account of the powerful stench. The evaporation of the filtrate *in vacuo* caused no trouble in the laboratory after we had installed an efficient sulphuric acid trap to prevent the escape of indole, skatole and the like. We thus obtained a syrupy mass which was mixed with sand and extracted with acetone. The pressor substances were then obtained in a mixture of acetone with some water, and after distilling off the acetone we had an aqueous solution giving a strong pressor action. To this we applied the ordinary methods for the isolation of alkaloids, such as had been employed long ago by Selmi in the study of his ptomaines, which he considered to be alkaloidal in character. We extracted the acidified aqueous solution with chloroform, which removed fatty acids derived from valine, leucine and other amino acids. On making alkaline and extracting once more with chloroform a good deal of the pressor substance was removed. Oxalic acid was then

[1] Compt. rend. soc. biol., 58, 463, 530 (1906).
[2] J. Physiol., 38, 343 (1909).

added to the concentrated chloroform solution and produced a crystalline oxalate which was analyzed. The formula suggested a salt of the base $C_5H_{13}N$ and on drying the salt above 100° a loss of weight was observed, due to partial volatilization of the base. The latter was next obtained pure by distilling the oxalate mixed with lime. The boiling point, odor, and other properties of the base showed it to be a primary amylamine, probably a mixture of the amines derived from the amino acids leucine and isoleucine by loss of the carboxyl group, in the main isoamylamine $(CH_3)_2CH.CH_2.CH_2.NH_2$. The vapor density of the amine was determined by the ordinary method of Victor Meyer, and the identification was completed by Dale when he showed that synthetic isoamylamine produced the same pressor effect, until then unsuspected.

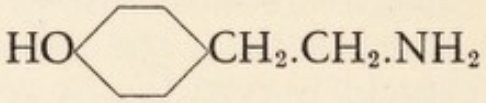
Continued extraction of our crude alkaline solution did not remove any further quantities of pressor substance, yet much remained behind in the aqueous layer. Evidently a second substance was present insoluble in chloroform. We next tried amyl alcohol and easily removed the whole of the pressor substance, but on repeating the experiment entirely failed to do so. I was greatly puzzled by this discrepancy, until I remembered that on the first occasion the solution had been made alkaline with sodium carbonate, and on the second with sodium hydroxide. This suggested a phenolic base, and remembering that the only phenol in protein is tyrosine, it seemed likely that we were dealing with the amine derived from this amino acid by decarboxylation, in the same way as the isoamylamine previously isolated resulted from leucine.

$$\underset{\textit{Tyrosine}}{HO.C_6H_4.CH_2.CH(NH_2)COOH} = \underset{\textit{p-Hydroxyphenylethylamine}}{HO.C_6H_4.CH_2.CH_2.NH_2} + CO_2$$

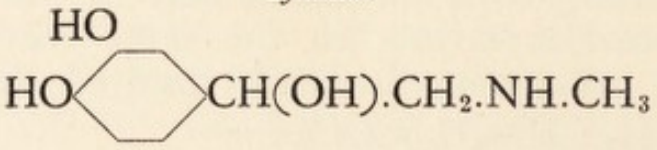
Our surmise was soon shown to be correct; the amine gave the Millon reaction; on benzoylation by the Schotten-Baumann method we easily got the known dibenzoyl derivative which crystallizes well, and finally a sample of *p*-hydroxyphenylethylamine (tyramine) obtained from tyrosine by heating *in vacuo* was found to have the same physiological activity.

We now began to suspect that this process of the decarboxylation of amino acids might be a general one; a search for phenylethylamine (from phenylalanine) resulted in the recognition of a third pressor substance in putrid meat.

The pharmacological properties of the various amines isolated were studied by Dale and Dixon.[1] Of them *p*-hydroxyphenylethylamine is by far the most active. Further, it most closely resembles adrenaline, both in physiological action and in chemical constitution. The action of adrenaline "simulates with considerable precision the effects of stimulating nerves of the true sympathetic system, being limited to muscle fibres and gland cells innervated by this system." Later, when we had shown that there is a whole range of bases thus simulating the effects of sympathetic stimulation, not only with varying intensity but with varying precision, Dale suggested the term "sympathomimetic" for the type of action common to these bases. All the amines then isolated from putrid meat had this sympathomimetic action, which shows itself, for instance, by a rise of blood pressure owing to constriction of the muscular coats of the arterioles. The chemical relationship between tyramine and adrenaline is evident from their structural formulas:



Tyramine



Adrenaline

The difference between them is that adrenaline has an additional phenolic hydroxyl in the benzene ring, an alcoholic hydroxyl in the side chain, and a methyl group attached to the nitrogen.

It seemed of interest to bridge this gap by the examination of compounds of intermediate structure, and to examine also compounds related to isoamylamine and

[1] J. Physiol., **39**, 25 (1909).

intermediate between it and tyramine. Dale and I began by an examination of the lower and higher homologues of amylamine,[1] which he and Dixon had shown to possess an action definitely of the sympathomimetic type.[2] In ascending the homologous series of straight-chain primary amines and injecting doses of 1 or 2 cu. cm. of a decinormal solution of their hydrochlorides into a cat, the action becomes evident with *n*-butylamine; *n*-amylamine is distinctly more active; *n*-hexylamine is the most active member of the series. The activity then declines, heptylamine being less active, and octylamine still less. Beyond this the comparison was impossible owing to increasing toxicity, but tridecylamine still shows a definite pressor effect. Pentadecylamine hydrochloride was too insoluble to be tested. Isobutylamine had practically no pressor effetc in the dosage indicated, while isoamylamine (1-amino-3-methylbutane) was slightly less active than the straight-chain compound. The branching of the chain therefore lowers the activity somewhat.

Among secondary amines, methylisoamylamine was only about half as active as isoamylamine itself and di-isoamylamine had very little action. Pentamethylene diamine (cadaverine), the only diamine examined, has an entirely different action from amylamine, causing a fall of blood pressure instead of a rise.

We next examined aromatic amines without phenolic hydroxyl, beginning with one of the pressor bases of putrid meat, β-phenylethylamine, $C_6H_5.CH_2.CH_2.NH_2$. This base is two or three times as active as isoamylamine and already possesses the carbon skeleton of adrenaline. Among fatty amines the optimum length of chain was found to be six carbon atoms. Among the fatty aromatic amines a side chain of two carbon atoms gave the greatest activity. Aniline, without any side chain, showed no specific activity, and with benzylamine, $C_6H_5.CH_2.NH_2$, having a side chain of one carbon atom, there was merely a trace of activity. Also $C_6H_5.CH(CH_3)NH_2$, α-phenylethylamine, was very much less active than its isomer with

[1] J. Physiol., 41, 19 (1910).
[2] J. Physiol., 39, 25 (1909).

TABLE I

Bases with the Carbon Skeleton of Adrenaline

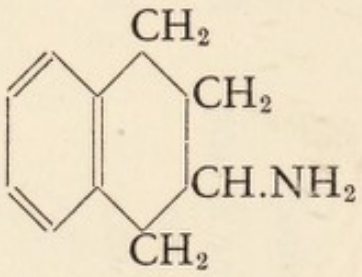
	Position of phenolic hydroxyls	First carbon atom of side chain	Nitrogen atom	Relative activity on blood pressure	Common Name
				1	Isoamylamine
1	none	CH_2	NH_2	2–3	Phenylethylamine
2	none	CH_2	NH Me	2–3	
3	none	CHOH	NH_2	2–3	
4	none	CHOH	NH Me	2–3	
5	4	CH_2	NH_2	10	Tyramine
6	3	CH_2	NH_2	10	
7	2	CH_2	NH_2	2–3	
8	4	CHOH	NH_2	2	
9	4	CO	NH_2	1	
10	4	CH_2	NH Me	10	
11	4	CH_2	NH Et	Much < 10	
12	4	CH_2	N Me_2	Slight (1?)	Hordenine

Table I (continued)

13	4	CH_2	$N(Me)_3I$	Nicotine action	Hordenine methiodide
14	3,4	CH_2	NH_2	20	
15	3,4	CH_2	NH Me	100	Epinine
16	3,4	CH_2	NH Et	30	
17	3,4	CH_2	NH Pr	5	
18	3,4	CH_2	$N(Me)_3$ Cl	Nicotine action	
19	3,4	CO	NH_2	30	
20	3,4	CO	NH Me	Much <30	
21	3,4	CO	NH Et	45	
22	3,4	CO	NH Pr	5	
23	3,4	CO	$N(Me)_3Cl$	Nicotine action	
24	3,4	CHOH	NH_2	1000	*d,l*-aminoethanolcatechol
25	3,4	CHOH	NH Me	700	*d,l*-adrenaline
26	2,4	CO	NH_2	Slight (1?)	
27	2,3,4	CO	NH_2	30	
28	2,3,4	CH_2	NH_2	20	

the longer side chain. Increasing the side chain beyond two C atoms in phenylpropylamine, $C_6H_5.CH_2.CH_2.CH_2.NH_2$, also lowered the activity considerably so that there appears to be a well-marked optimum at two carbon atoms. This same maximum was found later in iminazolylethylamine (histamine) which has an action of quite a different type. Nevertheless a two-carbon side chain may not be the optimum in every case. Generalization on this subject is particularly dangerous and it would be desirable to examine some other examples, especially with side chains of three carbon atoms.

Ac-tetrahydronaphthylamine,



which may be considered to have side chains of two, as well as of three carbon atoms, has actually a considerably stronger pressor action than β-phenylethylamine. From another point of view it is related to cyclohexylamine, and this latter base was found to have an activity similar to that of *n*-hexylamine. The effect of introducing a second ring likewise requires further examination. The rest of the investigation related entirely to amines having the carbon skeleton common to phenylethylamine and adrenaline (see Table I). To change the former into the latter the following groups must be introduced:

I. A phenolic hydroxyl in position 4 or para (*p*)

II. A phenolic hydroxyl in position 3 or meta (*m*)

III. An alcohol hydroxyl in the α position of the side chain (OH)

IV. A methyl group on the nitrogen (Me).

These modifications can be made one, two or three at a time, leading to the combinations: *p*; *m*; OH; Me; *pm*; *p* OH; *p* Me; *m* OH; *m* Me; OH Me; *pm* OH; *pm* Me; *p* OH Me; *m* OH Me. Of the fourteen compounds indicated all but four (*m* OH, *m* Me, *p* OH Me, and *m* OHMe)

have been examined. The introduction of hydroxyl groups into the *p* and *m* positions is much more important than that of hydroxyl into the side chain or of a methyl group on the nitrogen atom. In the absence of phenolic groups, the alcoholic hydroxyl and the *N*-methyl have no effect. (Compare numbers 2–4 of Table I, with number 1.) Introduction of OH yields (2), phenylethanolamine, $C_6H_5.CHOH.CH_2NH_2$; of Me yields *N*-methyl-β-phenylethylamine, $C_6H_5.CH_2.CH_2.NHCH_3$ (3); and of OH and Me together yields *N*-methyl-β-phenylethanolamine, $C_6H_5.CHOH.CH_2.NHCH_3$ (4). These three bases have all about the same pressor activity as phenylethylamine itself. It should be emphasized that the relative activities given in the table are only very approximate; accurate comparison is impossible, because the action is not, in all cases, of exactly the same character; moreover the ratio of the activities of two amines may change during the course of a prolonged experiment on the same animal, and even the order of activity may differ in different animals freshly prepared in the same manner. The activity of isoamylamine has been taken as unity; a direct comparison between it and the more active amines was not made, nor is it very feasible. Some approach to accuracy can be attained only among the catechol bases, but the order of magnitude of the activity will serve to illustrate the manner in which successive changes in the chemical constitution affect the physiological properties.

One phenolic hydroxyl, whether introduced into the *para-* or *meta*-position, increases the pressor activity of β-phenylethylamine three- to five-fold, but a phenolic hydroxyl in the *ortho*-position is without effect (7). Coming now to phenolic amines in which a further modification has been introduced, so as to make them approximate more closely to the structure of adrenaline, we find that no increase in activity results, unless this further modification is a second phenolic group (14), which roughly doubles the activity of tyramine. This is the case tabulated as *pm*. The combination *p* OH is represented by (8); the introduction of an alcoholic hydroxyl group into tyramine has lowered the activity, while the same modi-

fication enormously raises the activity in the corresponding catechol base (14 and 24). The introduction of an *N*-methyl group into tyramine, *p* Me (10), makes no difference in the activity. In certain catechol bases the activity is increased by the introduction of a keto group into the side chain and for this reason the base (9) was made, but with only one phenolic group in the molecule a keto group in the side chain lowers the activity even more than does a hydroxyl group in the corresponding position.

We now consider a third modification added to two hydroxyls in positions 3 and 4, that is, the combinations *pm* Me and *pm* OH, leading to the amines (15) and (24) respectively. In both cases the increase in activity is so great, and the action approximates so closely to the adrenaline type, that these bases are manufactured commercially as adrenaline substitutes. The *N*-methyl group, previously without effect, now increases the activity five times (14 and 15). The alcoholic hydroxyl previously made no difference or even lowered the activity; now, with a catechol nucleus present, it increases it fifty-fold (14 and 24).

The substance $(HO)_2C_6H_3.CHOH.NH_2$ is even more active on the blood pressure than adrenaline itself. The comparison was made on the racemic forms of the two bases. The pressor activity of natural *l*-adrenaline has been estimated by Schultz to be one-and-a-half times that of the racemic substance.[1] Dale found the ratio 6.5 : 10. These ratios imply that *l*-adrenaline is three or three-and-a-third times as active as the *d*-variety. Cushny later found by direct comparison that the *l*-variety is twelve times as active as the *d*-variety. On Schultz's ratio *l*-adrenaline should appear in the table with an activity of 1050; with Dale's ratio it would be 1077, with Cushny's 1300; *d*-adrenaline would have an activity of the order of 100–200, hardly more than the base (15) without alcoholic hydroxyl (at least if Cushny's figures are adopted). The great effect of the alcoholic hydroxyl in the catechol series is, therefore, closely connected with the stereochemical configuration. With a catechol base the proper kind of

[1] Hyg. Lab. Bull., No. 55 (Washington, 1909).

asymmetry can lead to a great increase in activity, but without the catechol nucleus the asymmetry cannot come into play. It would be interesting to resolve other amines with an alcoholic hydroxyl (3, 4, 8, and particularly 24) in order to see whether their enantiomorphs—which are all foreign to the body—show such a marked difference in activity as is the case with the adrenalines. Since aminoethanolcatechol (24) has not been resolved, *l*-adrenaline is still the most active pressor substance known, although it is conceivable that one of the enantiomorphs of (24) might be more active.

In the synthesis of adrenaline a ketone is its immediate precursor and Loewi and Meyer,[1] as well as Dakin,[2] found this ketone (20) possessed of some activity. The synthesis of ketones with other alkyl groups on the nitrogen atom was easily carried out, and one of these, ethylaminoacetocatechol $(HO)_2C_6H_3.CO.CH_2.NH.C_2H_5$, became a commercial product (21). This led us to investigate the effect of various *N*-alkyl groups in three series of amines (10–13, 14–18 and 19–23). In tyramine an *N*-methyl group makes little difference, an *N*-ethyl group greatly lowers the activity (11). Introduced into 3,4-dihydroxyphenylethylamine (14), a methyl and an ethyl group greatly increase the activity, the methyl still being the more powerful (15 and 16). Among the acetocatechol derivatives the effect is just the opposite of that with tyramine; the ethyl group increases the activity, the methyl group diminishes it (21 and 20 compared with 19). Here even the type of action is different; amino- and ethylaminoacetocatechol give a large and rapid rise of blood pressure, succeeded by a rapid fall; methylaminocatechol on the other hand raises the blood pressure less and more slowly, while maintaining it much longer. This difference in the character of the same sympathomimetic action prevents an accurate quantitative comparison of activities.

A propyl group on the nitrogen greatly lowers the activity (14 and 17; 19 and 22), and two methyl groups (12)—making a tertiary base—lower it still more. We have

[1] Arch. f. exp. Path. u. Pharmakol., **53**, 213 (1905).
[2] Proc. Roy. Soc. London, **76B**, 491 (1905).

already seen that additional alkyl groups lowered the activity of isoamylamine. The base (12) is the alkaloid hordenine isolated from malt germs by Léger and formerly used therapeutically. An analogous great reduction in pressor action, due to a dimethylamino group, is found in dimethylaminoacetocatechol (not included in the table) $(HO)_2C_6H_3.CO.CH_2N(CH_3)_2$ and had already attracted the attention of Loewi and Meyer.

Further methylation of (12) produces a quaternary salt, hordenine methiodide, $HO.C_6H_4.CH_2.CH_2.N(CH_3)_3I$, which once more causes a very sudden, powerful and evanescent rise in blood pressure, superficially resembling that of adrenaline. The action is, however, in reality of quite a different type, not sympathomimetic but of the nicotine type. This was established by an analysis of the pharmacological action of hordenine methiodide on other organs. An intravenous injection of 1 mg. of hordenine methiodide gives a pressor effect indistinguishable from that of 0.5 mg. of nicotine. As, according to Langley, there is a physiological antagonism between nicotine and curare, hordenine methiodide provides a notable exception to Crum Brown's and Fraser's generalization, referred to at the beginning of this chapter, that quaternary salts have a curare action. The related salts with catechol nuclei,

$(OH)_2.C_6H_3.CH_2.CH_2.N(CH_3)_3Cl$ (18)

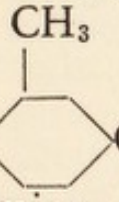
$(OH)_2.C_6H_3.CO.CH_2.N(CH_3)_3Cl$ (23)

have an action very similar to that of hordenine methiodide, (18) being stronger and (23) weaker. If we compare (3) with (14), and (13) with (18), we see that the introduction of a phenolic hydroxyl in the *meta*-position in each case increases the action, although the action in the first pair is sympathomimetic, and in the second pair of the nicotine type.

We have previously seen that a phenolic hydroxyl in the 2-position by itself does not result in increase of activity (7) and the same applies when it is present with an hydroxyl in the 4-position. The resorcinol derivative (26) is indeed much less active than tyramine (5). Similarly the pyrogallol derivatives (27) and (28) are some-

what less active then the corresponding catechol derivatives. It would, however, be interesting to examine amines with one *para*-hydroxyl and two *meta*-hydroxyls, in positions 3, 4, 5, therefore, which might more reasonably be expected to show a greater activity than the corresponding catechol derivatives.

Dale and I also investigated a number of bases not included in the table. In addition to α-phenylethylamine, $C_6H_5.CH(NH_2).CH_3$, already mentioned, we examined its *p*-hydroxy compound, $HO.C_6H_4.CH(NH_2)CH_3$ which, like the parent substance, has a very small activity; the side chain is too short. A methyl group in the benzene ring increases bactericidal power, the cresols being more potent than phenol. In arsenic derivatives of the salvarsan type, on the other hand, a methyl group lowers the therapeutic activity. It was the same in the present case,



o-cresylethylamine, $CH_2.CH_2.NH_2$, having only half the activity of tyramine. A similar effect of a methyl group is observed when it is introduced into the glyoxaline ring of histamine.

The examination of some forty amines, possessing a well-defined sympathomimetic action, should provide a good opportunity for drawing conclusions as to the relationship between chemical constitution and physiological action. The intensity of the pressor effect has, moreover, been expressed in a semi-quantitative manner. We have traced the action from simple aliphatic amines to that of adrenaline, by many intermediate stages, and it is unmistakable that the nearer we approach to the structure of adrenaline the more closely do we also approach to its activity. There is indeed a very obvious relationship capable of being discussed in detail. But what generalizations can be drawn from our results? They are, indeed, but few. All the sympathomimetic substances examined are amines (in contrast to the quaternary salts having a nicotine action). The amines are either entirely aliphatic or have at least an aliphatic side chain; the aromatic nucleus

merely serves to intensify the action. The most fundamental problem, therefore, would be to explain the action of the homologous aliphatic amines. Their only characteristic group is the amino group, giving rise to their common character of bases. If the basic character is abolished, the physiological effect disappears completely; the natural

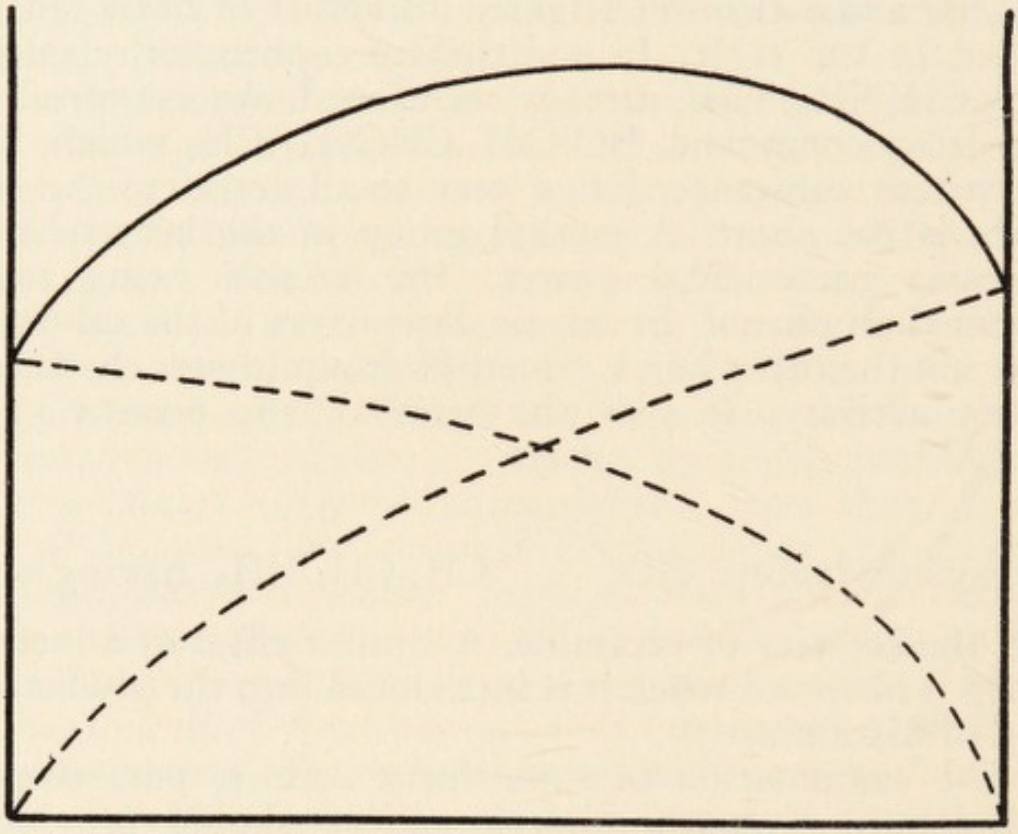


FIG. 2.
Summation of ordinates yields a maximum.

amino acids from which some of the amines are derived by decarboxylation, are wholly inert. Even tyrosine ethyl ester is inert, perhaps because it undergoes hydrolysis in the tissues. The basic function, which is the only chemical one we can invoke among the simple amines, is not enough to explain the action, and more particularly its rise to a maximum in *n*-hexylamine and its subsequent decline. This maximum in a homologous series can only be ascribed to the summation of at least two opposite effects which must be of a physical character. One of these effects might be continuously decreasing solubility in water, the other continuously increasing solubility in lipoids, as we ascend the series.

That a maximum may be produced by summation of two curves, neither of which has itself a maximum, may be illustrated in a formal manner by the accompanying diagram (Fig. 2). In a homologous series the strength of the amines as bases decreases continuously. The adsorption of the amines and their effect on surface tension, according to Traube's rule, would no doubt increase continuously. A careful study of the physical properties of the aliphatic amines might conceivably enable us to guess a combination of physical properties producing a maximum for *n*-hexylamine, which is almost the only physiological result that can be utilized here. Apart from this we can only infer that some physical property is involved.

The diminution in activity caused by the branching of a chain—isobutylamine compared with *n*-butylamine, isoamylamine with *n*-amylamine—seems quite analogous to the lowering of the boiling point in isomerides with branched chains, and it might be of interest to see whether this analogy extends further, for instance, to the various isomeric hexylamines. That sympathomimetic activity is lost by the introduction of another amino group, as in pentamethylene diamine, would appear to be connected with the great diminution of solubility in lipoids which this change seems to entail. In any case pentamethylene diamine is very much more soluble in water than in fat solvents such as chloroform or ether. Iminazolylethylamine (histamine) and guanidylbutylamine (agmatine) have similar solubilities and are not sympathomimetic either.

The connection between activity and lipoid solubility of the aliphatic amines is, *a priori*, not unlikely for, apart from the single amino group, these bases consist of nothing but an alkyl residue. Yet the greater activity of the fatty-aromatic amines is associated with a much decreased solubility in ether and presumably also in lipoids. While phenylethylamine can be readily extracted from an alkaline solution by ether, tyramine is extracted very slowly only and adrenaline not at all. Yet adrenaline is also very little soluble in water (the solubility is variously given as 1 in 3700 and 1 in 10500). With three hydroxyls a very much

greater solubility might be expected, which is indeed possessed by related substances with the same three hydroxyls, such as the ethyl ester of 3,4-dihydroxymandelic acid, $(OH)_2C_6H_4.CHOH.COOC_2H_5$. Tyramine with only one hydroxyl group is enormously more soluble in water than adrenaline.

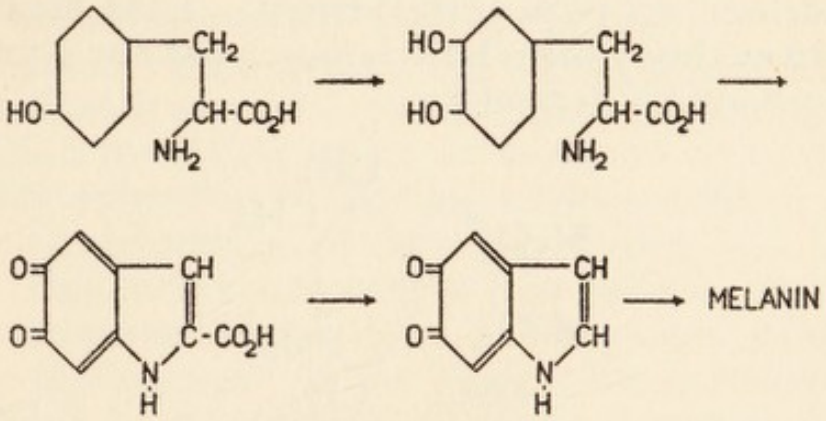
It might be considered that the instability of the catechol nucleus to oxidizing agents is connected with the greater activity of the more potent amines, but several facts are in conflict with this view. Ewins[1] found in the series 14–17 and 19–22 of Table I, that the methylamino bases are the most readily oxidizable, the amino bases the most stable; the propylamino bases were intermediate in stability between these two, whereas in activity they are much the weakest. Moreover, the very unstable pyrogallol bases (27 and 28) have no greater activity than their more stable catechol analogues.

INHIBITORY ACTION OF AMINES

We have, so far, discussed pressor activity only; but the rise of blood pressure, although mainly due to a stimulation of the muscular coat of the arterioles, is also affected by variations in the action of the heart and is not quite so simple an effect as might be desired. There are, however, a number of other sympathomimetic actions, some inhibitory instead of stimulative. A number of the amines were examined as to their power of inhibiting the spontaneous contractions of the isolated uterus, *i.e.* of causing its relaxation. While in general the amines, when tested by this method, fall in the same order of activity as their pressor actions, the concordance is not complete. Thus among the aminoacetocatechols the methylamino base (20) has a far greater inhibitor effect on the isolated virgin uterus than has the amino base (19), which is opposed to the order of their (stimulant) pressor activity. Of the two bases (14) and (15) the methylated one has both the stronger stimulative and the stronger inhibitory action. An explanation of the anomalous behavior of the ketone bases is impossible at present.

[1] J. Physiol., 40, 317 (1910).

In the few cases examined, it is quite clear that a side chain of two carbon atoms is much more effective than a shorter one; it seems also more effective than a longer one, but here in particular more evidence is desirable. One such piece of additional evidence was lately provided by an examination of 3,4-dihydroxyphenylpropylamine, $(OH)_2C_6H_3.CH_2.CH_2.CH_2.NH_2$, which Dr. J. H. Burn reports to have only one-third of the activity of its lower homologue, 3,4-dihydroxyphenylethylamine. That the amino group must be at a minimum distance from the phenyl group, so as to escape its influence, may be explicable on chemical grounds; but that a removal beyond this point should affect the activity adversely, is hardly sus-

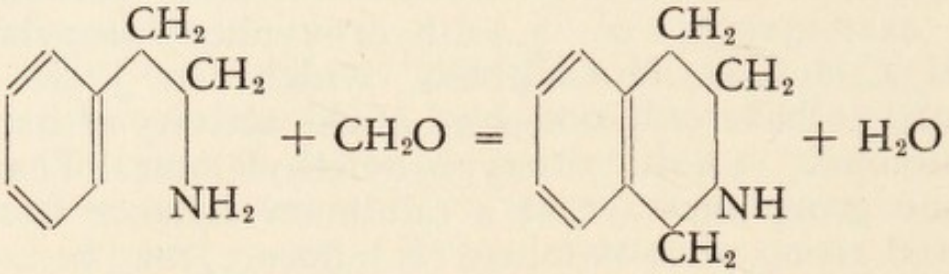


TYROSINE INTO MELANIN

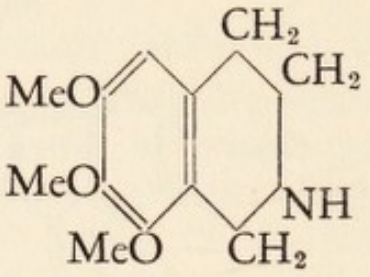
ceptible of a similar explanation. It would almost seem that the optimum side chain of two carbon atoms insures an optimum fit to some other molecular complex. It is significant that the optimum length of side chain is that occurring pre-formed in nature in the cyclic amino acids phenylalanine, tyrosine, tryptophan and histidine. This shows itself, of course, in the superior physiological activity of the amines derived from these amino acids. The side chain of two carbon atoms is, however, also the right length for forming a heterocyclic ring of five or six atoms. Thus Raper has recently shown that an oxidase converts tyrosine, by a most interesting series of reactions, into the orthoquinones of dihydroxyindolecarboxylic acid and of dihydroxyindole, and into melanin as shown above.[1]

[1] Biochem. J., **20**, 735 (1926); **21**, 89 (1927).

More frequently an additional carbon atom enters the heterocyclic ring, as in the case of many isoquinoline alkaloids. Phenylethylamine and formaldehyde yield tetrahydroisoquinoline.

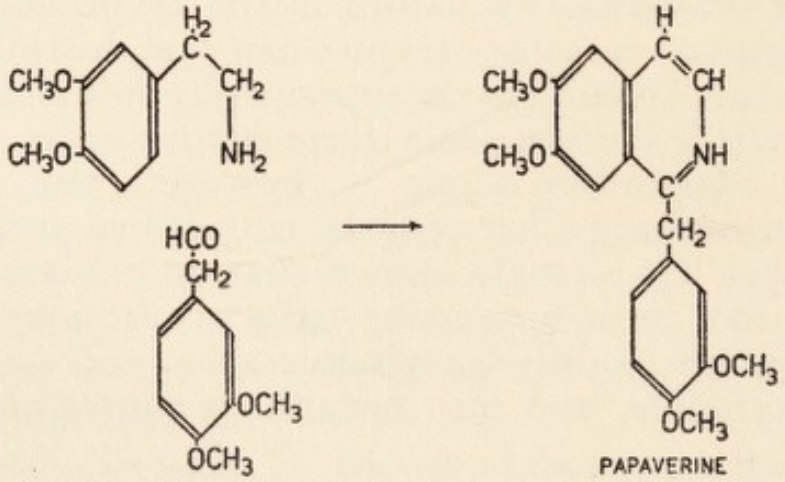


The numerous alkaloids of mescal buttons are either of this type or phenylethylamine derivatives which have not yet condensed up with formaldehyde. Thus mescaline is β-3,4,5-trimethoxyphenylethylamine, and the corresponding isoquinoline derivative



is the monomethyl ether of another alkaloid, anhalamine, occurring in the same plant.

Papaverine, an opium alkaloid, is built up from a more complicated aldehyde, 3,4-dimethoxyphenylacetaldehyde:



It might be thought, therefore, that amines with a side chain of two carbon atoms are physiologically active because they yield isoquinoline derivatives, not indeed with any simple aldehyde, because the known compounds of this type are hardly active, if at all.

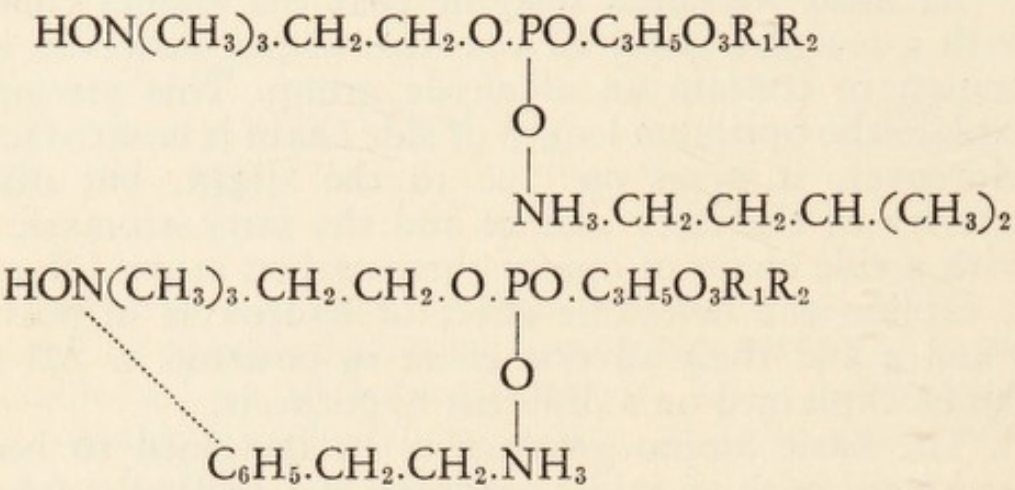
At most we could imagine that the amines condense with a reactive group of a protein which, however, is not known to contain an aldehyde group. This attempt to explain the optimum length of side chain is unsatisfactory. Moreover, it gives no clue to the slight, but distinct activity of the fatty amines and the fatty aromatic ones with a side chain of one or three carbon atoms. Nor does it explain the favorable effect of hydroxyls in positions 3 and 4 and their adverse effect in position 2. All these can be explained on a different hypothesis.

The basic amino group may be imagined to become associated with an acidic grouping in a molecule, whether by a union involving principal valencies or by means of secondary valencies or residual affinity, such as are considered to determine adsorption. Among the aliphatic amines this would constitute the whole process; but among the aromatic amines the acidic phenyl group would increase the strength of the association (combination or adsorption) by proximity to a basic group at a definite distance from the acidic one, and this distance would determine the optimum length of side chain of the amines. In a protein the grouping NH.CO.CHR.NH.CO does not correspond to the conditions laid down. A β-amino acid would be required, but the only one known to occur in the body (β-alanine, a constituent of carnosine) can hardly be invoked. Glutamic acid provides a better example of a basic group sufficiently remote from an acidic one, *e.g.* the glutaminyl group in a peptide,

$$\begin{array}{l} \text{NH.CO.}\underset{\displaystyle |\atop \displaystyle \text{NH}_2}{\text{CH}}\text{.CH}_2\text{.CH}_2\text{.COOH;} \end{array}$$

but a better example, and one more suitable for nervous tissue, is to be found in phosphatides such as lecithin and the latter substance may be used to illustrate the hypothesis.

In the case of isoamylamine we are concerned only with the attraction of the acidic phosphoric acid grouping for the amino group. In the case of phenylethylamine the attraction is increased by the affinity between the basic group of the lecithin and the phenyl group which has a



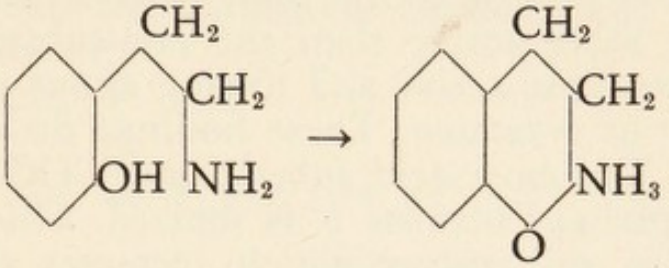
slightly acidic character. The optimum length of side chain would therefore be determined by the length of the choline grouping, each forming in space roughly half a hexagon. The acidic character of the phenyl group is well known to organic chemists through such reactions as the action of sodium on benzyl cyanide, $C_6H_5.CHNa.CN$, where it replaces one of the hydrogen atoms of the methylene group. Such a replacement takes place only if the methylene group is situated between two acidic groupings, *e.g.*

$CN.CH_2.COOEt$, $CH_3.CO.CH_2.COOEt$,
$CH_3.CO.CH_2.COCH_3$, $EtOOC.CH_2.COOEt$,

all of which yield sodium compounds.

The acidic nature of the phenyl nucleus, however, is greatly enhanced by a hydroxyl, as shown by the synonym for phenol, "carbolic acid." The greater sympathomimetic action of tyramine as compared with phenylethylamine, would therefore be due to the more strongly acidic nature of the hydroxylated benzene ring of the former compound. A second hydroxyl in the 3-position increases the effect. Catechol is known to form a number of coördination compounds, is precipitated by lead acetate, and the like. A hydroxyl in the 2-position, however, has the opposite

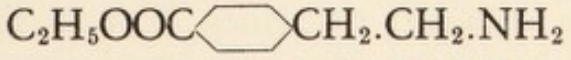
effect for it has a tendency to combine with the amino group of the amine to form a phenol betaine,

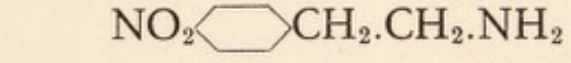


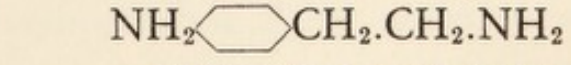
so that the affinity of the amine for the phosphoric acid portion of lecithin is decreased. Lecithin will also serve as an example of an asymmetric molecule which is fitted better by *l*-adrenaline than by the *d*-variety. A model shows that in the one case the alcoholic hydroxyl of adrenaline points away from the fatty acid residues of lecithin, in the other and perhaps less favorable arrangement, it points towards them. From what is known of the behavior of a film of oleic acid on a water surface it has been deduced that the hydroxyl (carboxyl) group is directed towards the water and the hydrocarbon chain is directed away from it.

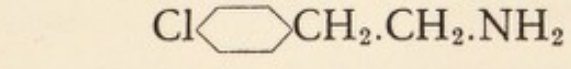
We must, finally, consider the manner in which the *N*-alkyl groups modify the activity of the amines. These groups probably determine the strength of the amines as bases. In this respect the amino group is known to be the strongest, the propyl group the weakest. Since the phenolic groups make the molecule more acid, the same *N*-alkyl group may not always produce the base of optimum strength, when phenolic hydroxyls are absent or present. In order to test the above hypothesis of the factors which determine the physiological activity of phenylethylamine derivatives I have had the following prepared (some are new):

(1) 

(2) 

(3) 

(4) 

(5) 

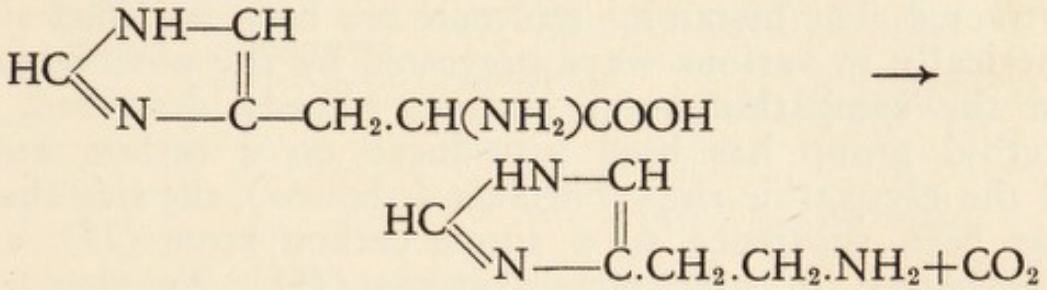
Dr. J. H. Burn has kindly examined their pressor action, and finds that the first has no pressor action; (2) and (3) have a smaller pressor action than phenylethylamine; (4) and (5) are more active than the non-substituted base, (5) being the most active and having about one-third of the activity of tyramine. These findings do not support the theory. The most acid substituent (COOH) leads to inactivity, perhaps because it is ionized. Chlorine is the only negative substituent which increases the activity of a phenyl nucleus (and that much less than a phenolic hydroxyl). Moreover the enhanced activity of the phenyl group, due to a basic amino group, is contrary to the theory outlined above. Other factors must be at work, which at present escape us.

AMINES WITH A CYCLIC NUCLEUS OTHER THAN PHENYL

After the recognition of the physiological activity of the amines already discussed, Dale became impressed by its similarity to the pressor effect of certain ergot extracts and we succeeded in isolating small quantities of isoamylamine and tyramine from them. Since some ergot extracts also show a powerful stimulant action on the isolated uterus, of a nature different from that due to the characteristic alkaloid ergotoxine, and since, as we have seen, the pressor amines have an inhibiting effect on the uterus, we continued our search for active principles.[1] The substance in question could not be extracted from aqueous solution by amyl alcohol which, as we have seen, removes tyramine and similar amines. The use of solvents immiscible with water being of no avail, we had recourse to precipitation methods. After removal of the ergotoxine, the uterine stimulant could still be precipitated as phosphotungstate, showing it to be a base, and after regenerating from its phosphotungstate it could be precipitated with mercuric chloride and by silver nitrate in alkaline solution. It was thus obtained in the so-called histidine fraction as a silver compound, from which a crystalline dipicrate was finally obtained, giving the color reaction with sodium diazobenzenesul-

[1] J. Chem. Soc., 97, 2592 (1910).

phonate, found by Pauly to be characteristic of histidine. Our previous work led us to suppose that we were dealing with β-iminazolylethylamine, formed from histidine by decarboxylation:

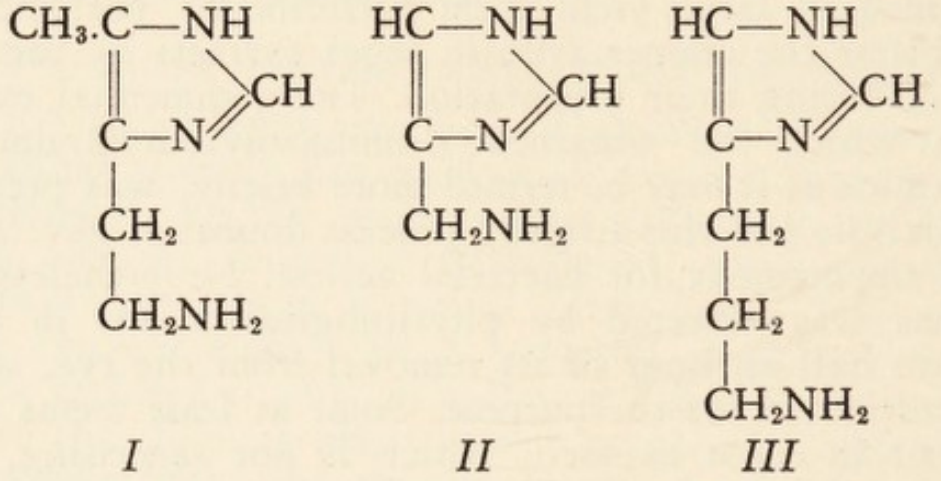


The supposition was soon confirmed by comparison with a substance which, just at that time, Ackermann had obtained in large yield from histidine by putrefaction. Doubtless the amines arise in ergot extracts by bacterial action during their preparation. The commercial extract from which we obtained β-iminazolylethylamine, or histamine as it may be termed more briefly, was prepared by dialysis and this lengthy process doubtless gave abundant opportunity for bacterial action. Nevertheless histamine was detected by physiological means in ergot within half an hour of its removal from the rye, which we cultivated for the purpose. Some at least seems to be present in ergot as such, which is not surprising, considering that ergot, a fungus, differs in its chemistry from the green plants and approaches more nearly to bacteria. Moreover, it was shown much later by Abel and Kubota that histamine occurs in the higher animals;[1] the lungs contain a notable amount;[2] muscle also contains some.[3]

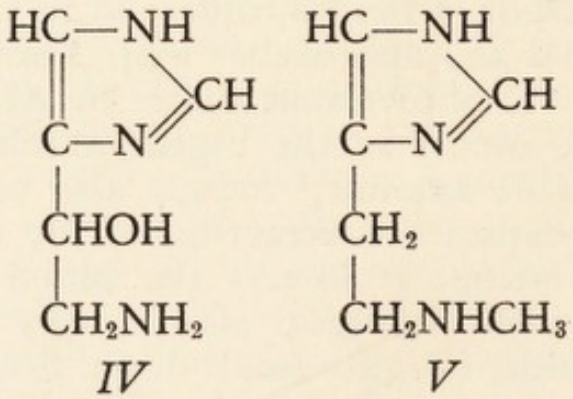
Histamine causes a contraction of the isolated uterus at extreme dilution; it lowers the blood pressure, and produces other physiological effects, very different from those of tyramine, in quite small doses. Strangely enough the substance was first obtained synthetically (by Windaus and Vogt),[4] and its physiological action remained unsuspected until it was found in ergot. Unlike the sympathomimetic amines, histamine is almost insoluble in

[1] J. Pharmacol., 13, 243 (1919).
[2] Best, Hale, Dudley and Thorpe, J. Physiol., 62, 397 (1927).
[3] Thorpe, Biochem. J., 22, 94 (1928).
[4] Ber., 40, 3691 (1907).

lipoid solvents but very readily soluble in water. On the hypothesis outlined above, the iminazolyl (glyoxaline) nucleus would not be attracted by the basic grouping of lecithin in the same way that a benzene nucleus might be attracted. The histamine molecule has been modified synthetically in various ways suggested by the observations on the sympathomimetic amines already described. A methyl group has been introduced on a carbon atom of the glyoxaline ring (Formula *I*, below), the side chain has been shortened to a single carbon atom (*II*), and lengthened to three carbon atoms (*III*). An alcoholic hydroxyl has also been introduced into the side chain (*IV*), and a methyl group has been added to the nitrogen of the



I *II* *III*



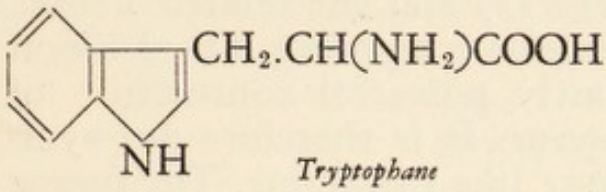
IV *V*

side chain (*V*). All these, and other modifications prepared more recently by van der Merve,[1] were much less active than histamine itself or quite inactive.

Tryptophane has been decarboxylated by Ewins and Laidlaw[2] to indolylethylamine, and the base has been

[1] Z. physiol. Chem., 177, 301 (1928).
[2] Proc. Chem. Soc., 26, 343 (1910).

synthesized by Ewins.[1] It resembles phenylethylamine and



Tryptophane

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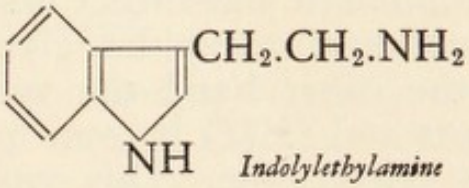
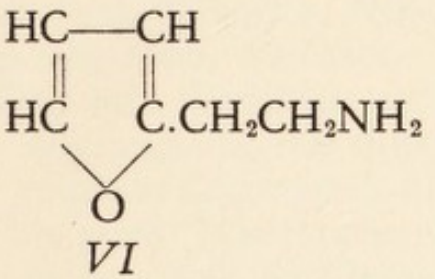


Indolylethylamine

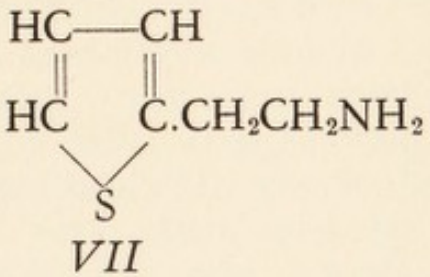
tyramine in its activity to the extent that it causes a rise of blood pressure, but in other respects behaves differently. It has been manufactured in Japan for medicinal use. Chemically it resembles the derivatives of phenylethylamine, since the indole nucleus is non-basic. Lysine is decarboxylated in putrefaction to pentamethylene diamine, and arginine is represented by agmatine (guanidylbutylamine) which occurs in fresh herring roes and in ergot. The physiological activity of these bases is slight and has not been fully investigated; it is certainly not sympathomimetic.

Quite a number of amines consisting of a heterocyclic nucleus and a side chain of two carbon atoms, have been synthesized in analogy to tyramine and histidine, but their physiological activity is very slight or has not been investigated.

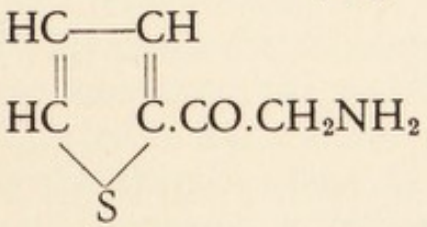
Thus Windaus and Dalmer[2] prepared furylethylamine



VI



VII



VIII

[1] J. Chem. Soc., **99**, 270 (1911).
[2] Ber., **53**, 2304 (1920).

(Formula *VI*) and Easson recently synthesized, in my laboratory, thienylethylamine (*VII*) and the related ketone (*VIII*). Furylethylamine causes a temporary fall of blood pressure in the cat and a fairly powerful contraction of the isolated guinea-pig's uterus. It is therefore not sympathomimetic but rather more like histamine. The furane nucleus here resembles glyoxaline rather than benzene. On the other hand the two thiophene derivatives were pressor and (*VII*) is even quantitatively quite similar to phenyethylamine. This would suggest that the action dependls on physical properties, in which, as is well known, thiophene derivatives closely resemble the corresponding benzene compounds.

CHAPTER IV

Chemotherapy

As the name implies, chemotherapy literally means healing by chemistry; it would therefore seem to include most kinds of treatment in which material substances are administered and would exclude only physical methods of healing, such as electrical or light treatment, remedial gymnastics, and the like. In practice, however, the term chemotherapy is restricted to the administration of a class of drugs on which, in most cases, a great deal of chemical work has been done; they are predominantly synthetic substances, or complicated alkaloids and their derivatives. This chemical restriction excludes simple inorganic salts and crude plant extracts. There is, however, a further and more important therapeutical restriction. Chemotherapy does not aim at merely combating symptoms or stimulating particular organs; the administration of antipyrine to lower the body temperature, or of strophanthin to stimulate the action of the heart, is not chemotherapy as usually understood, for the above mentioned therapeutical restriction limits it to an attack on the specific parasites, mostly protozoa or bacteria, which cause infectious diseases. The substances used in chemotherapy are therefore antiseptics, and since they must enter the body of the host, they differ greatly from ordinary external antiseptics like mercuric chloride or phenol.

The term "chemotherapy" was introduced by Paul Ehrlich and may be illustrated by his great achievement, the discovery of salvarsan for the treatment of syphilis. Salvarsan is a synthetic substance, the result of much chemical work, and when injected it has a toxic effect on the microorganism which is the specific cause of the disease.

Chemotherapy is, therefore, a peculiar branch of pharmacology based on the coöperation of organic chemistry with protozoology and bacteriology. Like other branches

of pharmacology, chemotherapy suggests a comparison between the chemical constitution of its drugs and their pharmacological action. It may be said at once that here this comparison leads to hardly any general relationships between the two sets of properties, chemical and physiological. In the older pharmacology these relationships have often been exaggerated; yet there are a number of well-marked cases where a particular action must be attributed to a particular group and the result of changes in the rest of the molecule may be traced in a systematic manner, giving at least some scope for prediction, and in any case resulting in the marketing of a large number of synthetic drugs. Thus nearly all local anesthetics are bases containing an ester grouping, and the number which have found practical application at one time or another is large. With chemotherapy in the restricted sense the case is very different. Salvarsan is stated to have been No. 606 in a series of substances which were made and tested in a research of which it was the only practical result, and in spite of much further work the number of arsenicals introduced into medical practice remains very much smaller than that of the local anesthetics, hypnotics or external disinfectants. The reason is to be sought in the limitations which were indicated in defining the scope of chemotherapy. Here we are not concerned with action on any particular structure (sensory nerve ending in the case of local anesthetics) or action on a bacillus outside the body (wound disinfection). In both these cases the toxic effect on the patient is restricted by limited absorption.

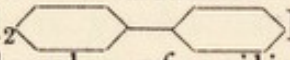
Chemotherapy sets itself the problem of combating a parasite in the tissues of the body; the therapeutic agent must be absorbed and must reach the parasite, yet its toxicity to the host must be small. We require a preferential attack on the parasite. As Ehrlich put it, the agent must be parasitotropic as much as possible, and organotropic as little as possible. The minimum curative dose C necessary to exterminate the parasite must exceed the maximum dose T tolerated by the host, as much as possible; the ratio C/T must be small. It is called the thera-

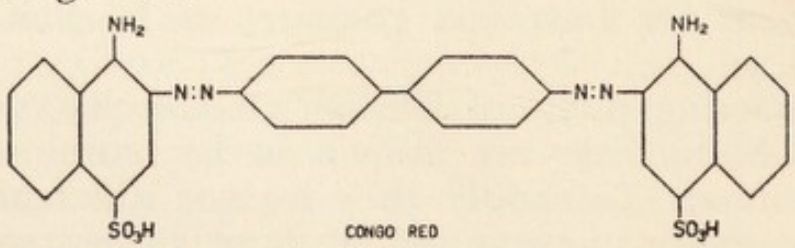
peutic index. (As it is more convenient to deal with whole numbers than with fractions it might have been better to use the reciprocal of this number, T/C, which should be as large as possible). We know little about the manner in which chemical constitution influences the toxicity to the parasite; we are likewise very ignorant about the effect of this constitution on the toxicity to the host. Hence it is not surprising that our ignorance concerning the effect of chemical constitution on a ratio involving both toxicities is profound.

There are other complications. Substances are known which have a very powerful antiseptic action on bacteria in ordinary culture media such as peptone broth. Malachite green acts thus in a concentration 1 in 10 millions on *Staphylococcus*. These substances are, moreover, not very toxic to the host. It would seem that not much more than half a milligram of malachite green would suffice to kill all the staphylococci in the 5 liters of blood circulating in an adult. Yet this is by no means the case, for in the presence of serum the concentration required for antisepsis is 250 times as great. Likewise mercuric chloride has to be 100 times more concentrated in serum than in water, in order to kill off staphylococci. No doubt the serum proteins adsorb the antiseptic very largely so that it is much more active *in vitro* than *in vivo*. In other cases the opposite is true. Many arsenic compounds have little or no action on protozoa *in vitro* and yet are effective *in vivo*. It seems probable that they are converted by oxidation (or by reduction) into the much more toxic arsine oxides, and for this conversion the tissues of the host are responsible. This change in the antiseptic, due to the host, introduces yet another complication into chemotherapy and shows that it is not merely a question of the relative affinities of the therapeutic agent for host and parasite, as Ehrlich at first imagined.

The starting point of Ehrlich's researches was vital staining. Many dyes are known to be harmless to the higher animals. Generally they do not differentiate between the various organs and are fugitive, but some dyes

pick out particular structures and stain them for a long time. On killing the animal, the special affinity of the dye for particular cells is thus demonstrated *ad oculos*. Robert Koch had already found malachite green to be an antiseptic and when Ehrlich and Guttmann[1] found that methylene blue, a vital stain, strongly dyes the malarial parasite, the idea suggested itself of finding a vital stain which would kill protozoa by its great affinity for them. The best results were obtained with trypanosomes and azo dyes derived from benzidine and related to the well-known indicator Congo red. In general azo dyes are formed by the union of a diazonium compound with an amine (or phenol); they therefore consist of two benzene rings united directly by the azo group —N=N— which is called chromophoric since it confers color on the molecule. Thus azobenzene, $C_6H_5.N{:}N.C_6H_5$, has a deep red color, but since it has no acidic or basic group attached, it is not a dye; *p*-aminoazobenzene, $NH_2.C_6H_4.N{:}N.C_6H_5$, formed by the union of aniline with benzenediazonium hydroxide, $HON{:}N.C_6H_5$, however, is already a very simple azo dye.

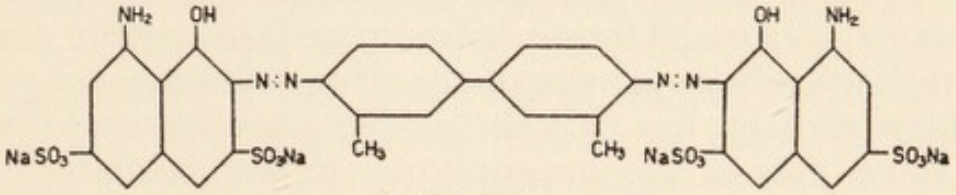
Congo red is built up on the same principle. Instead of a diazonium salt from aniline we use a substance in which this grouping occurs twice, for instance, tetrazotized benzidine. The base (*p*,*p*′-diaminodiphenyl),  , may be regarded as made up of two molecules of aniline, by the loss of two hydrogen atoms. When benzidine is treated with hydrochloric acid and sodium nitrite there results the tetrazotized substance $ClN{:}N.C_6H_5.C_6H_5.N{:}NCl$. Now when the latter is combined in alkaline solution with two molecules of an amine, a bisazo dye results and if we choose 1-aminonaphthalene-4-sulphonic acid (naphthionic acid) as amine, we obtain Congo red:



CONGO RED

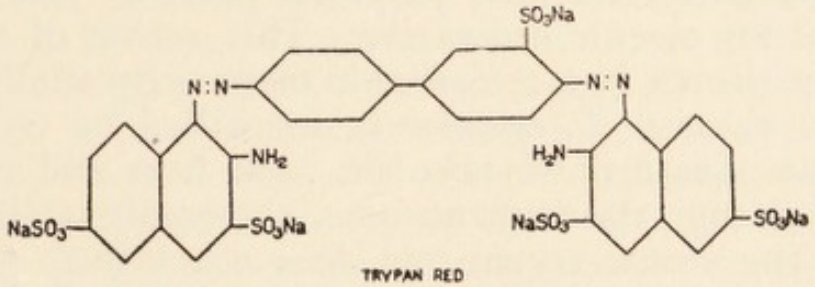
[1] Berl. klin. Wochschr., p. 953 (1891).

Congo test paper is impregnated with the red sodium salt, from which mineral acids liberate a blue acid. The first dyes to be useful against trypanosomes were trypan blue and trypan red. The former is derived not from benzidine, but from its dimethyl derivative tolidine, and the other component is 8-aminonaphthol-3,6-disulphonic acid



TRYPAN BLUE

The latter, like Congo red, is derived from benzidine; the naphthalene component is 3-aminonaphthalene-2,7-disulphonic acid, which introduces four sulphonic acid groups into the molecule, but this was not enough and a fifth one was introduced into the benzidine nucleus.



TRYPAN RED

We see that the dyes of this class have a high molecular weight; they are colloids or semi-colloids. Some are readily precipitated from solutions by electrolytes, they are hydrophobic or suspension colloids, like colloidal gold. Others are more soluble; they form true solutions, or they are hydrophilic colloids like starch. It is only the latter which can serve as vital stains. We see that in trypan blue greater solubility (as compared with Congo red) has been attained by the introduction of two extra sulphonic acid and two phenolic groups, and in trypan red by three extra sulphonic acid groups. The colloidal properties may, however, be modified by trifling changes in the position of the substituent groups in the naphthalene nucleus, one isomeride being hydrophilic and a vital stain, the other hydrophobic and incapable of vital stain-

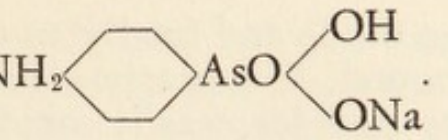
ing. We have here a fairly obvious connection between physiological and colloid (physical) properties, but how the latter depend on chemical constitution remains obscure. It is therefore very difficult to deduce the physiological properties of a drug from its molecular architecture. Influenced by the older and purely chemical theory of dyeing, Ehrlich attributed pharmacological action to a chemical union between certain specific anchoring or haptophore groups of the drug and receptor groups of the organism, but this theory becomes less useful when the same groups are haptophores in one position in the molecule and cease to be so in another position.

While it is difficult to predict whether a dye will be a vital stain, and still more difficult to foresee whether it will then kill trypanosomes, notable results have been achieved by the method of trial and error. Trypan red is inactive on trypanosomes *in vitro* but kills *T. equinum* in mice. Evidently the host plays its part, as was already indicated for arsenic derivatives. This action of the host is further shown in a remarkable manner by the fact that the same parasite *T. equinum* is not killed by trypan red in the rat. Again if we take the same host and the same dye and change the trypanosome, the result is also negative; in the mouse trypan red does not kill *T. brucei* (of nagana). Trypan blue has to some extent been used in curing dogs and cattle of a parasite infecting the red blood corpuscles (*Piroplasma*) but the practical value of these dyes has been slight. They were chiefly important as a starting point for further work leading to salvarsan and Bayer 205.

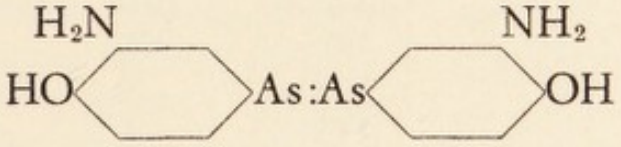
After studying dyes, Ehrlich turned his attention to arsenic derivatives, and more particularly to a supposed anilide of arsenic acid, discovered long ago by Béchamp[1] and later found to have some effect in temporarily driving trypanosomes out of the blood in sleeping sickness. Ehrlich and Bertheim[2] established in 1907 that the commercial substance atoxyl has not the constitution:

[1] Compt. rend., **56**, I, 1172 (1863).

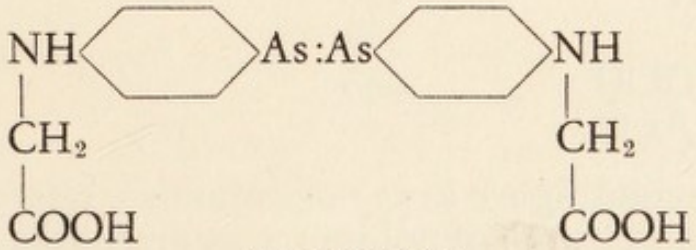
[2] Ber., **40**, 3292 (1907).

$C_6H_5.NH.AsO(OH)_2$, but is  . It is

not hydrolyzed by acids, as an anilide should be, but has the arsenic directly united to carbon in the *para*-position to the amino group. This chemical discovery opened up the possibility of modifying the structure of the atoxyl and many modifications were tested during the next few years resulting in the discovery of salvarsan. Atoxyl has hardly any action on trypanosomes *in vitro* and Ehrlich considered that its action *in vivo* might be due to a more active reduction product. Arsenious trioxide is much more toxic than arsenic pentoxide. The first stage of reduction of an arsonic acid, $R{\cdot}AsO(OH)_2$, leads to a substituted oxide, $R{\cdot}AsO$, in which the arsenic has become trivalent, and the substances of this class were invariably found to be much more toxic than the corresponding arsonic acids, more toxic to the host as well as to the parasite. The ratio of curative to tolerated dose C/T did not become sufficiently low, however, and so Ehrlich proceeded to a still further reduction, resulting in a new class of substances $R{\cdot}As{:}As{\cdot}R$ called arseno compounds in analogy to $R{\cdot}N{:}N{\cdot}R$, the azo compounds. $C_6H_5{\cdot}As{:}As{\cdot}C_6H_5$ is arsenobenzene. Two members of this class have proved particularly useful:



Salvarsan



Arsenophenylglycine

The synthesis of salvarsan is carried out by nitrating oxalyl atoxyl, removing the oxalyl group, exchanging the amino group for hydroxyl, and reducing to the hydroxy

amino oxide and finally to the arseno stage. The resulting compound, like nearly all arseno compounds, is an amorphous powder, easily oxidized and insoluble in water. For injection it is usually converted before use into the hydrochloride; the phenolic sodium salt, carefully protected from oxidation, is also manufactured. Solubility may further be conferred on the molecule by changing one or both of the amino groups to $NaSO_2.CH_2.NH$, methylene sulphoxylate. A mixture of the two compounds which thus result is known as neosalvarsan.

The toxicity of the arseno compounds is usually intermediate between that of the corresponding arsonic acids and oxides; occasionally the arseno compound is the least toxic of the three stages of oxidation. In the most favorable cases the ratio C/T becomes low enough for practical use (C/T for rabbit's syphilis: 1/10 for salvarsan, 1/2 for arsenophenylglycine). The following Table II[1] gives the lethal dose of elementary arsenic in milligrams for one kilo of mice, and the amount of arsenic present in various states of oxidation, viz., as arsonic acid, as the corresponding oxide and as arseno compound. R is the group $C_6H_4.AsO_3H_2$ (or $C_6H_3.AsO_3H_2$).

TABLE II

Lethal Dose of Elementary Arsenic per Kilogram of Mice

	Arsonic acid mg.	Oxide mg.	Arseno compound mg.
R OH	305	2	30
R NH_2	115	2	50
R $NH.CH_2.CO_2H$	900	20	315
R $(NH_2)OH$	530	8	90
R CO_2H	270	2	25
R (NH_2) COOH	640	—	60
Arsenic as As_2O_3	—	10	—

Thus the second figure (115) of column 1 refers to atoxyl, the third figure (315) of column 3 to arsenophenylglycine, the fourth figure of column 3 to salvarsan. It will be seen that the oxides are much the most toxic group, and become less so both by oxidation and by reduction. A given

[1]Sieburg, Z. physiol. Chem., 97, 53 (1916).

quantity of arsenious oxide can be rendered five times as toxic in some organic combinations, and may have its toxicity reduced to 1/90 in another. The differences in the toxicity of these three classes of arsenic compounds has led most investigators to assume that both the acids and arseno compounds are converted, in the body, into the oxides. Levaditi found that the acids mixed with liver substance are transformed into a substance trypanocidal *in vitro*.

Voegtlin has put forward an interesting theory according to which the great toxicity of the arsenoxides is due to their combination with sulphydryl groups, particularly with glutathione (glutaminylcysteine), which is thereby prevented from fulfilling its function in tissue respiration. The reaction which takes place is probably:

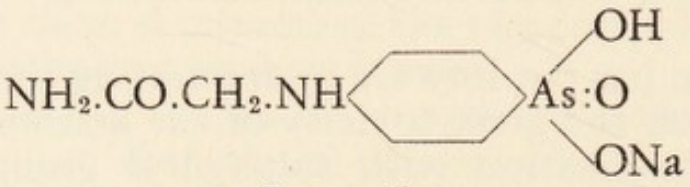
$$R\,As{:}O + 2\,HSG = R\,As(SG)_2 + H_2O$$

where G stands for

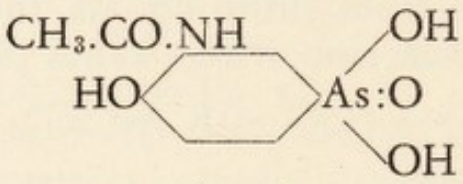
$$CH_2.CH(COOH)NH.CO.CH_2.CH_2.CH(NH_2)COOH.$$

He finds that arsenoxides kill much less rapidly when injected together with a sulphydryl compound like sodium thioglycollate, than when injected alone. Feeding an animal with fairly large amounts of the two constitutents of glutathione, cysteine and glutamic acid, will give considerable protection against a lethal dose of arsenoxide, given a few hours later. So much for the effect on the host. Trypanosomes also have sulphydryl groups, judging from a positive nitroprusside reaction, and the injection of reduced glutathione or other compounds with the SH group protects the SH groups of the trypanosomes, so that they disappear much more slowly from the blood. The effect of the arsenic compound is delayed, as is the case normally with salvarsan. There can be no doubt that when salvarsan is injected hypodermically it is precipitated at the site of injection and remains there as a depot from which a toxic substance is slowly given off, holding the parasite in check. If the injection is made into the tail of an infected rat, and the tail is later amputated, the parasite once more gains the upper hand.

We have seen that Ehrlich soon gave up attempts to find a suitable arsonic acid and passed on to reduced compounds. The low toxicity of arsonic acid is, however, no objection; it is the therapeutic index which matters. Of late years an extensive systematic study of arsonic acids has been undertaken by Fourneau[1] and his collaborators, resulting in the discovery of two useful agents:



Tryparsamide

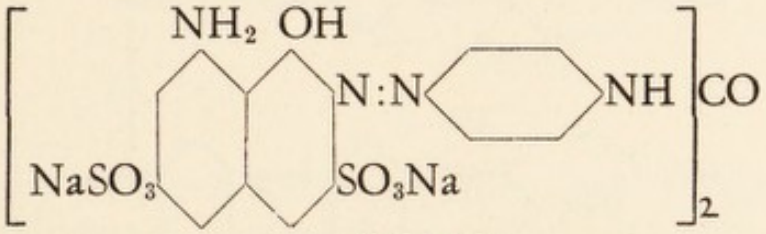


Stovarsol

As Fourneau points out, salvarsan fortunately proved to have an activity possessed by none of its isomerides, but this activity should not preclude a search among the arsonic acids, which have moreover the great advantage of being crystalline substances readily purified. The amorphous arseno compounds may be contaminated with varying amounts of oxide, according to the method of preparation, so that the results recorded in the literature are not always reliable. Fourneau insists on the importance of the phenolic hydroxyl for therapeutic action; this is present (in the *p*-position) in salvarsan and in stovarsol. For stovarsol it is claimed that it cures syphilis on oral administration; it has also been used in amoebic dysentery. Salvarsan, although chiefly used against the spirochaete of syphilis, is also active in some trypanosome infections, such as yaws, relapsing fever and ratbite fever. It is not efficient in sleeping sickness which disease is also due to a trypanosome; here the best arsenical seems to be tryparsamide referred to above, but the most promising drug of all seems to be Bayer 205 or Germanin.

[1] Ann. Inst. Pasteur, 37, 551 (1923); 38, 81 (1924).

The promise of Bayer 205 lies not so much in its actual performance as in its being a new point of departure in chemotherapy. The substance does not contain arsenic or any poisonous element, and in this respect resembles the dyes trypan red and trypan blue, the latter having already been used in certain cattle diseases. It is said that since stained meat is unsaleable, the idea suggested itself of producing colorless substances of the type of trypan blue. Here the several parts of the molecule are joined by the grouping —N=N— which confers on the molecule the undesirable color, but has nothing to do with the trypanocidal action. Hence the grouping NH.CO was used instead. This grouping had already been used in a dye, trypan (or afridol) violet,

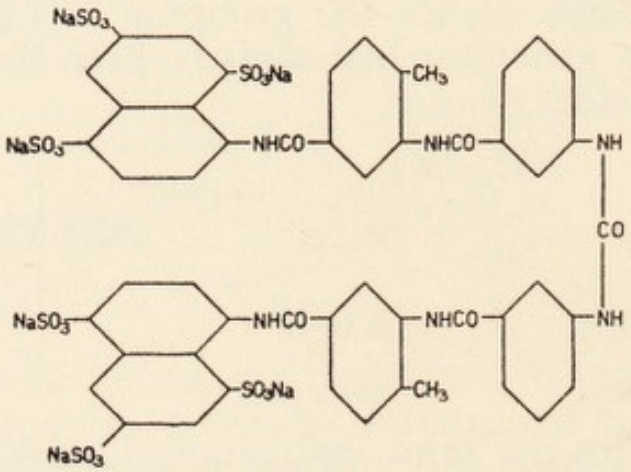


Afridol violet

which contains the same aminonaphtholdisulphonic acid as trypan blue, but differs in having as central nucleus diphenylurea and not tolidine. The new principle evolved in Bayer 205 is to substitute also the azo groups by an amide grouping, and from 1912 onwards appeared a large number of patents referring to substances obtained by condensing aminonaphthalenesulphonic acids with the chlorides of nitrobenzoic acid. In the resulting compound the nitro group is then reduced, and the amino group thus generated may be again combined with a nitrobenzoyl chloride, from which a further amino compound is obtainable. Finally, by the action of phosgene two amino compounds are united to a urea.

After the war, in 1920, it became known that the Bayer works, after a long series of tests, had obtained a substance No. 205, which was stated to be a powerful trypanocide with an extremely low therapeutic coefficient (1/100 or less in small animals). The constitution of this substance was kept secret. At the time the reason for this

secrecy, previously not observed in medical publications, was stated to be the violation of patent rights by Germany's late enemies, particularly by the United States, but now such secrecy seems to be connected with the fact that in certain European countries a therapeutic substance is not patentable. Later Fourneau examined a large number of substances of the kind indicated in the German patents, and found one which in physiological and chemical properties very closely resembles Bayer 205 and is now generally regarded as identical with it. Its constitution is as follows:



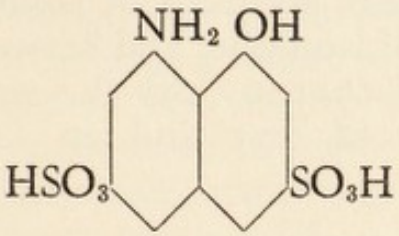
FOURNEAU 309

Fourneau 309 = Bayer 205

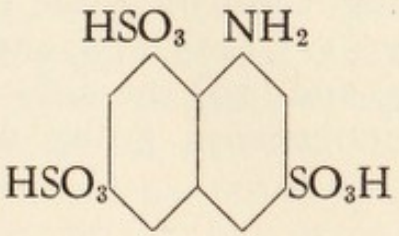
Since there are no German publications indicating how this substance was arrived at, I tabulate (Table III) some of Fourneau's experiments which led to its identification, in order to give at least some idea of the nature of this branch of chemotherapeutical research.

The substance is a symmetrical urea and a polypeptide. On hydrolysis it yields three different amino acids, as well as carbon dioxide. One of the amino acids is an amino-napthalenesulphonic acid belonging to a very numerous group of substances of great technical importance for the production of azo dyes. So numerous is this group that only a small number of expert dye chemists are really familiar with it. (We have seen that Bayer 205 arose out of an attempt to make "colorless trypan blue"). Of the azo dyes to which I have already referred, Congo red, trypan red, and trypan blue all contain different naphthalene-

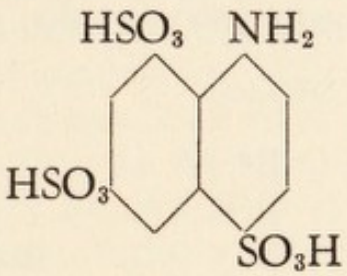
sulphonic acids. The one from the last-named dye, also present in trypan violet, is the common 8-aminonaphthol-3,6-disulphonic acid, usually called "H" acid, and this



'H" acid



Koch's acid



"B" acid

Fourneau used as a starting point. Later he used 1-amino-naphthalene-3,6,8-trisulphonic acid (Koch's acid) and finally 1-aminonaphthalene-4,6,8-trisulphonic acid which, since it occurs in Bayer 205, we may term "B" acid. The experiments to be quoted refer to the coupling of these naphthalene acids with *meta-* or *para*-amino derivatives of the following benzene acids: benzoic (B), benzene-sulphonic (S), phenylacetic (P), *p*-hydroxybenzoic (H), *p*-toluic (T). Where the amino group is in the para position to the acid grouping, we distinguish it by an asterisk; thus B* stands for *p*-aminobenzoic acid. The *meta*-amino derivatives appear to be more active and were mostly employed by Fourneau; these we represent by a single letter, without asterisk: thus T stands for 3-amino-4-methylbenzoic acid. Since all the compounds under discussion are symmetrical ureas, we write the groupings in one half of the molecule only.

It will be seen that the optimum number of benzene acids in each side of the molecule appears to be two. Exceptionally (10) is slightly better than (9). In general the *para*-nitro acids are less efficient than the *meta* ones. The methyl group in toluic acid seems to have a favorable effect (it

is also present in trypan blue; in some simple synthetic drugs its effect is distinctly adverse). The hydroxy group (in 17) produced low toxicity. The most important feature brought out by Table III is the great influence of isomerism; (11), (13), (19) and (20) are all isomeric, and between (19) and (20) the only difference is that in (19) the *meta*-aminobenzoyl group was introduced first and in (20)

TABLE III

Trials leading to Identification of Bayer 205 (Fourneau 309) (= No. 20)

	Constituents of compound		C/T	Toxic dose for a 20-gm.-mouse mg.
1	"H" acid	B* B*	1	12
2	"	B B	5/12	12
3	Koch's acid	B	inactive	—
4	"	B B	1/3	15
5	"	B* B*	inactive	—
6	"	B* B* B	inactive	—
7	"	B* B	1/3	15
8	"	B B*	inactive	15
9	"	S B	1	10
10	"	S B B	1/2	10
11	"	P B	inactive	10
12	"	T T	> 1/3	15
13	"	T B	1/12	12
14	"B" acid	T T	1/2	10
15	"	T T T	inactive	10
16	"	T T B	hardly active	10
17	"	H B	slightly active	25
18	"	B B	1/12	12
19	"	B T	1	4
20	"	T B	1/160, 1/300	10

last. Number 20 (Fourneau 309) is regarded as identical with Bayer 205 or Germanine. Here the chemotherapeutic coefficient has suddenly dropped to a very low value. It should be borne in mind that this is for mice and nagana (*T. brucei*), the host and parasite most convenient for laboratory infections. For other trypanosomes the

ratio C/T may be higher. Wenyon gives 1/60 to 1/100 for *T. equiperdum* in mice; and *T. lewisi*, a non-pathogenic trypanosome, is not seriously affected by the drug. It is commonly found that C/T increases with the size of the host. It may amount to 1/10 for the rabbit. Hence when it comes to the main problem of treating sleeping-sickness (*T. gambiense*) in man, a higher ratio C/T and less satisfactory results are to be expected. At first human sleeping-sickness was stated to be cured completely, but more recently all the patients originally treated are said to have died so that, as not infrequently happens, the first estimate of the drug's value may have been too favorable. Yet a very distinct advance has been made.

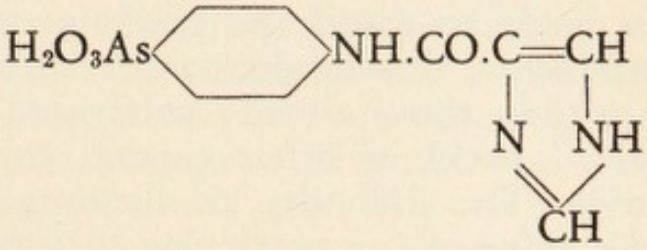
In contradistinction to compounds containing arsenic and antimony, Bayer 205 does not eliminate trypanosomes from the blood very rapidly; their disappearance may take 48 hours, and does not seem due to a direct lethal effect so much as to an inhibition of reproduction (fission). Incompletely divided individuals are found in the blood. The drug is eliminated very slowly and may persist in the blood for weeks, the serum showing a trypanocidal action; thus it is possible to confer some degree of passive immunity on an animal. A glance at Table III, will show that the toxicity to the host, of the 20 compounds tabulated, does not vary enormously; the great range in the chemotherapeutic quotient is therefore primarily due to variations in the effect on the parasite. It is interesting to speculate on the nature of this effect. It can hardly be determined by an ordinary chemical union through haptophoric groups; the only groups which could act in this sense are the sulphonic acid groups, but in the last two compounds tabulated they are both equal in number and arranged in identical fashion in the naphthalene nucleus. The whole difference between (19) and (20) lies in the two methyl groups, which are attached to different benzene rings in these two isomerides. Ordinary chemical forces will not account for the great biological difference, which reminds one of the specificity of enzyme action and of adsorption phenomena. It would appear that the whole molecule must have a par-

ticular shape, to fit some structure of the trypanosome, and that the activity of Bayer 205 is closely connected with its colloidal properties. That the substance is colloidal may be inferred at once from its molecular weight ($C_{51}H_{34}O_{23}N_6S_6Na_6$ = 1428; the free acid = 1296) which is greater than that of Fischer's synthetic octadecapeptide. Further, like peptone, Bayer 205 makes the blood less readily coagulable, and it precipitates certain basic colloids (clupein) at very great dilution. It might become possible by some such physical test as this to reject, without animal experiment, unsuitable compounds in future researches on this subject. An analogy to this problem is provided by the case of dyes from tetrazotized benzidine, such as Congo red and trypan blue. These are substantive dyes, *i.e.* they dye cotton without a mordant; here also it is impossible to point to chemical union between the dye and cellulose. Although there is among these dyes none corresponding to the specificity of Bayer 205, their colloidal properties may be completely changed by small changes in chemical constitution; one of two closely related isomerides may be hydrophobic, the other hydrophilic.

The above outline has been given mainly to indicate such hints at generalization as seem possible. The generalizations are necessarily very tentative; the number of substances studied, although considerable, is very small compared with the possibilities which readily suggest themselves. The above outline involved the mention of three naphthalene and of five benzene acids. These alone could furnish $3 \times 5 \times 5 = 75$ ureas of the type discussed. It has already been pointed out that the number of technical naphthalenesulphonic acids is enormous; the number of benzene acids could be increased by introducing halogen, methoxy, ethoxy, and many other groups in various positions. Many thousands of ureas could be readily imagined still adhering closely to the type of Bayer 205. King and Murch[1] have used atoxyl instead of aminonaphthalenesulphonic acids, without any success, however, probably because the resulting compounds are too little

[1] J. Chem. Soc., **125**, 2595 (1924); **127**, 2632 (1925).

soluble. Balaban and King[1] combined atoxyl with glyoxalinecarboxylic acid; the resulting compound

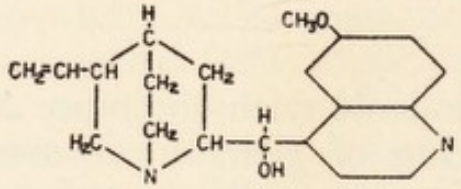


cured mice infected with *T. equiperdum*. It is not clear whether the effect is of the salvarsan or of the Bayer type. The only connection with the latter is the NH.CO grouping, which may have no direct therapeutic significance and is merely a means of uniting an amine to an acid. Other forms of union are conceivable, as are many types of compounds which could replace the naphthalene and benzene constituents of Bayer 205. The absence of a specifically poisonous element from the latter drug opens possibilities of chemotherapeutic research in many divisions of organic chemistry. It is not conceivable that the components of Bayer 205, and particularly the naphthalene acids, have any special biological importance. They have merely been used because they were readily available in the dye industry.

So far we have dealt with the more or less recent and successful combating of general protozoal infections by means of synthetic agents. We must now mention two much older forms of chemotherapy, the treatment of malaria with cinchona and other barks, and the treatment of (amoebic) dysentery with ipecacuanha root. The therapeutic effect on both diseases is due to alkaloids. Of these some twenty are present in cinchona bark and for the last half-century one of these, quinine, has been considered preëminently effective. The selective cultivation of cinchona, almost wholly in Java, has aimed at a maximum yield of quinine. Now it is becoming pretty clear, however, that several other alkaloids are at least as good and instead of the expensive pure quinine, the crude

[1] J. Chem. Soc., 127, 2701 (1925).

mixed alkaloids are being used. Quinine is one of the drugs of greatest commercial importance, the sales amounting to many millions of dollars annually, and if any serious attempt were made to assail the preëminent position of the Dutch producers, this might conceivably be done by varieties other than those already cultivated, or even by so-called "false" barks of other genera, containing different alkaloids. The difficulty of forming an accurate estimate of the value of any particular anti-malarial agent arises from the fact that the actual infection cannot be transmitted to laboratory animals. Tentative conclusions may be drawn from the effect on a related form of infection in birds (bird malaria; canaries are usually employed), but ultimately one is dependent on clinical trials. These can be carried out on an adequate scale in the tropics only, and are complicated by the existence of three distinct forms of malaria (benign and malignant tertian, quartan) caused by three distinct species of protozoa inhabiting the red corpuscles. Quinine is not in the unique position in which it was supposed to be, and its activity cannot be attributed to various features of its constitution which are absent in other alkaloids of similar activity. Its activity



QUININE

cannot be entirely attributed to the vinyl group nor to the methoxy group nor to the stereochemical arrangement, and this opens up the possibility of making synthetic quinine substitutes. Quinine itself has not yet been synthesized in the laboratory and, even if it had been, its industrial synthesis would in any case be too expensive. The problem of finding a cheap and efficient quinine substitute is one of very great economic importance, for malaria causes an enormous waste of human efficiency and has even determined the fate of empires. It is interesting, therefore, that the Bayer Company, in 1926, announced

the preparation of a relatively simple compound, an "ethylaminoquinoline," termed plasmoquin ("Plasmochin") which was stated to have a specific action on the malarial parasite. Its exact constitution has not been published and as usual its claims seem to have been somewhat exaggerated, but it may prove to be the first step into a new field of chemotherapy. The idea underlying the synthesis of plasmoquin is evidently to retain the readily accessible quinoline nucleus and to simplify the complicated piperidine residue. The recent use of salvarsan and stovarsol in benign tertian malaria, as well as that of mercurochrome (dibromohydroxymercurifluorescein) also suggests the possibility of attacking this problem along other lines. Research would be greatly facilitated if the human infection could be transmitted to ordinary laboratory animals.

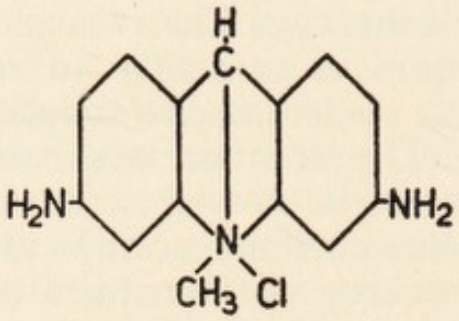
The second example of chemotherapy by natural agents is almost as old as the use of cinchona bark. Ipecacuanha root was introduced as a specific against dysentery more than 250 years ago, but that the action was due to the alkaloids of the drug was not fully recognized until recent years. Early in the present century "de-emetinized" root was used against dysentery, *i.e.* the root from which emetine had been extracted. We now know that any activity left in the root must have been due to imperfect extraction of the alkaloids, and that its emetic and anti-dysenteric properties are inseparable.

We may consider the use of emetine in amoebic dysentery an example of chemotherapy because the alkaloid attacks the specific cause of the disease, *Entamoeba histolytica*, in the intestine. Dale and Dobell found the toxicity to the parasite to be very slight *in vitro*, but the explanation of this is not the same as that given for salvarsan. When Dobell and Laidlaw had succeeded in cultivating the *Entamoeba* it became evident that emetine does not so much affect the vegetative phase as inhibit reproduction.

BACTERIAL DISEASES

So far we have dealt exclusively with the chemotherapy of diseases caused by protozoa. Against bacteria very little progress has been made, perhaps because of their resistant

cell wall. An antiseptic which could be introduced into the blood stream in a general septicaemia, such as plague, must of course be non-poisonous to the host. A few such antiseptics are known, for instance trypaflavine, or 3,6-diaminomethylacridinium chloride,



Trypaflavine (Acriflavine)

a dye originally introduced by Benda[1] against trypanosomes, as the name implies. During the late war Browning demonstrated that it is more valuable against bacteria and it is manufactured in England under the name of acriflavine; proflavine is the corresponding tertiary base. The quaternary salt was introduced with a view to securing solubility in alkaline fluids. Browning and his collaborators found that, unlike most disinfectants, trypaflavine preserves its antiseptic power in the presence of blood serum and even claim that this power is increased by such presence.[2]

Table IV gives the antiseptic dilution of some disinfectants against *Staphylococcus*.

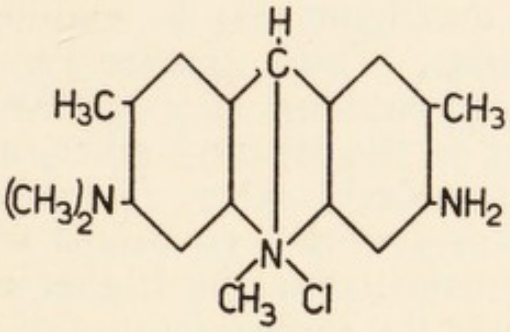
TABLE IV

Antiseptic Dilutions against Staphylococcus

Disinfectant	Peptone	Serum
Chloramine T	2000	250
$HgCl_2$	1 000 000	10 000
Iodine in KI	10 000	700
Brilliant green	10 000 000	30 000
Malachite green	10 000 000	40 000
Crystal violet	4 000 000	400 000
Trypaflavine	20 000	200 000

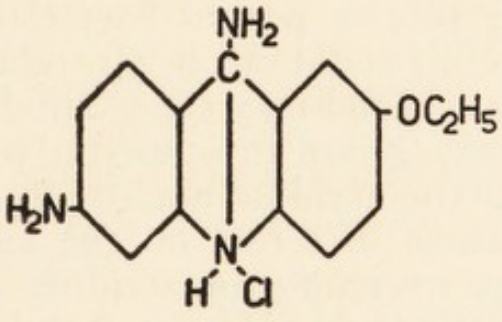
[1] Ber., 45, 1787 (1912).
[2] Browning, Gulbransen, Kennaway and Thornton, Brit. Med. J., 1917-i, 70.

Browning, Cohen, Gaunt and Gulbransen[1] have examined a number of acridine and phenazine derivatives in the same way, and have come to the conclusion that activity in serum is favored by the introduction of amino groups,[2] and is most strongly developed in the quaternary salts (methochlorides). Trypaflavine satisfies both these conditions; so does flavicide or 2,7-dimethyl-6-dimethylamino-3-amino-10-methylacridinium chloride, which is very active against the diphtheria bacillus, and was introduced



Flavicide

by Langer.[3] Rivanol is the hydrochloride of 2-ethoxy-6,9-diaminoacridine, and has been largely recommended



Rivanol

as a wound antiseptic by Morgenroth.[4] The introduction of the ethoxy group was suggested by Morgenroth's work on the ethers of dihydrocupreine.

Cupreine is a phenolic alkaloid occurring in the bark of *Remija cuprea*; quinine is its methyl ether. Long ago Grimaux had made homologues of quinine such as ethyl-

[1] Proc. Roy. Soc. London, **93B**, 329 (1922).
[2] Compare also Fairbrother and Renshaw, J. Soc. Chem. Ind., **41**, 134 (1922).
[3] Z. ges. exptl. Med., **27**, 174 (1922).
[4] Deut. med. Wochschr., **45**, 505 (1919); **47**, 1317 (1921).

cupreine, by alkylating cupreine. Since cupreine is now no longer obtainable, and since it cannot be prepared from quinine, Morgenroth had recourse to dihydroquinine in which the vinyl group is reduced to an ethyl group. This confers stability on the molecule, so that it becomes possible to eliminate the methyl group without causing any other change. There results dihydrocupreine which can now be alkylated, giving a series of ethers with powerful antiseptic properties. With increase in the size of the alkyl group, the activity increases to a maximum and then diminishes. The maximum may be variously situated for different organisms. Thus against *Pneumococcus* ethyldihydrocupreine is the most effective, against the diphtheria bacillus it is the isoamyl ether, against Streptococcus it is the isoöctyl ether. There is considerable specificity which is doubtless connected with a difference in physical properties; thus the higher members of the series greatly lower the surface tension of their solutions.

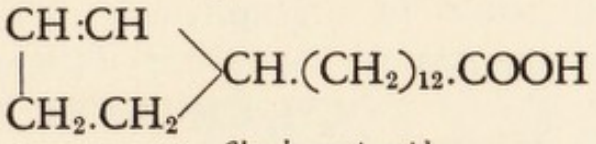
The most valuable scientific result of Morgenroth's work was probably his demonstration that ethyldihydrocupreine ("optochin") can cure experimental infections of *Pneumococcus* in mice. This was the first chemotherapeutic laboratory success with a bacterial infection. Unfortunately the result could not be reproduced in man; the alkaloid is very toxic and may cause blindness. It was the effect of the ethoxy group in optochin which led to its introduction into the acridine nucleus in rivanol.

The adverse effect of serum on the antiseptic power, which effect was overcome in acridine compounds, already made itself felt in an older research by Bechhold and Ehrlich[1] who found that the antiseptic power of phenol, already enhanced by a methyl group in the cresols, could still be increased enormously by the introduction of halogen. Thus tetrabromo-*o*-cresol is 250 times as powerful as phenol, and only half as toxic. In this research various bacilli were found to show great specific differences in their susceptibility to some phenols, *e.g.* bromonaphthols. In the presence of serum these phenols had very little activity however.

[1] Z. physiol. Chem., 47, 173 (1906).

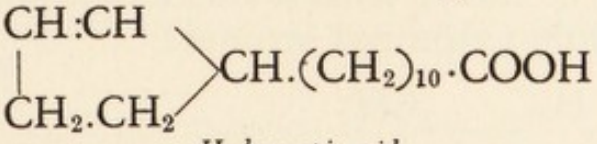
More recently similar attempts at modifying the action of resorcinol seem to have been more successful. T. B. Johnson and F. W. Lane[1] have prepared butylresorcinol, and later hexylresorcinol has come into use as an intestinal antiseptic. The long alkyl side chain probably serves to make the compound more readily soluble in lipoids. The use of chaulmoogra oil in leprosy may depend on the same property.

Chaulmoogra oil is obtained from the seeds of *Taraktogenos Kurzii*, an Indian tree; Power and Gornall[2] isolated from it a peculiar fatty acid $C_{18}H_{32}O_2$, present as glyceride. The so-called chaulmoogric acid contains four hydrogen atoms less than stearic, yet contains only one double bond. Evidently it must contain a ring. The acid is further peculiar in being optically active, and from these facts and a study of the oxidation products, Barrowcliff and Power[3] arrived at the following constitution:



Chaulmoogric acid

The oil of several species of *Hydnocarpus* (related to *Taraktogenos*) was found, by Power and Barrowcliff,[4] to contain a lower homologue of chaulmoogric acid, which they named "hydnocarpic acid"; it has the composition $C_{16}H_{28}O_2$ and is similarly constituted, with a shorter side chain. It is also present in chaulmoogra oil.

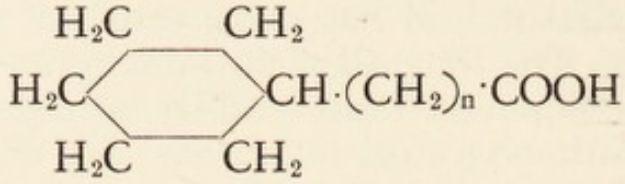


Hydnocarpic acid

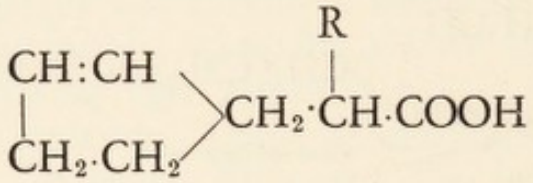
The specific action of these oils has recently led Roger Adams and his pupils[5] to investigate the action of a large

[1] J. Am. Chem. Soc., 43, 348 (1921).
[2] J. Chem. Soc., 85, 838 (1904).
[3] J. Chem. Soc., 91, 557 (1907).
[4] J. Chem. Soc., 87, 885 (1905).
[5] Shriner and Adams, J. Am. Chem. Soc., 47, 2727 (1925); Noller and Adams, *ibid.*, 48, 1080, 244 (1926); Hiers and Adams, *op. cit.*, 1089, 2385; Van Dyke and Adams, *op. cit.*, 2393; Sacks and Adams, *op. cit.*, 2395; Adams, Stanley, Ford and Peterson, *ibid.*, 49, 2934 (1927); Arvin and Adams, *op. cit.*, 2940; Adams, Stanley and Stearns, *ibid.*, 50, 1475 (1928); Yohe and Adams, *op. cit.*, 1503.

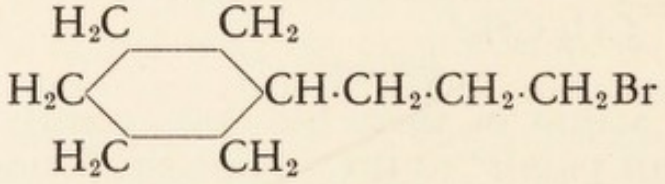
number of synthetic derivatives. In the first place the series of cyclohexyl aliphatic acids was investigated, of the type shown below.



Here the action on *Bacillus leprae* begins with n = 2, gradually increases as the side chain is lengthened to 9 carbon atoms, and again declines until with 13 carbon atoms there is again hardly any action. Chaulmoogric and hydnocarpic acids have side chains of 13 and 11 carbon atoms, respectively, attached however to an unsaturated cyclopentene ring, which has so far prevented their synthesis and the exploration of the homologous series of which they are members. Isomerides of this series of the general formula:



with a branched chain can be obtained (by introducing cyclopentenyl and alkyl (R) radicals into malonic ester). The bactericidal effect of the sodium salts on *Bacillus leprae* increases with increase of the alkyl group, until when R is nonyl, the bacillus is killed at 1:150 000. By use of cyclopentyl or hexyl with a side chain ending in halogen, *e.g.* γ-cyclohexylpropyl bromide



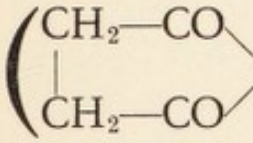
the alkyl radical R in the last but one formula can be displaced to various positions remote from the cyclic complex, whereby the number of isomerides is greatly increased.

The general conclusion is reached that the bactericidal power does not depend very much on the distance of the

carboxyl group from the cyclic complex, but on the total number of carbon atoms in the molecule; 16–18 is the optimum. Evidently this number implies an optimum physical property; cyclohexyl, cyclopentyl, and the unsaturated cyclopentenyl (present in chaulmoogric and hydnocarpic acids) are about equally effective. The only essential difference from palmitic and stearic acids is, therefore, the terminal ring structure. Why this should make all the difference is not at all clear.

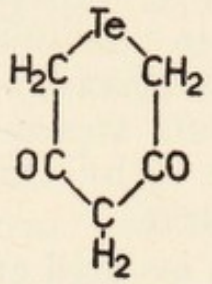
Various more or less isolated attempts have been made to utilize the bactericidal action of the heavy metals. Colloidal silver preparations, protected by various colloids, have been used against *Gonococcus*. A complex sodium aurothiosulphate, $Na(AuS_2O_3)Na_2S_2O_3$, has been introduced by Möllgaard under the name of "sanochrysin" as a remedy in tuberculosis; it is still on clinical trial. Among antimony compounds sodium p-acetylaminophenylstibonate, the analogue of acetyl atoxyl, is used in some tropical protozoal infections and antimonyl potassium or sodium tartrate is a specific remedy against certain tropical worms (*Bilharzia*) which inhabit the large blood vessels. The analogous bismuth compound and other compounds such as the oxide, have of recent years found extended application in syphilis. These are, as a rule, given hypodermically, suspended in oil, and their action seems to depend on the splitting-off of metallic bismuth, which perhaps unites with a constituent of the cells to form the same active agent whatever the compound of bismuth administered.

Many organic compounds of mercuric have been made. In some the mercuric is firmly attached to a benzene ring and has lost its specific toxic properties. In others it can ionize more or less readily as in mercury succinimide



Hg. Mercurochrome (dibromohydroxymercurifluorescein) is an example of the first group and has been used intravenously in malaria and plague.

Selenium compounds were at one time considered by Wassermann to be useful in cancer. Certain tellurium compounds of cyclopentanedione examined by Morgan,



Cyclotelluropentanedione-3,5

Cooper and Corby,[1] have a very powerful antiseptic action *in vitro* which is not possessed by analogous compounds with other metals. These substances do not appear to have been used therapeutically.

In conclusion, the present position of chemotherapy may be said to be as follows: Considerable success against protozoa has been achieved with organic compounds of arsenic. A partial success, very suggestive and hopeful, has been obtained with Bayer 205, also against protozoa. Against bacteria some good external antiseptics have been found, as well as indications that a septicaemia may ultimately be curable by such means; but at present no cures have been effected in man. The whole subject of chemotherapy makes generalization extremely difficult, and presents great scope for the united labors of the organic chemist and the biologist.

[1] J. Soc. Chem. Ind., 43, 304 (1924).

CHAPTER V

Blue Adsorption Compounds of Iodine

The remarkable blue substance produced from starch and iodine has been the subject of investigation for more than a century, and even during the last decade papers on this subject have appeared at the rate of one or two per annum. Starch long remained a more or less isolated example of a reaction with iodine which is now known to take place in similar fashion with a large number of substances. In most cases it has become possible to correlate the formation of a blue iodine compound with chemical constitution, and by a study of the composition and colloidal properties of the analogues of "starch iodide" much light has been thrown on this substance itself.

ADSORPTION OF IODINE BY STARCH

The formation of a blue compound with starch was observed very soon after the discovery of iodine itself. The date of the latter event is sometimes given as 1811, but the first half of 1812 would appear to be nearer the mark. In B. Courtois' paper[1] it is stated that the discovery was communicated by Clément to the Institute on December 6, 1813, and that the "new substance" (iodine) was first observed some eighteen months previously (in 1812 therefore, not in 1811). Less than four months after the announcement to the Institute, Colin and Gaultier de Claubry communicated to the same body a paper "Sur les combinaisons de l'iode avec les substances végétales et animales".[2] They remark that since Clément, Gay-Lussac, and Davy had restricted themselves to the investigation of the action of iodine on inorganic compounds, they themselves had made a systematic survey of its action on various classes of organic substances; the most remarkable

[1] Découverte d'une substance dans le vareck, Ann. chim., 88, 304–310 (1813).
[2] Ann. chim., 90, 87–100 (1814).

reaction observed was one with starch, which reaction they discuss in detail. A translation of the paper appeared in Gilbert's Annalen der Physik[1] and it was not until the next year that Stromeyer (incidentally one of the discoverers of cadmium) published a paper on the subject: "Ein sehr empfindliches Reagens für Iodine, aufgefunden in der Stärke (*Amidon*)".[2] Stromeyer refers to Colin and Gaultier de Claubry; evidently he has no claims to priority in this matter, although the discovery of starch iodide was subsequently attributed to him, *e.g.* by Andrews and Goettsch in a paper[3] which gives an extensive bibliography of the older literature.

The final sentence of Stromeyer's paper is of some interest: "Ich möchte überhaupt die Iodine-Stärke nicht zu der Klasse der wahren Verbindungen zählen, sondern sie nur als eine blosse Auflösung betrachten".[4] If we interpret "Auflösung" as solid solution, or as adsorption compound, we see that Stromeyer at once arrived at a view which after long debate seems to have met with general acceptance only in the present century, but even now may require modification.

Liebig[5] refers to an early analysis of starch iodide by Lassaigne, who considered that one equivalent of starch combined with one atom of iodine; but since Lassaigne's preparation showed crystals of iodine under the microscope, Liebig agreed with Payen that the two constituents do not combine in any simple proportion. He wrote (*loc. cit.*): "Das Verhalten des Jods gegen Amylon in seinen verschiedenen Zuständen der Löslichkeit, so wie die Farbe der Verbindung selbst, welche ganz dieselbe ist, wie die des Joddampfes, schien den meisten Chemikern auf einer ähnlichen Ursache zu beruhen, wie das Gefärbtwerden von Pflanzen- und Thierstoffen, von Leinwand, Baumwollen- und Seidenzeugen, deren Oberfläche sich mit Farbstoffen verbindet und gefärbt erscheint, ohne eine, im eigentlichen Sinne chemische Verbindung zu bilden. Den

[1] Ann. Physik, **48**, 297 (1814).
[2] Ann. Physik, **49**, 146–153 (1815).
[3] J. Am. Chem. Soc., **24**, 865 (1902), citing Heron in Thorpe's *Dictionary of Applied Chemistry*, III, p. 565 (London, 1893).
[4] Stromeyer, *loc. cit.*
[5] Ann., **42**, 306 (1842).

meisten Chemikern also schien das Jodamylon nichts anderes zu seyn, wie Amylon gefärbt durch Jod. Die Farbe selbst gehörte, ihrer Ansicht nach, dem Jod an."

This comparison to the dyeing process already foreshadows the current conception of starch iodide as an adsorption compound. Although chemical union is not entirely excluded in either case, we must not look for a stoïchiometric relationship. Such a relationship was indeed denied not only by Liebig, as above mentioned, but by Béchamp,[1] Blondlot,[2] Pohl,[3] R. Fresenius,[4] Personne,[5] Duclaux,[6] Brukner[7] and others.

Pohl laid great stress on the fact that starch iodide is decomposed by the mere addition of alcohol; and Fresenius, in discussing Pohl's paper, remarks emphatically that the latter's view is the only correct one. Fresenius already pointed out that the dissociation of starch iodide is counteracted by increasing the concentration of the starch as well as that of the iodine. Thus on adding a dilute starch solution to 20 cu. cm. of 1/330,000 aqueous iodine solution, the blue color does not appear until after several drops have been added, and does not reach its maximum intensity until after the addition of 2–3 cu. cm. Personne regarded starch iodide as a lake. Duclaux considered that the equilibrium between iodine, starch and water resembles equilibria in which charcoal is involved and thus in effect compared the reaction to an adsorption.

There were others, however, who regarded starch iodide as a definite compound, *e.g.* Fritzsche[8] and Guichard.[9] Bondonneau[10] considered that starch iodide always has the composition $(C_{12}H_{10}O_{10})_5I$ or in modern symbols, $(C_6H_{10}O_5)_5I$. Rouvier[11] finally went as far as assuming the

[1] J. pharm. chim., [3] **27**, 406 (1855); **28**, 303 (1855).
[2] Ann. chim. phys., [3] **43**, 225 (1855).
[3] J. prakt. Chem., **83**, 38 (1861).
[4] Ann., **102**, 184 (1857); Z. anal. Chem., **1**, 34 (1862).
[5] Compt. rend., **61**, 993 (1865).
[6] Compt. rend., **74**, 533 (1872); Ann. chim. phys., [4] **25**, 264 (1872).
[7] Monatsh., **4**, 906 (1883).
[8] Pogg. Ann. Physik Chem., **32**, 153 (1834).
[9] Bull. soc. chim., **1863** 115, 278.
[10] Bull. soc. chim., [2] **28**, 452 (1877).
[11] Compt. rend., **114**, 749, 1366 (1892); **117**, 281, 461 (1893); **118**, 743 (1894); **124**, 565 (1897).

existence of four distinct iodides $(C_6H_{10}O_5)_{16}I_n$ where n = 2, 3, 4, and 5, and this in spite of the fact that he observed a gradual change in the composition of the iodide, in which, over a certain range, the iodine content was proportional to the square root of the concentration in the aqueous phase. According to Dhar,[1] altogether thirteen different molecular formulas have been suggested for starch iodide, and there has been at least one other since he wrote. The papers of the older authors who assumed a definite compound to exist, are not impressive, particularly as regards analytical data, and their opponents might well have won an early victory had it not been for Mylius[2] who, in a paper that is undoubtedly one of the most important on this subject, assigned to starch iodide the formula $\{(C_6H_{10}O_5)_nI\}_4,HI$ and later $(C_{24}H_{40}O_{20}I)_4,HI$. Mylius was led to investigate starch iodide by his discovery of a closely analogous compound between iodine and cholalic acid which can be obtained in blue crystals. The analytical figures for these crystals were in (moderate) agreement with the formulas $(C_{24}H_{40}O_5I)_4,HI$ and $(C_{24}H_{40}O_5I)_4,KI$ according as the iodine was dissolved in hydriodic acid or potassium iodide. Another important contribution of Mylius was his discovery that hydriodic acid or an iodide enters into the composition of the blue compounds, both of starch and of cholalic acid. That "starch iodide" is not simply starch+iodine was already indicated by some previous observers. Guichard[3] claimed to have prepared a colorless starch iodide by boiling the blue substances. After evaporation and precipitation with alcohol this colorless "iodide" became blue on addition of oxidizing agents, doubtless owing to some unchanged starch being present. More important was Guichard's observation that a small quantity of silver nitrate can decolorize "immense" quantities of the blue iodide. Mylius was led by this result, and by his own observation that the blue crystals from cholalic acid contain hydriodic acid, to demonstrate that starch is not colored blue by iodine in

[1] J. Phys. Chem., 28, 125 (1924).

[2] Z. physiol. Chem., 11, 306 (1887); Ber., 20, 688 (1887).

[3] *Loc. cit.*

the absence of iodide ions. An aqueous iodine solution free from such ions is most conveniently prepared by pouring an alcoholic solution into slightly acidulated water. (Acidulation is necessary to prevent the action of the alkali of glass, which otherwise forms iodide and iodate). With such a solution starch remains colorless, but at once becomes blue on the addition of potassium iodide or hydriodic acid. Aqueous solutions of iodine prepared by heating crystals in water are apt to contain a little hydriodic acid, which may be eliminated by adding chlorine or iodic acid, and both these reagents prevent the formation of the blue iodide, as do silver nitrate, mercuric chloride, etc. While silver nitrate at once decolorizes preformed starch iodide, iodic acid does so less readily, but when the starch iodide is decolorized by heat in the presence of iodic acid the color does not return on cooling.

That iodide ions favor the formation of starch iodide has been confirmed by many subsequent observers. Stocks[1] at first denied that hydriodic acid was necessary, but a year later was converted to the view of Mylius. Lonnes[2] gives a table of the first appearance of a blue color when an aqueous iodine solution is added to 100 cu. cm. of 0.02-per-cent starch solution.

KI added (mg.):	0	0	0.008	0.04	0.08	0.2	0.4	1.0
I added (mg.):	0.81	0.74	0.53	0.48	0.36	0.23	0.20	0.10

This shows the favorable effect of iodide in a simple manner. Lonnes found 3.75 to 4.30 equivalents of iodine per equivalent of hydriodic acid, when he estimated the iodide ions by difference (as Mylius did), and 2.98, 2.99, 3.27 equivalents by a direct method, when he washed the starch iodide by repeated centrifuging with dilute acid and water. Padoa and Savarè[3] found by conductivity measurements, that the ratio iodine:iodide ion in starch iodide varied from 1:3 to 1:9 and therefore was not exactly 1:4 as Mylius supposed it to be. As regards the ratio of iodine to starch they agreed with $(C_{24}H_{40}O_{20}I)_4 \cdot KI$, his for-

[1] Chem. News, **56**, 212 (1887); **57**, 183 (1888).

[2] Z. anal. Chem., **33**, 409 (1894).

[3] Atti accad. Lincei, [5] **14**, i, 467 (1905); Gazz. chim. ital., **36**, i, 313 (1906).

mula. The necessity of the presence of iodide ions was disputed by Meineke[1] who found that potassium iodide may be replaced by potassium, sodium, or ammonium chlorides, of which salts, respectively, 200, 2000, and 500 times as much is required as of potassium iodide. In any case, therefore, the iodide ion is much more efficient. Lonnes[2] was inclined to explain the effect of chlorides by slight formation of iodide ions and chlorine, by double decomposition; but it seems more likely that the iodide ion is merely required to change the charge on the starch particles by being adsorbed thereon, as first indicated by Katayama.[3]

Other anions can do the same, only they differ greatly in regard to the readiness with which they are adsorbed, and the concentration in which they are effective. Tinkler[4] detected the formation of periodides by characteristic ultra-violet absorption bands, and concluded that there is some tendency for the formation of $KClI_2$ for instance. These bands disappear from a solution of potassium triiodide, when starch is added, owing to the triiodide being adsorbed by the starch, as was later inferred by Lottermoser (see p. 136) on other grounds.

In any case Mylius' formula $(C_{24}H_{40}O_{20}I)_4 \cdot HI$, supported by the analogous formula for the iodide of cholalic acid, appeared to his contemporaries so plausible that it was adopted in the third edition of Beilstein. It was, however, rendered doubtful by the very careful work of Küster,[5] whose paper stands out in the older literature of the subject. Küster's experiments were mainly concerned with the composition of the precipitate produced in a filtered starch solution, to which iodine in potassium iodide and dilute sulphuric acid were successively added. The iodine was present in widely different concentrations, ranging from $N/2$ to $N/1600$, but its absolute amount was always greatly in excess of that required to convert the whole of the starch into the iodide of Mylius; the sulphuric

[1] Chem. Ztg., **18**, 157 (1894).
[2] Z. anal. Chem., *loc. cit.*
[3] Z. anorg. Chem., **56**, 209 (1907).
[4] J. Chem. Soc., **91**, 996 (1907).
[5] Ann., **283**, 360 (1894).

acid was merely added, in constant amount, to flocculate the starch iodide. The iodine in the supernatant liquid was determined, which gave the iodine in the starch by difference, and in order to secure accuracy Küster used of his most dilute solutions as much as four liters, employing two liters for the titration of the iodine remaining in the water.

TABLE V

Concentrations of Iodine in Starch (a) *at different Concentrations in Water* (c), *calculated from Küster's results.*

c	a (found)	a (calc.)
0.491	0.460	0.456
0.231	0.416	0.422
0.121	0.393	0.395
0.0412	0.351	0.353
0.0277	0.325	0.339
0.0198	0.319	0.328
0.0139	0.316	0.316
0.00998	0.312	0.305
0.00716	0.294	0.295
0.00574	0.293	0.288
0.00490	0.289	0.284
0.00363	0.284	0.275

One series, of the twelve most concentrated solutions ranging from $N/2$ to $N/200$, shows a quite gradual change in the iodine content of the starch iodide from 26.51 per cent to 18.20 per cent and Küster tabulated the ratio of the 10th root of the concentration in water to the concentration in the starch; this ratio is practically constant (extreme variations 5.24–5.59) down to $N/200$ iodine, and then increases somewhat at extreme dilutions. This behavior is entirely characteristic of an adsorption equilibrium, of which Küster's results form an excellent example. It seemed worth while to recalculate the results according to the formula employed by Freundlich, $a = \alpha c^{1/n}$, where a is the concentration of iodine in the starch, in gram-atoms per gram equivalent of starch ($C_6H_{10}O_5 = 162$), c the concentration in the water, in gram-atoms per liter, and where

α and 1/n are constants (Table V). By plotting logarithms the constants were obtained leading to the equation

$$a = 0.491c^{0.103}$$

from which the concentration of iodine in the starch was calculated. The agreement is very good and with logarithmic plotting the points are practically on a straight line (Fig. 3, Curve I).

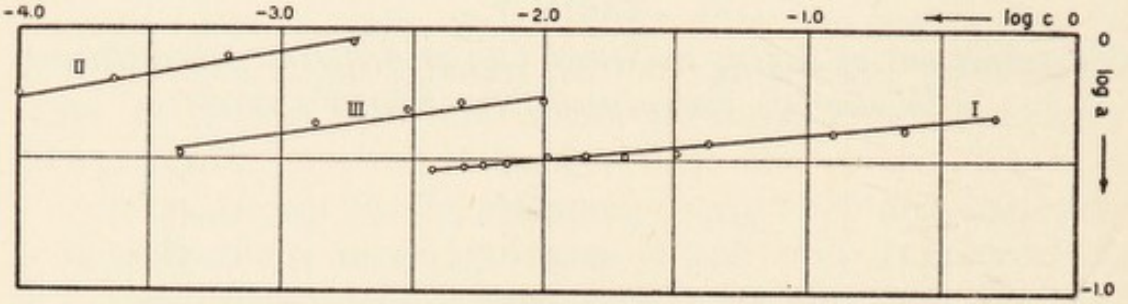


FIG. 3.

Adsorption of Iodine by: Starch (I), amorphous Cholalic Acid (II), and Saponarin(III)

Küster concluded from his experiments that blue starch iodide is a solution of iodine in starch. The experiments of Seyfert,[1] who declared for the constant composition $(C_{24}H_{40}O_{20})_6.I_7$, were all made in such a way that the resultant concentration of the iodine in water was practically the same, and the composition demanded by the above formula corresponds closely with a particular point on Küster's curve. This must have been also the case with Mylius' experiments, relating mostly to another small range of final concentrations of iodine in water. In a private communication to Küster, Mylius stated that this was always below $N/100$. Küster's results also agree closely with those of Rouvier[2] whose interpretation, however, was at fault when he assumed the existence of four compounds in varying proportion. Küster did not investigate the proportion of the "free" to the "combined" iodine in the precipitated starch, but quotes results by A. Meyer, obtained under varying experimental conditions, which results range from 3.12:1 to 4.27:1 instead of being exactly 4:1, as demanded by Mylius' formula.

[1] Z. angew. Chem., 15, 126 (1888).

[2] Rouvier, Compt. rend., *loc. cit.*

Küster's successors mostly agreed with his conclusions, and many investigated the subject by the methods of of physical and colloidal chemistry. Tóth[1] adhered to the view that starch iodide is a definite compound and considered iodide ions unnecessary for its formation. Friedenthal[2] used the depression of the freezing point, but misinterpreted his results in favor of chemical combination.

Rodewald and Kattein[3] measured the osmotic pressure of starch iodide, Andrews and Goettsch[4] the (very small) vapor pressure of iodine in starch solutions. The last named prepared starch solutions by heating to 152° or even to 175°, which renders the results not quite comparable to those of other observers, and then shook the solutions for days with finely powdered iodine at room temperature; even after 17 days equilibrium does not seem to have been reached in several cases. The amount of iodide (from the glass) was not controlled, and little importance can be attached to the formula $(C_6H_{10}O_5)_{12}I$ suggested. The low percentage of iodine merely shows the important part played by iodide ions. In other experiments starch and iodine were heated in sealed tubes to 100°, and the formula $(C_6H_{10}O_5)_{12}I_2$ obtained in this case is quite fortuitous, more hydriodic acid having been formed than in the cold. Andrews and Goettsch drew the conclusion that "the remarkably low vapor tension of the iodine . . . while quite natural if the iodine is combined, is exceptional and difficult to understand on any other basis." It might equally well be argued that when a vacuum is produced by charcoal, the gases form definite compounds with carbon.

The equilibrium between iodine, starch, and potassium iodide in dilute solutions was investigated by Katayama[5] by means of colorimetry, on the assumption that intensity of the blue color is a measure of the concentration of starch iodide. According to Padoa[6] this assumption is not valid,

[1] Chem. Ztg., **15**, 1523, 1583 (1891).
[2] Centr. Physiol., **13**, 54 (1899).
[3] Z. physik. Chem., **33**, 586 (1900).
[4] J. Am. Chem. Soc., **24**, 865 (1902).
[5] Z. anorg. Chem., *loc. cit.*
[6] Atti accad. Lincei, [5] **17**, i, 214 (1908).

because the color also depends on the size of the particles. Katayama concluded that the amount of starch iodide in the (very dilute) solutions employed by him, was proportional to the concentrations of both the starch and the iodine, but dependent on the 2d and 3d power of the concentration of the potassium iodide; he considered that starch iodide is a solid solution.

A number of subsequent authors investigated the partition of iodine between starch and organic solvents such as carbon tetrachloride (Lottermoser,[1] Murray[2]), chloroform (Firth and Watson[3]), benzene (von Euler and Myrbäck[4]), toluene (von Euler and Landergren[5]) and tetrachloroethane (Berczeller[6]). These investigations mostly led to the conclusion that starch iodide has no definite composition, or is an adsorption compound. Von Euler and Myrbäck indeed proposed at first the formulas $(C_6H_{10}O_5)_{12}I_2$ and $(C_6H_{10}O_5)_{12}I_4$ and afterwards the formulas $(C_6H_{10}O_5)_{18}I_2$ and $(C_6H_{10}O_5)_{18}I_4$, but later expressed themselves very guardedly. Murray considered that in very dilute solution, starch iodide contains the ion $(C_6H_{10}O_5)_nI'_5$ where n is approximately 15 (so that the composition would correspond approximately to that deduced by Mylius).

Of the partition experiments those of Lottermoser seem to be the most valuable; they confirmed other important experiments of the same author[7] in which the triiodide of the aqueous phase was determined by a potentiometric method, also employed by Murray. Lottermoser concluded that the triiodide ion is most strongly adsorbed from dilute solution, but that the iodide ion, as well as undissolved KI and KI_3 are also adsorbed. He thus confirmed the effect of the iodide ion which participates, if only temporarily, in adsorption. This also follows from the work of Firth and Watson who found that when starch solutions containing increasing quantities of potassium

[1] Z. angew. Chem., **37**, 84 (1924).
[2] J. Chem. Soc., **127**, 1288 (1925).
[3] J. Soc. Chem. Ind., **42**, 308 (1923).
[4] Ann., **428**, 1 (1922); Archiv Kem. Mineral. Geol., **8**, No. 9, 1 (1922).
[5] Kolloid-Z., **31**, 89 (1922).
[6] Biochem. Z., **133**, 502 (1922).
[7] Z. angew. Chem., **34**, 427 (1921); Z. Elektrochem., **27**, 496 (1921).

iodide are shaken with a solution of iodine in chloroform, the amount of iodine transferred to the starch increases proportionally to the potassium iodide up to 0.192 gm. per liter of the latter; with more concentrated iodide solutions the effect on the adsorption of iodine falls off. Similarly von Euler and Bergman[1] found that the solubility of iodine in starch solution containing potassium iodide is greater than the sum of the solubilities in starch solution and in potassium iodide solution taken separately.

There is thus abundant evidence of the part played by the iodide ion, at the low concentration with which most of the later investigators have been concerned. It does not figure prominently in Küster's investigations with more concentrated solutions, and according to Angelescu and Mirescu[2] the adsorption of iodine is independent of potassium iodide when the latter is between 2 and 15 per cent.

The iodide ion is, however, not specific. Meineke[3] found long ago that it could be replaced by a much greater concentration of chloride ion. Gorbatschev and Vinogradova[4] recently placed anions in the following order:

$$\overline{Ac}' > I' > Br' > NO_3' > Cl' > SO_4''$$

showing that acetates are even more active than iodides. Apart from this the order is approximately lyotropic. The difference between cations is less marked. The following explanation of the phenomenon is given, and was already foreshadowed by Katayama.[5] Starch is a negative colloid, and iodine is regarded as positive. When the negative charge has been nearly neutralized, adsorption of iodine ceases, the residual charge being due to hydrogen ions. The addition of easily adsorbed anions removes the inhibiting hydrogen ions, so that adsorption of iodine can proceed. At low concentrations of these anions the adsorption of iodine has a positive temperature coefficient (up to about

[1] Kolloid-Z., **31**, 81 (1922).
[2] Bul. Chim., Soc. române stiinte, **27**, 59 (1924).
[3] Chem. Ztg., **18**, 157 (1894).
[4] Z. physik. Chem., **127**, 93 (1927); J. Russ. Phys. Chem. Soc., Chem. Pt., **59**, 433 (1927).
[5] Z. anorg. Chem., *loc. cit.*

40°) due to decrease in the adsorption of hydrogen ions. When these have been removed, there is a true adsorption with a negative temperature coefficient.

The color of starch iodide is not always pure blue. In the first place starch is a mixture of amylopectin—probably an ester of phosphoric acid—which is colored a bluish purple by iodine, and of amylose, electrically neutral, more highly disperse and giving a pure blue color with iodine.[1] Hence Castoro[2] infers that the color depends on the size of the particles; Samec and Klemen[3] also conclude that the color is influenced by the size of the particles, and not by combination with phosphoric acid or the cations bound to amylopectin. It is further a well-known fact that high concentrations of potassium iodide change the blue iodide of starch through purple to crimson. Mylius (*loc. cit.*) and Hale[4] both found for the blue iodide the ratio 4I:HI and for the crimson one 2I:HI. On dilution with water the crimson iodide again becomes blue, and according to Hale the transformation is a chemical one:

$$\underset{blue}{KI \cdot I_4} + KI = \underset{crimson}{2KI \cdot I_2}$$

Barger and Field[5] however regard the effect as a physical one, for whereas the change to crimson was brought about by a final concentration of 0.40 molar potassium iodide, it was brought about by 0.28 molar potassium thiocyanate, and by a saturated solution of potassium bromide. This indicates a lyotropic effect, which will be further illustrated in a subsequent section by the analogous case of saponarin. Mylius[6] found that starch paste, suspended in concentrated zinc iodide solution, or in concentrated sulphuric acid, is colored brown by iodine, and becomes blue only on dilution with water. He considered that the brown iodide is $C_{24}H_{40}O_{20}I_2$, analogous to the brown iodide of

[1] Compare Freundlich, *Colloidal and Capillary Chemistry*, p. 635, (London, 1925) and papers by Samec, there quoted.
[2] Gazz. chim. ital., 39, i, 603 (1909).
[3] Kolloidchem. Beihefte, 21, 55 (1925).
[4] J. Am. Chem. Soc., 28, 433 (1902).
[5] J. Chem. Soc., 101, 1403 (1912).
[6] Ber., 28, 385 (1895).

cholalic acid. Küster[1] objects to this conception of the brown iodide of starch, on physico-chemical grounds; starch iodide with much iodine is not a mixture of two compounds. The different colors of starch iodide were discussed rather fully by Harrison,[2] who considered this substance analogous to the purple of Cassius. A sol of starch iodide is regarded as a colloidal iodine solution, in which starch plays the part of a protective colloid. When a dilute solution of an iodide and an iodate is acidified there is a momentary blue coloration, but almost at once minute grey crystals of iodine separate. Harrison considers that the various colors of starch iodide are due to the different sizes of the particles of iodine, as is the case of sols of gold and many other substances. According to W. Ostwald[3] the largest particles are blue, and violet, crimson, pale red, orange and yellow ones are successively smaller in size. The effect of alcohol is to diminish the protective action of starch and to increase the size of the particles.[4] Potassium iodide has the opposite effect. Harrison found by ultramicroscopic observation that the size of the particles is indeed increased by alcohol, and decreased by potassium iodide. Lottermoser[5] considers that similar color changes in the case of lanthanum acetate (to be discussed later) also depend on the size of the particles—of the acetate, however, and not of the iodine. An objection to Harrison's very simple conception of starch iodide seems to be the specificity of the protective colloid and the effect of its chemical constitution, which is not observed in the case of gold sols. The color of the iodine compound of the erythrodextrins is in agreement with the fact that these substances are simpler than starch and presumably have smaller particles.

In the case of cholalic acid (*q.v.*) Mylius[6] insists on the importance of water, as well as of iodide, for changing

[1] Ber., 28, 783 (1895).
[2] Kolloid-Z., 9, 5 (1911).
[3] Kolloidchem. Beihefte, 2, 409 (1911).
[4] Cf. the effect of alcohol on the stability of agar sols in the presence of small quantities of electrolytes. Kruyt and Bungenberg de Jong, Z. physik. Chem., 100, 250 (1922); Rec. trav. chim., [iv] 3, 437 (1923); [iv] 5, 35 (1924).
[5] Kolloid-Z., 33, 271 (1924).
[6] Ber., 28, 385 (1895).

the brown iodide into the blue, and applies these results to starch, which in the absence of iodides (presence of iodic acid, chlorine, silver nitrate or mercuric chloride) is colored brown by iodine. Starch grains in a concentrated solution of zinc iodide or in moderately strong sulphuric acid are colored reddish brown by traces of iodine, and become blue on dilution with water. The dried iodide of saponarin is crimson, and also becomes blue when moistened. These observations together with the lyotropic effect of anions, observed by Barger and Field and above referred to, seem to indicate that hydration plays a considerable part in determining the color of adsorption compounds with iodine.

Similar views were developed by Lange[1] who discusses the lyotropic influence of salts, but emphatically declares that iodide ions are unnecessary. Harrison's view of an extremely lyophobic colloid (iodine) protected by a lyophilic one (starch) is perhaps better inverted, by considering that the starch has become more lyophobic by having iodine particles on its surface, the very converse of a protected gold sol. For this there is a good deal of evidence. Barger and Field (*loc. cit.*) found that the flocculation of starch iodide (a negative colloid, wandering to the anode, faster indeed than amylopectin) depends on the valency of the cation. A given sol was flocculated by a final concentration of 0.18 molar potassium chloride, and by 0.03 molar barium chloride. It was also flocculated by all concentrations of methylene blue, a positive dye, in excess of 0.07 per cent; and showed with another positive and more highly colloidal dye, night blue, a well-marked optimal zone of precipitation. Mixtures were made of 4 cu. cm. 1-per-cent starch, 1 cu.cm. $N/100$ iodine in potassium iodide and 1 cu. cm. night blue of varying concentration. With 0.4-per-cent night blue there was slight flocculation, with 0.5-per-cent greater, with 0.8-per-cent complete, with 1.0-per-cent and 2.0-per-cent night blue no flocculation. Negative dyes, such as azo blue, do not flocculate at all. Similar effects were shown more clearly by the less lyophilic saponarin. Since starch without iodine is not flocculated under the

[1] Biochem. Z , 95, 61 (1919).

above conditions, it follows that the iodine has made it more lyophobic. This also follows from the observation by Vanino and Schinner[1] that starch iodide is completely carried down by freshly precipitated barium sulphate, which carries down starch alone very incompletely.

The color of starch iodide is destroyed by sunlight, ultra-violet light and X-rays,[2] and Sen[3] attributes this to the reaction

$$3I_2 + 3H_2O = 5HI + HIO_3$$

This hydrolysis of iodine has been investigated by Bray,[4] who deduced that the true solubility of iodine in water is 1.32 millimols I_2 per liter at 25°.

The investigations of the last forty years leave no doubt that iodine is adsorbed on starch in varying proportions, depending on the iodine and iodide content of the solution, and it has generally been concluded that this rules out the existence of a chemical compound such as was postulated by Mylius. This conclusion is, however, not binding. As Bergmann[5] has pointed out, the problem is not necessarily one of "adsorption or chemical union." Both kinds of combination may well occur together; a chemical compound with a fixed proportion of iodine may well adsorb more iodine in varying amounts. Of course even the blue crystalline molecular compounds, with a definite iodine content, are different from the ordinary colorless iodine compounds of organic chemistry. They are more or less readily dissociated, and do not exist in (molecular-disperse) solution; the iodine is comparable to water of crystallization and hence Küster applied the name *Krystallstrukturverbindung*. The chemical forces involved in the formation of such a compound are not very different from those involved in adsorption; the difference is quantitative, rather than qualitative. The existence of a compound between starch and iodine has been inferred by J. Mellanby[6]

[1] Arch. Pharm., **253**, 47 (1915).
[2] Bordier, Compt. rend., **205**, 291 (1916).
[3] Chem. News, **129**, 194 (1924).
[4] J. Am. Chem. Soc., **32**, 932 (1910).
[5] Ber., **57**, 755 (1924).
[6] Biochem. J., **13**, 29 (1919).

on other grounds. After adding small quantities of iodine in potassium iodide to starch solution, precipitating with magnesium sulphate and filtering, the filtrate may contain either excess of starch or excess of iodine, except at a certain proportion of the two constituents, which proportion is uninfluenced by dilution and is approximately $10C_6H_{10}O_5 : I$. For starch granulose the proportion is more like $5C_6H_{10}O_5 : I$. Since ordinary starch is not a chemical individual Mellanby refrains from deducing a definite formula, but considers that some kind of compound exists (with much less iodine than that required by Mylius' formula). Bergmann and Gierth[1] have contributed the most recent and, to my mind, the most satisfactory discussion of starch iodide, in a paper dealing with simple cyclic semi-acetals of known constitution. These form crystalline addition compounds with iodine, evidently due to the presence of a bridge-oxygen atom, which atom is also postulated in starch. Since various amyloses obtained by bacterial fission of starch by Schardinger,[2] also yield, according to Pringsheim and Eissler,[3] crystalline iodine compounds, there is a further reason for believing that similar compounds are formed by starch itself, although their isolation in a state of purity may be quite impossible.

The iodine compounds of the amyloses and of the cyclic acetals prepared by Bergmann will be discussed in later sections.

OTHER SUBSTANCES GIVING BLUE IODINE COMPOUNDS

The many substances now known to react with iodine more or less like starch, are mainly of two types:

I. Colloids, either (a) markedly hydrophilic and chemically related to starch or (b) hydroxides and basic acetates of certain metals.

II. Many crystalline substances, of smaller molecular weight and varying in chemical constitution; cholalic acid is one of the best known examples. Two well-defined

[1] Ann., 448, 57–60 (1926).
[2] Z. Untersuch. Nahr. Genussm., 6, 784 (1903).
[3] Ber., 46, 2968 (1913).

semi-colloids, saponarin (a glucoside) and euxanthic acid (a glucuronic acid), occupy an interesting transitional position between these two groups.

The best known example of Group I is isolichenin, an amylose from Iceland moss (*Cetraria islandica*). Berzelius[1] already compared this lichen substance with starch paste. Berg[2] found that on standing the jelly separates into two carbohydrates; the more abundant of these is soluble only in hot water and is not colored by iodine; it retained the original name lichenin; the other was later called isolichenin, is soluble in cold water, and alone is colored blue by iodine;[3] both have the composition $C_6H_{10}O_5$. A partial separation of the two carbohydrates was effected by Hönig and Schubert,[4] a more complete one by Pringsheim[5] who regarded isolichenin as closely analogous to the amylose constituent of starch. The amylopectin of starch is, according to Pringsheim, closely related to glycogen, both substances giving a reddish-brown color with iodine.

It is interesting to note that according to Bergmann and Ludewig[6] acetyl starch with 46–48 per cent of acetyl still adsorbs iodine, and also potassium iodide. (Ratio of I : KI in two experiments = 7.1 : 1 and 4.4 : 1). The polyamyloses, simple fission-products of starch, form crystalline blue and brown addition compounds with iodine and are discussed in Group II.

Although cellulose itself is not colored by iodine, it is changed by treatment with concentrated acids into a substance which behaves like starch. Gmelin[7] showed that fuming nitric acid transforms paper into a substance which is colored blue by potassium triiodide. Schleiden[8] discovered the blue iodine reaction of cell walls after treatment with sulphuric acid. He believed that the cell walls were thus transformed into starch, an error

[1] Schweigg. J., 7, 317 (1813); Ann. chim., 90, 227 (1814).
[2] Jahresber. Chem., 1873, 849.
[3] Errera, Dissertation, Brussels (1882).
[4] Monatsh., 8, 452 (1887).
[5] Ber., 57, 1594 (1924).
[6] Ber., 57, 961 (1924).
[7] Schweigg. J., 58, 377 (1830).
[8] Pogg. Ann., 43, 391 (1838).

which was corrected by Liebig.[1] The latter pointed out that, although starch is a specific reagent for iodine, the converse does not hold, a fact more obvious today than in Schleiden's time. Liebig showed that when the mixture of cellulose and sulphuric acid is diluted with water, the addition of an alcoholic iodine solution only causes precipitation of iodine, and the blue substance is no longer formed. The fission-product of cellulose formed by sulphuric acid evidently has a smaller molecular weight than starch and probably forms a molecular-disperse solution in water.

A carbohydrate giving a claret color with iodine was discovered in mace (the arillus of nutmeg seeds) by Henry[2] and described as "*Amylodextrinstärke*" by Tschirch and Schklowsky[3]. There are doubtless a number of other starch-like carbohydrates of this type.

The best known example of Group I(b) is basic lanthanum acetate. Damour[4] dissolved lanthanum carbonate or oxide in excess of acetic acid, diluted with much water, added a slight excess of ammonia and collected the gelatinous precipitate, which he found gave a blue compound with iodine. Damour already discussed the question whether the blue substance was "une véritable combinaison ou bien une simple diffusion de l'iode entre les molécules du sel gélatineux." He considered the latter alternative more likely since the color is identical with that of starch iodide and disappears on gentle heating. Quantitative adsorption experiments were carried out by W. Biltz,[5] who found that over a certain range the iodine-content of the gel was approximately proportional to the concentration of the iodine left unadsorbed. With a sol of the lanthanum salt the ratio was less constant, relatively more iodine being adsorbed from dilute solution. I have plotted logarithmically (Fig. 4, Curve IV) the results obtained with the sol, and calculated them according to the equation $a = 2.837c^{0.75}$. The results appear in Table VI.

[1] Ann., **42**, 306 (1842).
[2] J. pharm., **10**, 283 (1824).
[3] Arch. Pharm., **253**, 102 (1915).
[4] Compt. rend., **43**, 976 (1856).
[5] Ber., **37**, 719 (1904).

Biltz considers that the blue substance obtained from basic lanthanum acetate is similar to starch iodide, and that both are adsorption compounds. The adsorption is much more pronounced in the latter case, however, as shown by the much smaller exponent of c.

Lottermoser[1] finds that lanthanum acetate, precipitated by very dilute ammonia and washed, gives a brown color with iodine, and a blue one when the basic acetate has been kept for 12 hours or warmed to 40–50°; heating to 70° gives a substance colored dirty green by iodine, and

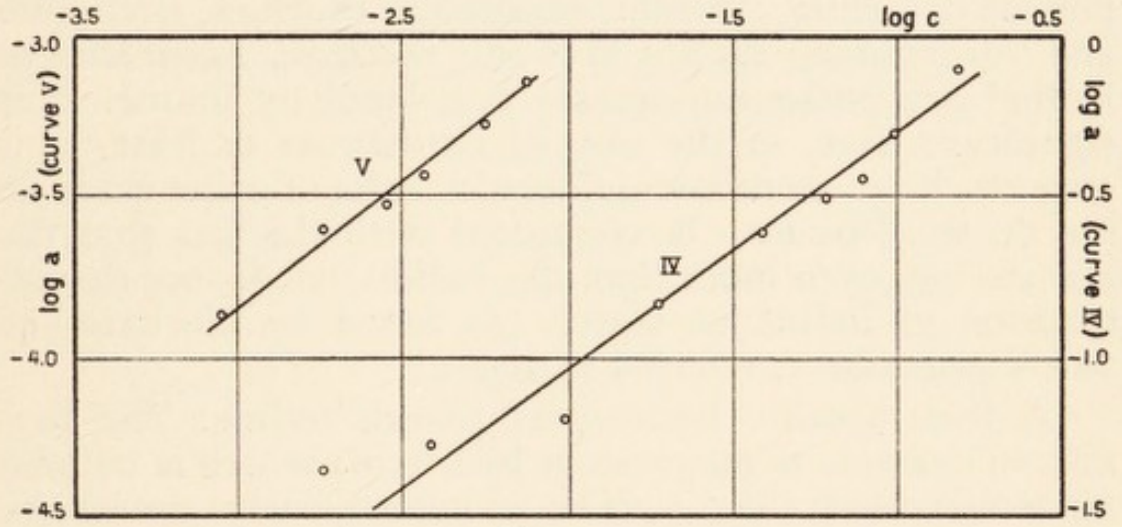


FIG. 4.

Adsorption of Iodine by: basic Lanthanum Acetate (IV), and Zirconium Hydroxide (V).

TABLE VI

Adsorption of Iodine by basic Lanthanum Acetate

(Calculated from the results of Biltz)

Gram-atoms I per liter c	Grams of iodine per gram of La_2O_3 a (found)	a (calc.)	c/a (found)
0.156	0.80	0.71	0.195
0.0976	0.49	0.50	0.199
0.0783	0.36	0.42	0.217
0.0609	0.31	0.35	0.196
0.0389	0.24	0.25	0.161
0.0194	0.143	0.147	0.136
0.00969	0.063	0.078	0.154
0.00375	0.054	0.043	0.070
0.00180	0.045	0.025	0.040

[1] Kolloid-Z., 33, 271 (1924).

boiling yields one which is merely turned yellow. On keeping for four weeks crystals are formed which no longer adsorb iodine. All the amorphous varieties adsorb much more iodine than iodide, and at high concentrations the iodine displaces the iodide entirely. This is quite different from starch and cholalic acid, which take up potassium iodide quite definitely.

According to Orlow[1] basic praseodymium acetate behaves in similar fashion; the gelatinous precipitate is colored violet-blue by iodine. The corresponding compounds of cerium, yttrium, aluminum, thorium, zirconium and "didymium" do not give this reaction. According to Kolbe[2] dry potassium acetate is colored by iodine. It is significant that, in the case of lanthanum at least, it is only the basic acetate which reacts; salts of other acids do not do so. This may be connected with the fact that the acetate ion, even more than the iodide ion, favors the adsorption of iodine on starch (as found by Gorbatschev and Vinogradova, referred to above).

A few metallic hydroxides adsorb iodine. The best known example is magnesium hydroxide which is colored reddish-brown; the reaction is conveniently shown by adding less than one equivalent of sodium hydroxide to a dilute solution of magnesium sulphate and then adding potassium triiodide. This reaction requires further investigation.

The adsorption of iodine by zirconium hydroxide was studied by Wedekind and Rheinboldt.[3] The pasty substance was shaken for several hours with potassium triiodide solutions of various concentrations, and then completely precipitated by adding potassium sulphate solution; the iodine in the clear supernatant liquid was estimated. The concentrations of iodine which would have resulted had no adsorption taken place, ranged in one series of experiments (II) from $N/125$ to $N/1000$, and the ratio of adsorbed iodine to non-adsorbed from 9.0 to 5.7. Wedekind and Rheinboldt considered that the general equation for ad-

[1] Chem. Ztg., 31, 45 (1907).

[2] Kolbe, *Organische Chemie*, v. I, p. 622 (Braunschweig, 1854).

[3] Ber., 47, 2142 (1914).

sorption is not followed and that zirconium hydroxide closely resembles lanthanum acetate, investigated by Biltz (*loc. cit.*). The latter conclusion is certainly correct, but I am unable to agree with the former. The relationship between adsorbed and non-adsorbed iodine is not strictly linear but exponential, and for the series referred to is more nearly expressed by the equation $a = 0.0362c^{0.80}$, where a is expressed in grams of iodine per gram of ZrO_2 and c in gram-atoms of iodine per liter (see Fig. 4, Curve V). Of

TABLE VII

Adsorption of Iodine by Zirconium Hydroxide

(Calculated from results of Wedekind and Rheinboldt)

c	a (found)	a (calc.)
0.00742	0.00073	0.00072
0.00561	0.00051	0.00057
0.00370	0.00038	0.00041
0.00276	0.00030	0.00032
0.00178	0.00025	0.00023
0.00088	0.00013	0.00013

course the exponent 0.80 is abnormally high; the other series of Wedekind and Rheinboldt's experiments requires an exponent of 0.65, and that of Biltz with lanthanum acetate, previously referred to, fits an exponent 0.75. In these cases the relationship is much more nearly linear than with starch, amorphous cholalic acid and saponarin, where the observed exponents range from 0.103 to 0.168.

According to Wilks[1] iodine is adsorbed from solution in carbon tetrachloride by dry calcium hydroxide, forming a violet or black powder; the equilibrium is approximately expressed by the equation $a = c^{\frac{1}{3}}$ which also expresses that with bromine. According to Lottermoser[2] adsorption is here simulated by a slow chemical reaction with the hydroxide and it is calcium oxide which shows a true adsorption of iodine.

Thallous salts give a reaction with iodine which, in several respects, simulates that given by organic com-

[1] J. Chem. Soc., 101, 366 (1912).

[2] Kolloid-Z., 33, 271 (1924).

pounds. In particular iodide ions are necessary for this reaction. Thus if to 2 cu. cm. of a cold saturated solution of thallous chloride, 2 drops (= 0.04 cu. cm.) of a $N/5$ alcoholic iodine solution are added, the resultant solution is clear and has a pale brown color. Addition of a few drops of potassium iodide solution at once produces an intense grayish-black precipitate which disappears on warming and returns on cooling, as in the case of starch iodide.[1] The precipitate doubtless consists of the higher iodide Tl_6I_8, for which the conditions of stability were investigated by Abegg and Maitland.[2] It is not obtained at all readily with pre-formed thallous iodide, but only when this iodide is precipitated in the presence of free iodine. This substance resembles the mixed crystals of iodine and coumarin in color, but apparently differs from the latter in having a constant composition.

CHOLALIC ACID

Cholalic acid is the best known of those substances which yield a crystalline blue addition compound with potassium triiodide of the formula 4MI,KI, where M is a molecule of the organic compound. That pre-formed crystals of narceine are colored by iodine was known at an earlier date, but they have not as yet been further investigated. The case of cholalic acid was discovered by Mylius.[3] He showed that the blue compound is formed when cholalic acid crystallizes from a mixture of alcohol and water in the presence of iodine, and that the concentration of the iodine must be within certain limits; with very dilute iodine solutions crystals of unchanged cholalic acid separate, with too concentrated ones a brown product is formed.

The blue crystals can be washed and have the composition $(C_{24}H_{40}O_5I)_4KI,H_2O$. Compounds in which hydrogen or barium replaces the potassium, were also prepared.

The equilibrium between iodine and cholalic acid was studied more closely by Küster[4] who found that when

[1] Barger and Eaton, J. Chem. Soc., **25**, 2412 (1924).
[2] Z. anorg. Chem., **49**, 341 (1906).
[3] Z. physiol. Chem., **11**, 306 (1887).
[4] Z. physik. Chem., **16**, 156 (1895).

cholalic acid in 70-per-cent alcohol is mixed with increasing amounts of aqueous potassium triiodide, and then diluted with water, the most dilute iodine solutions yield only pale yellow crystals of cholalic acid. With rather more iodine the precipitate is greenish and contains particles of a blue substance; when the final concentration of the iodine is 0.2 gm. per liter the precipitate consists entirely of blue crystals and further augmentation of the amount of iodine at first merely increases the quantity of these crystals, without raising the iodine content of the solution, which remains at 0.2 gm. per liter ($N/635$) over a certain range, before ultimately increasing.

The constant concentration of iodine corresponds to the dissociation pressure of a solid and implies the formation of a chemical compound.

Küster dissolved 4.0064 gm. of cholalic acid (containing alcohol of crystallization) in 100 cu. cm. of 70-per-cent alcohol, and for each experiment added to 10 cu. cm. of this solution various volumes of an aqueous solution containing 12.196 gm. of iodine per liter with potassium iodide. The mixtures were then all made up with water to 100 cu. cm.; next day they were filtered and the iodine in the filtrate was estimated; the iodine in the cholalic acid is obtained by difference.

TABLE VIII

Iodine in crystalline Cholalic Acid

(Küster's results)

Iodine added cu. cm.	Total iodine in filtrate gm.	Total iodine in cholalic acid gm.	Percentage iodine in precipitate.
0.5	0.0050	0.0011	——
1	0.0106	0.0016	——
1.5	0.0144	0.0039	——
2	0.0200	0.0044	——
3	0.0198	0.0168	——
5	0.0198	0.0412	——
10	0.0200	0.1020	28.0
15	0.0750	0.1079	29.6
20	0.1337	0.1102	30.2
25	0.1920	0.1129	31.0

It will be seen that over the range indicated by the brace, the iodine concentration of the solution does not increase, the additional iodine added all going into the cholalic acid until the precipitate reaches the composition required by Mylius' formula (27.5 per cent I). After this most of the additional iodine remains in solution, a little going into the precipitate. The composition of the latter was obtained indirectly, after titrating the small amount (about 10 per cent) of the cholalic acid which went uncombined through the filter. The iodide of cholalic acid is therefore in equilibrium with a solution of iodine containing 0.2 gm. per liter, or $N/635$. Küster's experiment was repeated by Barger and Field[1] with similar results, except that a less definite and lower dissociation pressure was found ($N/2500$—$N/5000$). In a further series of experiments sodium cholalate and iodine in aqueous solution were mixed with hydrochloric acid, when the cholalic acid, separating in an amorphous condition, takes up much more iodine than in the crystalline. Up to a certain concentration of iodine the precipitate is blue, and an adsorption formula expresses its composition; at higher concentrations it becomes black and finally brown, taking up much more iodine and deviating from the exponential equation. In Table IX, c is the final concentration of iodine in gram-atoms per liter, and a the composition of the precipitate in atoms of iodine per molecule of cholalic acid. The cal-

TABLE IX

Adsorption of Iodine by amorphous Cholalic Acid

(Barger and Field)

c	a		color
	found	calc.	
0.000099	0.55	0.55	blue
0.000227	0.62	0.63	blue
0.00063	0.78	0.75	blue
0.00185	0.89	0.89	blue
0.00425	1.26	1.03	black
0.00766	1.81	1.14	black
0.0130	2.09	1.24	brown

[1] J. Chem. Soc., 101, 1404 (1912).

culated values of a were obtained from the equation $a = 2.58c^{0.168}$ (see also Fig. 3, Curve II; and Fig. 5, reproducing the results of Barger and Field).

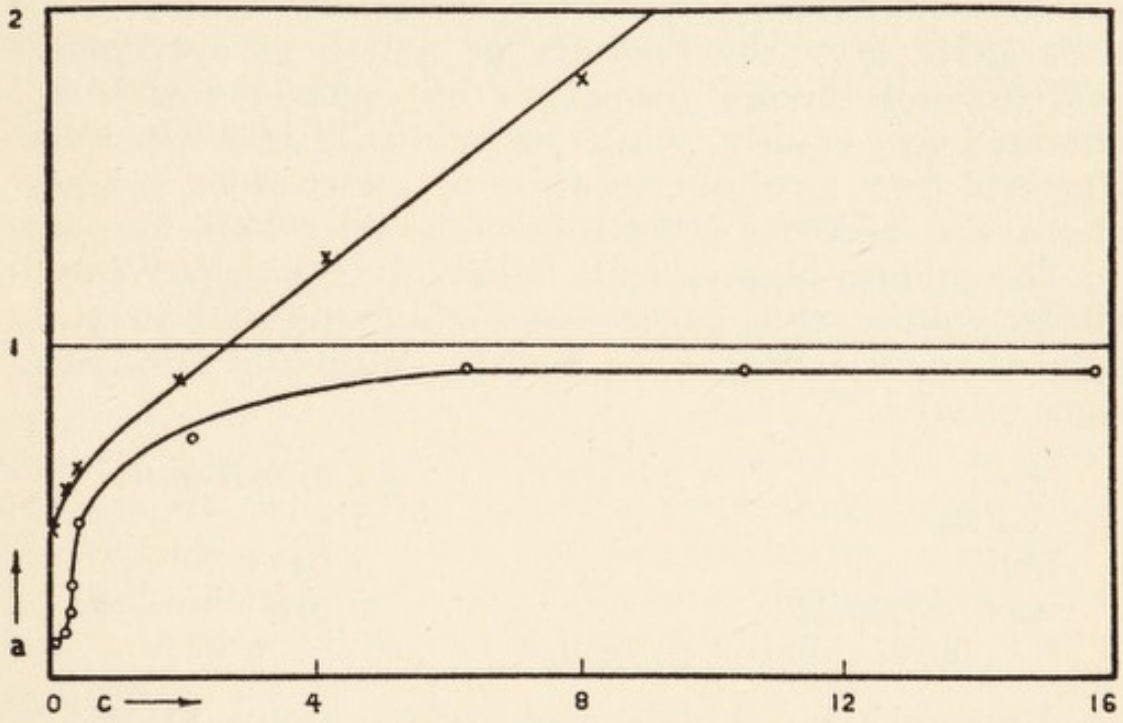


FIG. 5

Iodine content of amorphous and crystalline addition compounds of Cholalic Acid.

The brown iodide contains more than one atomic proportion of iodine and becomes blue on addition of very little sodium thiosulphate. Mylius[1] finds the brown iodide of cholalic acid has the composition $C_{24}H_{40}O_5I_2$, without any metal. It can be made by mixing 0.2 gram cholalic acid in 7 gm. of 66 per-cent zinc iodide solution with 2 gm. of iodine dissolved in 18 gm. of the same solution, and diluting with water, or by diluting an alcoholic solution containing the two components in molecular proportion. It becomes blue when HI, KI, ZnI_2 or reducing agents are added.

Cholalic acid crystallizes with water, methyl, ethyl, propyl, and allyl alcohol, with ethylene glycol, acetone and phenol; its crystallization with iodine is apparently yet another example of its molecular compounds. When the acid is precipitated from the aqueous solution of its sodium salt, the iodide separates in a finely divided

[1] Ber., 28, 385 (1895).

(amorphous or microcrystalline?) condition and can then take up additional iodine by adsorption. There seems to have been a slight additional uptake of iodine even in Küster's experiments with the crystalline iodide. It is remarkable how the presence of iodine in appropriate concentration favors formation of crystals which are obtained very readily, while pure cholalic acid when precipitated from alcoholic solution by water alone is amorphous and becomes crystalline only after long keeping.

The minute blue crystals behave like a negative lyophobic colloid; thus Barger and Field found that a certain suspension was flocculated by the following concentrations of salts:

KCl	35.0 millimole
$BaCl_2$	0.5 "
$AlCl_3$	0.03 "
$Co(NH_3)_6Cl_3$ (luteo-cobaltic chloride)	0.016 "

A suspension of cholalic acid without iodine, formed by dilution of an alcoholic solution with water, behaves quite differently; it is not even flocculated by 166 millimolar lanthanum nitrate. The blue iodide crystals are also flocculated by dyes in the same way as saponarin (see below), but they differ from saponarin and starch iodides in not becoming crimson on the addition of much iodide or thiocyanate. This color change is given only by amorphous substances and seems to depend on a change in the size of the particles.

The alkaloid narceine was found by Neubauer[1] to be colored blue by dilute solutions of iodine in potassium iodide. The reaction is peculiar in that it is given by the pre-formed crystals of the alkaloid, which appear to be permeable to iodine. It is, however, also possible to obtain the blue narceine-iodine compound by adding light petroleum to a pyridine solution, or chloroform to a glacial acetic acid solution of the components.[2] If the aqueous iodine solution acting on the crystals is too concentrated,

[1] Z. anal. Chem., 9, 390 (1870).
[2] Barger and Starling, J. Chem. Soc., 107, 415 (1915).

a brown substance results, apparently similar to the ordinary periodides of other alkaloids; by careful addition of ammonia the excess may be removed and the color changed to blue. Narceine is a feeble base and contains a carboxyl group, in this respect resembling the ergot base ergothioneine which also forms a blue iodide existing only as crystals.[1]

SAPONARIN AND EUXANTHIC ACID

The epidermal cells of the leaves of certain flowering plants, belonging to various natural orders, have long been known to contain, dissolved in their cell sap, a substance which is colored blue by iodine. The color disappears on warming and returns on cooling, as is the case with starch; unlike starch, however, the color is not confined to well-marked grains but extends uniformly throughout the cell as a fine blue precipitate, and when the cell wall is ruptured a blue cloud passes out. The substance was regarded as an amorphous variety of starch by its discoverer Sanio,[2] who showed by plasmolysis of the leaf epidermis of *Gagea lutea* that the substance is confined to the cell sap. The publication of this observation led Schenk[3] to record similar observations in the closely allied genus *Ornithogalum*. Schenk doubted the identity of the substance with starch, because fragments of the epidermis of *Ornithogalum* leaves, colored blue by iodine, lost their color when placed in water. (The iodide readily dissociated and was most likely due to a substance capable of forming a molecular-disperse solution). The substance was next observed in *Ornithogalum* by Trecul[4] and later studied by Nägeli[5] who definitely declared against the identity with starch. It was then found by Kraus[6] in *Arum*; and finally a detailed account of "soluble starch" was published by Dufour,[7] who found it in about twenty species of phane-

[1] Barger and Ewins, J. Chem. Soc., **99**, 2336 (1911).
[2] Botan. Ztg., **15**, 420 (1857).
[3] Botan. Ztg., **15**, 497, 555 (1857).
[4] Bull. soc. botan. France, **5**, 711 (1858).
[5] Beitr. wiss. Botanik, **2**, 187 (1860).
[6] Botan. Mitt. Halle, 1885.
[7] Bull. soc. vaud. sci. nat., **21**, 227 (1885).

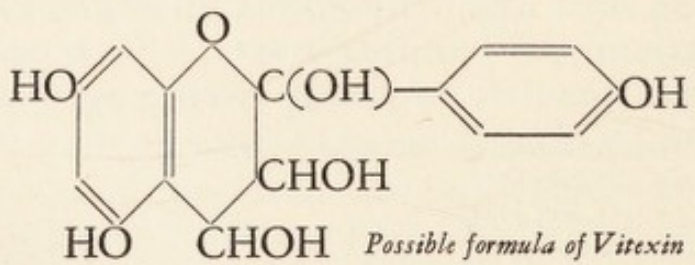
rogams but, like his predecessors, he did not isolate it. This was done by the writer[1] from *Saponaria officinalis*, a plant which under the microscope appeared to be relatively rich in the substance, and is grown on the Continent for pharmaceutical purposes so that large quantities are easily obtainable. This is necessary, since the substance is restricted to the single layer of epidermal cells.

The substance proved to be a glucoside and was named "saponarin". It is hydrolyzed by acids, according to the equation

$$C_{21}H_{24}O_{12} + H_2O = C_{15}H_{14}O_7 + C_6H_{12}O_6$$

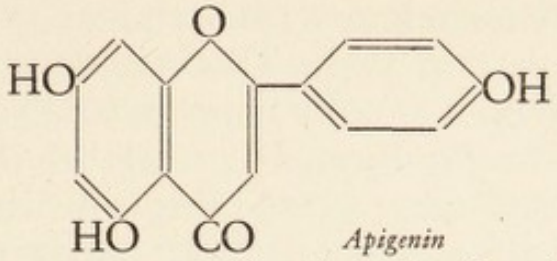
into glucose and vitexin, a coloring matter previously obtained by A. G. Perkin[2] from the dye-wood of the New Zealand tree *Vitex littoralis*. Perkin obtain vitexin by hydrolysis, but whether it is present as a glucoside, identical with saponarin, has not been ascertained, nor is it known whether the "soluble starch" of monocotyledons (*Gagea*, *Ornithogalum*, *Arum*) is identical with saponarin. It might quite well be some other flavone derivative, many of which behave with iodine in a similar fashion. While investigating saponarin I attempted to isolate the "soluble starch" from *Bryonia dioica*, which substance behaved in unexpected fashion and is probably not identical with saponarin. Nasse[3] found that an extract of acorns gives a blue reaction with iodine, which he considered due to quercetin (also a flavone derivative).

The constitution of vitexin has not been fully elucidated, but my own experiments and those of Perkin, who converted it into tetranitroapigenin, leave no doubt that it is closely related to the flavones. It may be a reduced flavanone of the constitution below.



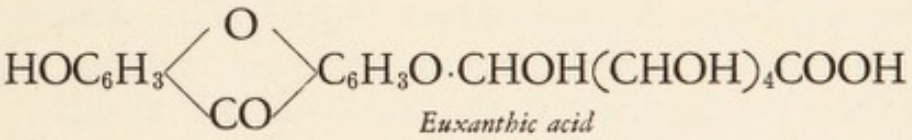
Possible formula of Vitexin

[1] Barger, J. Chem. Soc., **89**, 1210 (1906).
[2] J. Chem. Soc., **73**, 1030 (1898); **77**, 416 (1900).
[3] Ber., **17**, 1166 (1884).

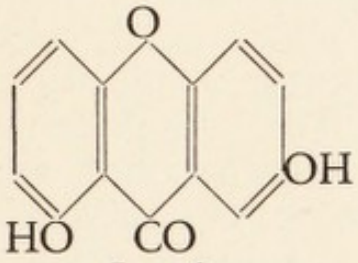


Apigenin
(1,3,4′-trihydroxyflavone)

When some insight into the constitution of saponarin had been obtained, I considered whether any of the six or eight substances then known to give blue compounds with iodine, had any similarities in their chemical constitution. It had been recorded by Graebe[1] that the esters of euxanthic acid, formed by heating the silver salt with alkyl iodides, formed blue iodine compounds. I soon found that this property is not confined to the esters, as Graebe stated, but also belongs to the acid itself, and to its fission product euxanthone (4,6-dihydroxyxanthone) which is combined with glucuronic acid to form euxanthic acid.



Euxanthic acid



Euxanthone
(4,6-dihydroxyxanthone)

It is not known which hydroxyl is united to the glucuronic acid. Now the saponarin and euxanthic acid are both derivatives of γ-pyrone. This led to the examination of synthetic pyrone derivatives, most of which were found to give blue compounds with iodine under suitable conditions, and later other synthetic substances were found to do the same; these will be discussed in a later section.

[1] Ber., 33, 3360 (1900).

Saponarin when shaken with water at 18° dissolves to the extent of 1 part in 7100. This solution is molecular-disperse and gives no coloration with potassium triiodide, nor do the pre-formed crystals of the substance. Colloidal solutions may be obtained by dissolving the glucoside in alkali and acidifying, or by dissolving in pyridine or formamide and diluting with water. Less concentrated ones (1:1000) are obtained by boiling with water and cooling. In these cases the saponarin does not crystallize out for some time. The "supersaturated" solutions are colloidal; they can be resolved under the ultramicroscope and show the Tyndall effect, but are not opalescent to the naked eye. They are colored blue by potassium triidiode and when ionized substances are present only in small concentration, a blue hydrosol results. When this is diluted with water the blue color vanishes more or less abruptly between 1:7000 and 1:8000, in the neighborhood of the true solubility. This shows conclusively that molecular-disperse solutions do not adsorb iodine. The same experiment can be made with euxanthic acid, where a sol is obtainable by acidifying the solution in alkali, or by rapidly cooling the hot saturated solution water. The sol is unstable and soon crystallizes; it gives a blue sol with potassium triiodide which on dilution becomes colorless at about 1:7000, the true solubility at room temperature being about 1:5510. The long yellow needles of euxanthic acid obtained from dilute alcohol, when placed in water, break up into an amorphous suspension which is colored blue by iodine and changes in a few minutes into small colorless lancet-shaped crystals. The fact that neither the crystals nor the molecular-disperse solutions of saponarin and of euxanthic acid are colored by iodine, but only their colloidal solutions and amorphous suspensions, finds no analogy with starch because the latter neither crystallizes nor forms molecular-disperse solutions. Cholalic acid, on the other hand, does both but it forms no colloidal solutions. Saponarin and euxanthic acid do all three, since they are intermediate between starch and cholalic acid. They are semi-colloids with one glucose residue, therefore

moderately hydrophilic, and yet provided with the pyrone nucleus which adsorbs iodine.

In the absence of electrolytes the blue hydrosol appears homogeneous and passes through filter paper. It is precipitated by electrolytes, and a concentrated sol sets to a thick jelly on the addition of iodine, so that the test tube containing it may be inverted without loss.

TABLE X

Minimum Final Concentrations of Electrolytes Flocculating within 1 minute

(Saponarin 1:2000 after addition of iodine and electrolyte)

	Millimols		Millimols
KCl	51	$La(NO_3)_3$	0.2
KNO_3	49	$\frac{1}{2}Al_2(SO_4)_3$	0.21
KI	60	$AlCl_3$	0.22
$\frac{1}{2}K_2SO_4$	53	$CO(NH_3)_6Cl_3$	0.1
$\frac{1}{2}$K tartrate	50	$Th(NO_3)_4$	0.2
NaCl	53	HCl	58
NH_4Cl	53	HNO_3	66
LiCl	75	$\frac{1}{2}H_2SO_4$	78
Aniline hydrochloride	50	CCl_3COOH	88
Morphine hydrochloride	no action	$CHCl_2COOH$	195
		$\frac{1}{3}H_3PO_4$	482
		$CH_2Cl \cdot COOH$	no action
$BaCl_2$	1.8	$CH_3 \cdot COOH$	no action
$SrCl_2$	2.25	$HOOC \cdot COOH$	no action
$CaCl_2$	2.6		

The iodide of saponarin is a negative lyophobic colloid, being particularly sensitive to multivalent cations as shown in Table X, which gives the minimum final concentrations of electrolytes that flocculated within one minute.[1]

The saponarin-iodine sol contained already potassium iodide corresponding to a final concentration of 2.5 millimols, but since 60 millimols of this salt were required to flocculate, the error is not larger than a few per cent. The average concentrations of uni-, bi-, and trivalent cations

[1] Barger and Field, J. Chem. Soc., 101, 1388 (1912).

are in the ratio 50:2.2:0.2, and afford a good illustration of Schulze's law. The negative character of the sol is also readily shown by cataphoresis, as in the case of starch. The lyophobic character is, however, much greater than with starch. Since saponarin itself is not precipitated by electrolytes, adsorption of iodine seems to have rendered it much less stable, unlike a gold sol which is rendered more stable by adsorbed starch or gelatin. The hydrosol of saponarin iodide is flocculated by positive dyes; methylene blue precipitates completely in all concentrations above a certain limit, but the more highly colloidal night blue shows a well-marked zone of optimal flocculation, so that more concentrated solutions are once more ineffective. Negative dyes (azo blue) are inactive in any concentration.

When dry the "blue" saponarin iodide is red, but is at once changed back to blue on the addition of water. The change from blue to red is also brought about by salts, the order of their activity being lyotropic, thus:

thiocyanate > iodide > bromide > nitrate > chloride and sulphate

The effect of the nitrate, not demonstrable at all with starch, is here so slight that a saturated solution of ammonium nitrate was required, the potassium salt not being sufficiently soluble. The chloride and sulphate were inactive.

The lyotropic order is also that of the salting-out of hydrophilic colloids,[1] thiocyanate salting-out least and peptizing most. The thiocyanate ion is regarded as the least hydrated of the series, and it is not clear why this ion should favor a color change, which in the case of saponarin is also brought about by desiccation. Water is necessary for the production of the blue iodide of starch and Graebe did not obtain any blue compounds of euxanthic acid esters until the reaction product of the silver salt and alkyl iodide was moistened with water.

It was shown in dealing with starch and cholalic acid, that a blue compound is not formed with iodine alone; potassium iodide or some other electrolyte must be present.

[1] Freundlich, *Capillary and Colloidal Chemistry*, p. 576 et seq. (London, 1925).

Likewise a solution of iodine in water does not color saponarin, but the addition of potassium iodide at once brings about coloration. Chlorides are much less effective and the required concentration depends on the valency of the cation. Two cu. cm. of saponarin sol, 1:1250, prepared in a quartz vessel, was mixed with 5 cu. cm. of saturated aqueous iodine solution and 1 cu. cm. of various chlorides was added, producing the following effects:

1 cu. cm. *M* KCl gave green in 38 minutes; later blue;
1 cu. cm. *M*/10 $BaCl_2$ gave deep green in 20 minutes; blue in 30 minutes;
1 cu. cm. *M*/100 $AlCl_3$ gave no effect;
1 cu. cm. *M*/100 $LaCl_3$ gave deep green in 10 minutes; blue and flocculated in 20 minutes;
1 cu. cm. H_2O gave very pale green in 53 minutes.

The inactivity of the aluminum chloride is probably due to hydrolytic dissociation. For the production of a blue color it seems to be necessary that some cation be adsorbed, and the sol rendered more positive, as was discussed in the section on starch.

Bayliss[1] showed that neutral salts increase the adsorption of Congo red and other negative colloids by filter paper, and that the adsorption of trypsin[2] by various adsorbents is favored by calcium sulphate. The explanation suggested for the effect of multivalent cations on iodine adsorption, implies that iodine sols are negative. It is noteworthy that Amann[3] has shown that many brown solutions of iodine in organic solvents are resolvable under the ultramicroscope.

Starch is not very suitable for showing the effect of the valency of the cation, but lanthanum chloride can readily be shown to be more effective than potassium chloride, even in this case. The best example of this phenomenon is euxanthic acid, where the acidic electro-negative character of the sol makes the superiority of multivalent cations very evident. It would be interesting to compare this effect in

[1] Biochem. J., 1, 195 (1906).
[2] Proc. Roy. Soc. London, 84 B, 88 (1911).
[3] Kolloid-Z., 6, 235 (1910).

the case of euxanthic acid with some neutral derivative, such as an ester. In general euxanthic acid deserves further investigation in this connection.

Finally it should be mentioned that in addition to the colloidal adsorption compound so far discussed, saponarin forms blue crystals (needles) when a solution in dilute acetic acid containing a little iodine is allowed to evaporate on a watch glass.

The composition of the iodide of saponarin was investigated by methods similar to those employed by others in the case of starch. In the first place Barger and Field (*loc. cit.*) showed that the conductance of a saponarin

TABLE XI

Adsorption of Iodine by Saponarin

(Barger and Field)

c	a (found)	a (calc.)
0.00042	0.317	0.337
0.00134	0.432	0.409
0.00294	0.491	0.466
0.00477	0.519	0.505
0.00623	0.527	0.528

solution and a solution of iodine in potassium iodide is less after mixing than before, proving that some of the potassium iodide is adsorbed. The "free" iodine was estimated by mixing measured volumes of 0.1-per-cent saponarin sol with iodine solutions of various concentrations and flocculating by adding half a volume of 10 per-cent sulphuric acid. The precipitate did not, however, settle down so well as in the case of starch; it was therefore filtered through glass wool, filter paper being objectionable because it adsorbs iodine. The final concentrations of non-adsorbed iodine in gram-atoms per liter (c) and atoms of iodine adsorbed per molecule of saponarin (a), both found and calculated according to the equation $a = 1.23\,c^{0.167}$, are given in Table XI (see Fig. 3, Curve III). The value $1/n = 0.167$ is practically the same as that found for amorphous cholalic acid, and similar to that of

Küster's experiments with starch and dilute iodine solutions. Since the saponarin could be employed only in very dilute solutions, the results are necessarily not very accurate and the concentration of the iodine extends over a rather small range; but it is evident that in this case also, as with other amorphous addition compounds, the composition changes quite gradually with increasing concentration of iodine.

SYNTHETIC PYRONES COLORED BLUE BY IODINE

As has been pointed out above, the fact that both euxanthic acid and saponarin are γ-pyrone derivatives led to an investigation of related substances of relatively small molecular weight. Since, like cholalic acid, they did not readily form stable colloidal solutions, and since iodine is only taken up by a disperse phase, Barger and Starling[1] adopted the method of mixing the solutions in some organic solvent (usually alcohol) with an aqueous solution of potassium triiodide, a method originally employed by Mylius in the case of cholalic acid. When dealing with substances soluble in alkali one can pour the alkaline solution into potassium triiodide containing excess of acid. In either case the precipitated substance may be colored blue, and there are two possibilities: blue crystals may be formed, or the amorphous precipitate may become blue. In the case of cholalic acid both these possibilities have been realized, the one by using a solution in alcohol, the other by using an alkaline solution. Saponarin also forms blue crystals, as well as a colloidal adsorption compound; but with euxanthic acid only the amorphous compound has been obtained. The ability to form mixed crystals, or a *Krystallstrukturverbindung* according to Küster, is possessed by certain substances only and has not been connected with their chemical constitution. Thus γ-benzopyrone-2-carboxylic acid forms mixed crystals with iodine (pleochroïc needles), but its 6- and 7-methyl derivatives do not; coumarin does, 4,6-dimethylcoumarin does not. Even isomerides may differ in this

[1] J. Chem. Soc., 107, 411 (1915).

respect: 6- and 7-methylflavone form blue filamentous needles, 8-methylflavone does not form mixed crystals, yet it forms a blue amorphous adsorption compound.

It seems that crystals containing iodine are of two kinds: the proportion of iodine may vary from traces upwards, or the amount is fixed as in the case of cholalic acid. Thus coumarin (the simplest α-pyrone) crystallizes from $N/500$ iodine in blue, from $N/5000$ in pale grey, from $N/20\,000$ in almost colorless needles. Here true mixed crystals or solid solutions of continuously varying composition are formed, as is the case with the mixed crystals of iodine and benzene.[1] Barger and Eaton[2] found the composition of the mixed crystals of iodine and coumarin varied continuously from 0.208 to 0.5 per cent atoms of iodine per molecule of coumarin, when the resultant concentration of iodine in solution changed from $0.007N$ to $0.164N$; acetocoumarin behaved in the same manner. Similar crystals result when coumarin and iodine are kept side by side in a partial vacuum at room temperature; and likewise crystals, of metallic appearance, are formed when flavone and iodine are sublimed together at 1 mm. pressure.

According to Bruni[3] it is not necessary that the components of a mixed crystal should be isomorphous. Pleochroïsm is particularly marked when iodine crystallizes with certain simple coumarins, particularly 3-acetyl- and 3-benzoylcoumarin, also with narceine. The composition of these and other crystalline addition products with iodine should be investigated in order to ascertain whether some have not a definite dissociation pressure, as Küster found for cholalic acid. Preliminary indications show that for 3-acetylcoumarin a concentration of about $N/600$ iodine is required for the formation of grey mixed crystals, whereas a transitory amorphous blue adsorption compound can be obtained with more dilute iodine solutions, but this rapidly changes to colorless crystals. With 3-benzoylcoumarin and $N/100$ iodine in 20–30 per-cent alcohol there first separate, from a warm solution, colorless large

[1] Beckmann and Stock, Z. physik. Chem., **17**, 120 (1895).
[2] J. Chem. Soc., **125**, 2411 (1924).
[3] Bruni, *Feste Lösungen und Isomorphismus*, p. 52, (Leipzig, 1908).

crystals and then from separate centers, clusters of much smaller, very thin, mixed crystals, indicating an abrupt change to a separate compound.

There is no definite indication of an ionic influence on the formation of the crystalline iodine addition compounds, and where—as in the case of coumarin and flavone—mixed crystals are obtained from the vapor phase, such influence is excluded. Ions, however, often greatly influence the formation of amorphous adsorption compounds and since most of the organic substances employed form electro-negative suspensions, adsorption is chiefly influenced by cations, whose effect is shown by an alteration in the concentration of the iodine required to produce a blue color. In a few cases an effect of multivalent cations was observed, similar to that on the adsorption of iodine by saponarin (referred to in an earlier section). Thus 2-phenyl-4,3-β-naphthopyrone is colored dark blue by $N/1000$ iodine solution after 30 minutes, when $N/1000$ aluminum chloride or $N/10$ hydrochloric acid is present, but not in the absence of such electrolytes. The adsorption of iodine by 4,3-β-naphthopyrone-2-carboxylic acid takes place much more readily when tap water (containing calcium) is used, instead of distilled water. The most sensitive of all the substances employed, α-naphthoflavone, gives a pure blue coloration even with $N/80\,000$ iodine; but in the presence of multivalent cations the reaction is about twice as delicate, and becomes considerably more delicate than the iodine reaction of starch, which is not so susceptible to the action of minute concentrations of multivalent cations (in these experiments there was always potassium iodide present, since aqueous potassium triiodide was employed). 1,4-Dimethylthioxanthone slowly becomes blue with $N/100$ iodine, but at once in the presence of calcium chloride; 1,4-dimethoxythioxanthone becomes green in a few seconds with $N/30000$ iodine, but in the presence of calcium chloride there is an immediate blue coloration.

The effect of cations is, however, most frequently illustrated by increased adsorption due to hydrogen ions. This is characteristic of many thiopyrones. Thus 5,8-

dimethylthioflavone gives, in neutral solution, an amorphous adsorption compound with $N/300$ iodine. Calcium chloride has no effect, but hydrochloric acid at once makes the reaction 100 times as sensitive; even $N/10000$ acid produces a definite blue color after half an hour, while without the acid the substance remains colorless. This concentration of hydrogen ions is much below that affecting Congo red. 6,8-Dimethylthioflavone and β-thionaphthoflavone are likewise 100 times more sensitive in the presence of acid, the 6-, 7-, and 8-methylflavones become 5 to 10 times as sensitive in normal HCl; 4,3-β-naphthopyrone is influenced by $N/1000$ hydrochloric acid. If, as has been suggested in the case of starch, cations act by diminishing the negative charge of the particles, multivalent anions should inhibit adsorption. There is some indication of this, although the effect is not pronounced. In the case of 4,3-β-naphthopyrone it is carboxylic acid, and of α- and β-naphthoflavones, sodium citrate and phosphate that slightly restrain adsorption.

In one case, that of 5-methyl-8-isopropylflavone, the effect of hydrogen ions and of multivalent cations was the opposite of that described above, and resulted in the delay or prevention of adsorption; on the other hand multivalent anions hastened it. When an alcoholic solution of this flavone is added to $N/30$ iodine in potassium iodide, the amorphous precipitate undergoes no obvious change for about 30 seconds and then becomes bluish-green with striking rapidity. With $N/100$ or $N/200$ iodine this change occurs after 5 minutes, with $N/500$ after 10 minutes. Multivalent cations delay or prevent the color change; but citrate, phosphate, ferricyanide, and sulphate hasten it, so that it is evident with $N/100$ iodine after a few seconds. The electrical properties of a suspension of this flavone require investigation: the presence of a large alkyl (isopropyl) group, in addition to methyl, is perhaps the cause of a change of sign.

The above effects on adsorption depend on the valency of the ions. A lyotropic effect is not clearly indicated; Barger and Eaton[1] examined the effect of potassium iodide,

[1] J. Chem. Soc., 125, 2411 (1924).

bromide, chloride, nitrate, sulphate, acetate, tartrate, citrate, and thiocyanate on the rapidity of adsorption of iodine by sols of 7-benzoyloxy-4-methylcoumarin, about to be discussed.

An alcoholic solution containing the coumarin derivative and iodine, and thus practically free from iodide ions, was poured into a 0.2 molar aqueous solution of the various salts to be examined. The blue color appeared first with the iodide but the salts did not fall into a lyotropic series. A similar negative result was obtained with benzylidenephthalide.

While mixed crystals, when formed at all, generally separate at once on mixing a sufficiently concentrated alcoholic solution of the organic substance with an aqueous solution of iodine of suitable strength, the formation of an amorphous adsorption compound may require several seconds or minutes, or even half an hour. This delayed adsorption is conveniently demonstrated by the case of 7-benzoyloxy-4-methylcoumarin, an easily accessible substance. A 0.1-per-cent solution in 90-per-cent alcohol is mixed with ten volumes of potassium triiodide in distilled water. With $N/1000$ iodine the mixture becomes almost immediately dark blue; with $N/3000$ iodine this takes a few seconds; with $N/5000$ iodine the white suspension acquires a blue tint after about 30 seconds and gradually becomes deep indigo blue; with $N/10\,000$ iodine the blue color appears after 50 seconds. 1,4-α-Naphthopyrone-2-carboxylic acid and many thioflavones also show slow adsorption which is generally hastened by the presence of hydrogen ions and multivalent cations.

When the small quantity of potassium iodide in the iodine solution is the only electrolyte present, the amorphous adsorption compounds form blue, negative, strongly lyophobic sols resembling that described in the section on saponarin. Thus the blue sol of the benzoyl derivative of 7-hydroxy-4-methylcoumarin, just referred to, is at once flocculated if the iodine solution has been prepared with tap water. It is possible, by adding barium chloride, to clot the sol to a thick gel, so that the test tube can be inverted, although the amount of the coumarin present is

not more than 1 : 1000. The gel is probably a network of filamentous crystals, like fibrin in the clotting of blood.

With substances of small molecular weight, which have a great tendency to crystallize, and do not form mixed crystals with iodine, the amorphous adsorption compound may have a very transitory existence; it soon changes to minute colorless crystals. This is so with acetocoumarin, with 4,6-dimethylcoumarin, with 7-hydroxy-4-phenylcoumarin. 1,4-α-Naphthopyrone crystallizes extremely rapidly. Hence with $N/20\,000$ iodine a blue flash can just be detected, before colorless crystals appear. With $N/1000$ iodine, on rapid cooling, blue flakes and colorless crystals appear together. The former slowly disappear, more slowly in the presence of gelatin which acts as a protective colloid. 4-Methyl-1,2-α-naphthopyrone also crystallizes very rapidly and the blue color is momentary. 7-Benzoyloxy-3,4-dimethylcoumarin is colored blue by $N/3000$ iodine in fifteen seconds, and in another ten seconds is transformed into colorless crystals. 7-Benzoyloxy-4-methylcoumarin crystallizes more slowly, and the blue adsorption compound may persist for half an hour. Other adsorption compounds persist for days or weeks but, since we are not dealing with substances of high molecular weight, there is always a tendency to crystallize. Rapid crystallization may render the detection of an adsorption compound difficult. Thus on slow cooling of a warm solution, the substance may crystallize without forming a blue compound, whereas the latter may appear on cooling rapidly (for instance with γ-benzopyrone-6-methyl-2-carboxylic acid and naphthopyrone). If the substance is soluble in alkali, the solution may be mixed with an acid iodine solution, thus utilizing more abrupt precipitation as well as the often favorable effect of hydrogen ions.

While in the vast majority of synthetic compounds examined the adsorption compound with iodine is a pure blue (as is typical of starch), crimson and pink colorations have occasionally been observed without obtaining any explanation for the aberrant color. In several cases the color is crimson or pink with very low concentrations of

iodine and becomes blue when more iodine is present. Indenoflavone, more sensitive to iodine than starch itself, gives a blue color when a few drops of its 0.1-per-cent solution in alcohol are added to 5 cu. cm. of iodine more concentrated than $N/10\,000$. At this limit the adsorption compound is crimson and at $N/40\,000$ to $N/60\,000$ pink; in the presence of multivalent cations a pink color is obtained at $N/125\,000$. If the solution is slightly alkaline a pink color is produced even with more concentrated iodine solutions. Thus Barger and Eaton found that with 0.2 cu. cm. of 0.05-per-cent indenoflavone in alcohol, 1 cu. cm. of $N/1000$ iodine in potassium iodide and 5 cu. cm. of a buffer mixture, the color of the adsorption compound was pink at pH = 10, violet at pH = 8.4, and pure blue at pH = 1.5. It seems that the pink color is due to a minimal adsorption which can be brought about both by extreme lowering of the iodine concentration and by lowering the concentration of the hydrogen ions; as was mentioned above, an increased hydrogen-ion concentration often increases adsorption. At such extreme dilutions there may be less than one part of iodine and less than one part of the flavone per million of water. γ-Benzopyrone and its 2-carboxylic acid give a crimson coloration; 6,8-dimethyl-γ-benzopyrone-2-carboxylic acid gives a crimson or purple coloration with $N/1000$ to $N/10\,000$ iodine, but with $N/500$ a blue one. In the presence of much potassium iodide the color is always crimson, resembling the lyotropic effect obtained with starch. The crimson color in these cases may have nothing to do with the pink color obtained with indenoflavone and naphthoflavone in the presence of extremely little iodine.

Brown periodides are frequently obtained with high concentrations of iodine, as with cholalic acid.

The change from blue to pink in the case of α-naphthoflavone, and the discovery by Barger and Starling that this substance is a more sensitive indicator for iodine than starch itself, has led to its practical application by Hahn, Schütz and Pavlidés.[1] They used a 0.1 per-cent alcoholic

[1] Über den Chlorungseffekt im Trinkwasser. Die Verwendung von α-Naphthoflavon als Indicator auf freies Chlor. *Z. Hyg. Infektions Krankh.*, 108, 439 (1928).

solution, obtainable commercially as "Chlortest-Kahlbaum" and consider it more delicate than starch for demonstrating the presence of chlorine in drinking water, after the addition of potassium iodide. It is also more convenient, since alcoholic solutions of α-naphthoflavone, unlike starch solutions, do not become less sensitive on keeping and can readily be used by foremen. Hahn, Schütz and Pavlidés recommend as end point the change from blue to pink, instead of the change to colorless. With colored impure water the latter change, in the case of starch, is not sharp. They found that α-naphthoflavone still gave a coloration with $N/30\,000$ iodine, starch with $N/15\,000$, so that the naphthoflavone is twice as sensitive as starch, in accordance with Barger's and Starling's observations. In testing for chlorine, however, the naphthoflavone was found to be about ten times as delicate as starch. With starch, less than 0.014 mg. of chlorine in 200 cu. cm. of tap water escaped detection, while with naphthoflavone 0.0014 mg. could still be recognized. With river water the ratio was even more favorable; starch may fail to reveal the presence of chlorine, when naphthoflavone still shows it.

OTHER ORGANIC SUBSTANCES. INFLUENCE OF CONSTITUTION

The substances discussed in the last section are all pyrone derivatives. Their investigation was suggested by the fact that both saponarin and euxanthic acid belong to this group, but blue adsorption compounds with iodine are formed by many other organic compounds, quinones, phthaleins, cyclic acetals, and the like, and this makes it very difficult to connect iodine adsorption with any particular grouping. The numerous pyrones show that there is at least some connection with chemical constitution, but it is of a fitful character, not unlike the connection between chemical constitution and physiological action, discussed in Chapter III.

At the outset the fact that basic lanthanum acetate behaves so very much like starch, would indicate that the adsorption of iodine—like physiological activity—may

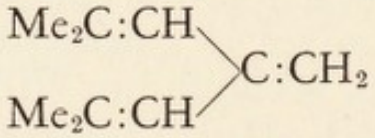
depend primarily on physical properties, which in their turn depend on chemical constitution. It has been shown that certain physical, and more particularly colloidal, conditions must be satisfied even if a chemical grouping—requisite for adsorption—is present. This is somewhat analogous to the theory of haptophore and toxophore groups.

If, nevertheless, an attempt be made to connect adsorption of iodine with chemical constitution it would seem, as a first generalization, that the molecule must have some residual affinity, usually, but not always, associated with oxygen. The vegetable pigments carotin and lycopin, however, are both hydrocarbons of the formula $C_{40}H_{56}$ and both form addition compounds with iodine. Carotin and iodine mixed in ethereal solution form deep violet crystals which, according to Willstätter and Mieg,[1] have the composition $C_{40}H_{56}I_2$. Another compound which Arnaud[2] obtained by adding iodine crystals to a benzene solution of carotin has, according to Willstätter and Mieg, the composition $C_{40}H_{56}I_3$. Lycopin (from tomatoes) was found by Willstätter and Escher[3] to yield an amorphous iodide in dark green gelatinous flakes, with 34–37 per cent of iodine, instead of 32 per cent calculated for the formula $C_{40}H_{56}I_2$. Since both these hydrocarbons are unsaturated, it might be that the iodine is bound by ordinary valencies, but on account of the color of the iodides it is more likely that the carotin compound contains iodine of crystallization, while lycopin forms an amorphous adsorption compound with an increased iodine content. The crystalline compounds of carotin are also apt to contain a slight excess of iodine, even when much less than the calculated quantity of iodine is added. This also applies to a crystalline addition product of xanthophyll, $C_{40}H_{56}O_2I_2$, prepared by Willstätter and Mieg, which seems to be regarded as a molecular compound. In the case of fucoxanthin, from brown algae, Willstätter and Page[4] also found a molecular compound, $C_{40}H_{56}O_6I_4$, forming violet-black prisms. It is interesting, in connection with

[1] Ann., **355**, 1 (1907).
[2] Compt. rend., **102**, 1119 (1886).
[3] Z. physiol. Chem., **64**, 47 (1910).
[4] Ann., **404**, 270 (1914).

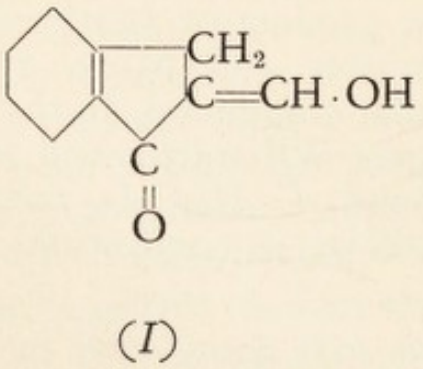
the discussion in the previous section, that Willstätter and Page considered the oxygen of fucoxanthin to be present in pyrone rings; the substance is basic and forms a hydrochloride, $C_{40}H_{56}O_4 \cdot 4HCl$.

The additive compounds with iodine present some analogy to the very interesting additive compounds of the alkali metals, described by Schlenk[1] and his pupils. These compounds are also highly colored; the only hydrocarbons that react are unsaturated, with benzene nuclei adjoining the double bond (tetraphenylethylene, *as*-diphenylethylene) or they are of the fulvene type (with a crossed conjugated bond), *e.g.*

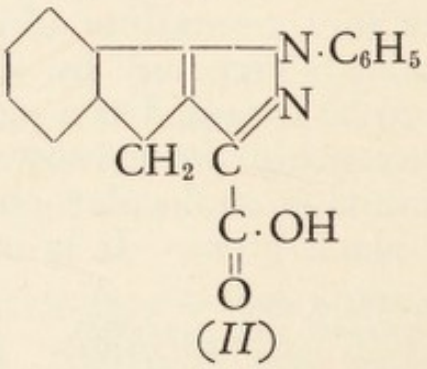


It would be interesting to see whether some synthetic hydrocarbons of this type, perhaps containing naphthalene nuclei, do not form addition compounds with iodine like carotin and lycopin. Fulvenes show an exceptional tendency to combine with alkali metal. Among the many oxygen compounds chromone (γ-benzopyrone) and xanthone add potassium, and residual valency is increased by the accumulation of benzene and naphthalene nuclei. This circumstance was also utilized by Barger and Starling, who employed aromatic nuclei to confer a high molecular weight and insolubility necessary in order to obtain colloidal solutions.

A crossed conjugated double bond is present in the γ-pyrones and in other substances which also give typical blue iodine compounds, such as (*I*) and (*II*) below:



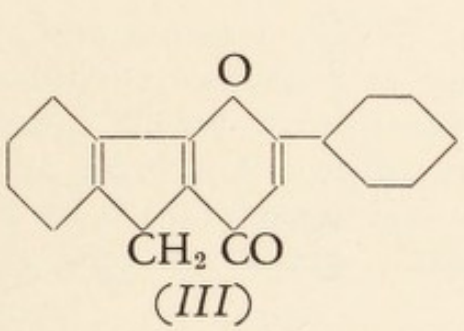
(*I*)



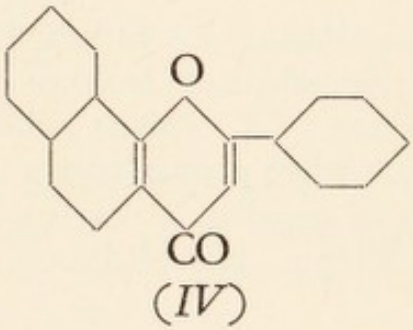
(*II*)

[1] Ber., **46**, 2840 (1913); **47**, 473, 1664 (1914); Ann., **463**, 1–322 (1928); **464**, 1–42 (1928).

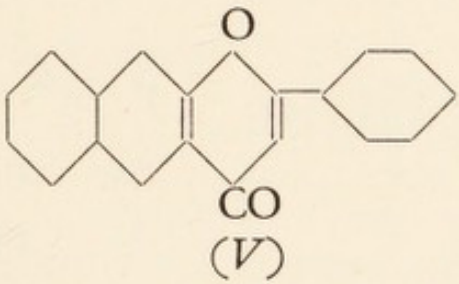
Therefore Barger and Starling attempted to connect this type of unsaturation with the power of adding iodine. The slight adsorptive power of β-naphthoflavone (*V*) (in neutral solution there is no typical coloration even with *N*/10 iodine; in acid solution there is a blue color up to *N*/10 000) as compared with the great reactivity of indenoflavone (*III*) and α-naphthoflavone (*IV*), is probably connected with the fact that, whether we adopt a naphthalene formula with alternating double bonds or the one suggested by Willstätter and Waser,[1] the pyrone ring does not have its usual double bonds in β-naphthoflavone. Moreover, if one of these bonds is reduced, as in the flavanones, the power of adsorbing iodine is abolished.



(*III*)



(*IV*)

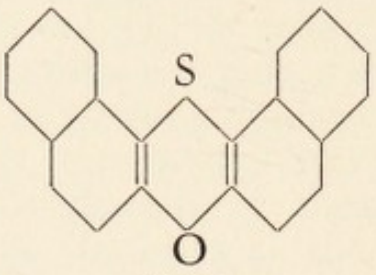


(*V*)

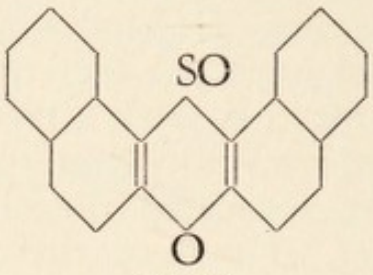
It is a question whether, in pyrones, the iodine is attached to the bridge-oxygen or to the carbonyl oxygen. The cyclic acetals of Bergmann, and starch itself, would suggest that it is the bridge-oxygen atom. Schlenk, however, found that alkali metals are attached to the carbonyl oxygen. Moreover, Barger and Starling found that naphthothionine (*VI*) does not form a blue iodine compound whereas the corresponding oxide (*VII*) does; naphthothionine dioxide (a sulphone), again, does not. Since all three compounds contain a bridge-oxygen and only the

[1] Ber., 44, 3431 (1911).

second one the SO group, analogous to carbonyl, it would appear that the carbonyl group is important, even if it does not actually bear the iodine. Carbonyl groups alone, even with a crossed conjugated double bond, do not cause adsorption of iodine. Thus Barger and Eaton found that

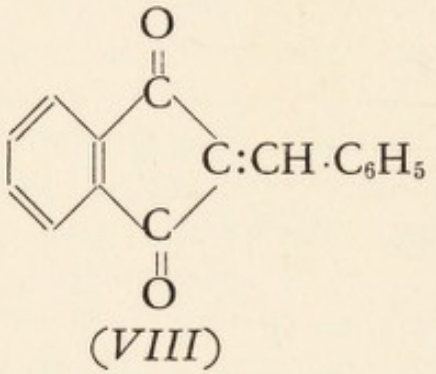


(*VI*)

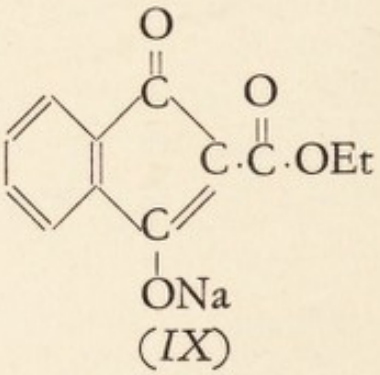


(*VII*)

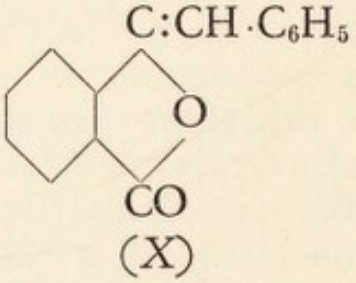
2-benzylidene-1,3-indanedione (*VIII*) does not add iodine, but the sodium salt of ethyl-1,3-diketohydrindenecarboxylate (*IX*) forms a blue precipitate, which disappears on warming and reappears on cooling. The bridge-oxygen atom is present in various phthalides examined by Barger and Eaton. Ethylidenephthalide gives no very charac-



(*VIII*)



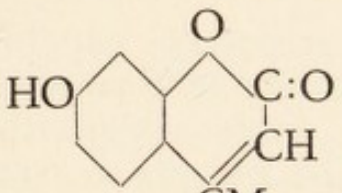
(*IX*)



(*X*)

teristic reaction with iodine, but the benzylidene compound (*X*) behaves very much like starch. A further increase in molecular weight in ethylenediphthalide,

fluorenephthalide, and diphenylphthalide, failed to give blue addition compounds. The exceptional position of the benzylidene compound depends on the fact that it alone, among the phthalides examined, forms a relatively stable colloidal solution even when very dilute alcoholic solutions ($N/250\,000$) are mixed with water. Here adsorption depends on a physical condition. A somewhat analogous case is that of the acyl compounds of 7-hydroxy-4-methylcoumarin (*XI*) examined by Barger and Eaton. The ben-



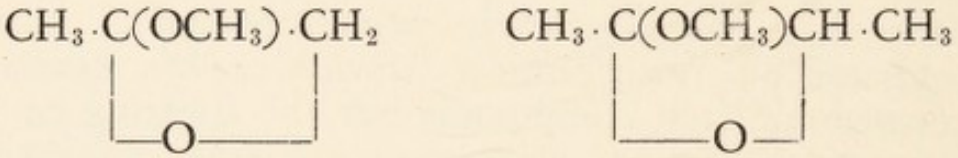
(*XI*)

zoyl derivative has been referred to already as forming a typical blue adsorption compound at great dilution. In addition the carbethoxy, phenylacetyl, cinnamoyl, β-naphthoyl, *o*-chlorobenzoyl, *m*-nitrobenzoyl, *p*-nitrobenzoyl, and phenylcarbimido derivatives were examined. All formed addition compounds but the limiting concentrations of the iodine were very different, respectively $N/40$, $N/100$, $N/500$, $N/1000$, $N/4000$, $N/5000$, $N/3000$, $N/10$, as compared with $N/10\,000$ for the benzoyl derivative. The phenylacetyl and the phenylcarbimido derivatives form bluish-black crystals, the others colloidal solutions of various stabilities. While the coumarin grouping, common to all nine derivatives, adsorbs iodine, the physical properties are variously influenced by the acyl group in the benzene nucleus—a further analogy to haptophore and toxophore groups.

I have synthesized a number of phenanthrene derivatives with pyrone and other groupings, in an attempt to obtain still more delicate reagents for iodine than α-naphthoflavone, but apparently this is not a suitable method of weighting the molecule with aromatic nuclei; better suggestions are obtainable from Gomberg's and Schlenk's work on radicals with trivalent carbon. A simple

type of iodine adsorbent resulted, however, in 3-methoxy-4-phenanthrene aldehyde. Still simpler examples exist in the iodides of benzamide, *p*-toluamide, *p*-bromobenzamide, phthalimide, benzenesulphonamide, various anilides and even succinimide, described by Moore and Thomas.[1] Benzamide forms green needles of the composition $3C_6H_5CONH_2 \cdot NaI \cdot I_2$ and there are analogous compounds containing barium, copper, cobalt, nickel, potassium, mercury and lead. Succinimide yields the compound $4C_4H_5O_2N \cdot KI \cdot I_3$.

Another simple structure, capable of adding iodine, was found by Bergmann[2] in certain cyclic acetals, which take us back to our point of departure, starch. In them, as in starch, the iodine appears to attach itself to a bridge-oxygen atom. They have a glucoside-like structure, and form bluish-black crystals of metallic appearance, analogous in composition to the iodide of cholalic acid. The methyl cycloacetals of acetol and of acetoin form rather strongly associated double molecules, for instance in

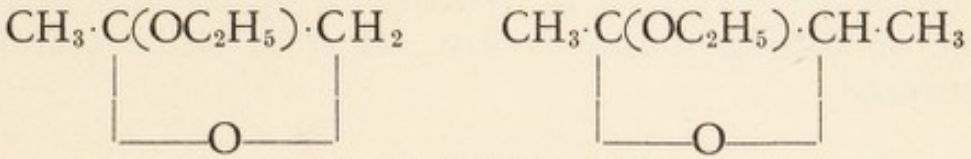


phenol solution (the vapor consists of single molecules). The association is doubtless due to the residual affinity of the bridge-oxygen atoms, and the same affinity appears to be concerned in holding the iodine of crystallization. The iodides are made by mixing alcoholic solutions with aqueous potassium triiodide, and have the composition $(C_4H_8O_2)_2I_4 \cdot KI$ and $(C_5H_{10}O_2)_2I_4 \cdot KI$, respectively.

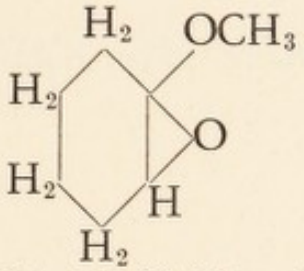
Additional compounds of this type were described by Bergmann and Gierth;[3] such as the ethyl acetals of acetol and acetoin and the methyl acetal of cyclohexanolone. Like the preceding, all three give crystalline iodides composed of two molecules of the acetal, four atoms of iodine and one molecule of potassium iodide. These acetals do not contain free hydroxyl groups, which are present in

[1] J. Am. Chem. Soc., **36**, 1928 (1914).
[2] Ber., **57**, 753 (1924).
[3] Ann., **448**, 48 (1926).

starch, but Bergmann and Ludewig[1] showed that acetylated starch, with 46–48 per cent of acetyl, still gives the blue reaction with iodine on shaking with potassium triiodide. The ratio I:KI varied here from 4.4:1 to 7.1:1. It would seem that the amorphous acetyl starch forms a compound of the type of the acetal iodides, which then adsorbs additional iodine.



Ethyl acetals of Acetol and Acetoin



Methyl acetal of Cyclohexanolone

In this connection the crystalline dextrins obtained by Schardinger,[2] by the action of *Bacillus macerans* on starch, are of interest. Schardinger already found that they form addition products with iodine, and these have been further described by Pringsheim and Eissler.[3] Pringsheim has termed these fission products of starch "amyloses". They all have the composition $C_6H_{10}O_5$ and fall into two series: the α-amyloses which on crystallization from a hot aqueous solution containing potassium triiodide form green needles, and the β-amyloses which form dark red prisms. The latter series is considered to be more closely related to glycogen. Pringsheim and Eissler give the following formulas:

α-series, iododiamylose $(C_6H_{10}O_5)_2 \cdot \frac{3}{4}I$, and iodotetraamylose $(C_6H_{10}O_5)_4 \cdot 4H_2O \cdot 1\frac{1}{2}I$;

β-series, iodotriamylose $(C_6H_{10}O_5)_3 \cdot 4\frac{1}{2}H_2O \cdot 1\frac{1}{2}I$, and triiodohexaamylose $(C_6H_{10}O_5)_6, 9H_2O, 3I$.

[1] Ber., **57**, 961 (1924).
[2] Z. Untersuch. Nahr. Genussm., **6**, 874 (1903).
[3] Ber., **46**, 2959 (1913); **47**, 2565 (1914).

The ionic iodine, if any, was not differentiated from the total. These amyloses also form orange or yellow crystalline addition compounds with bromine.

Since the composition of the amyloses is the same as that of starch (they appear as products of depolymerization) it may be inferred that they still contain bridge-oxygen atoms, of the type present in Bergmann's acetals. The latter are very readily hydrolyzed by acids, when the power of adding iodine is lost, as it is when starch is hydrolyzed.

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