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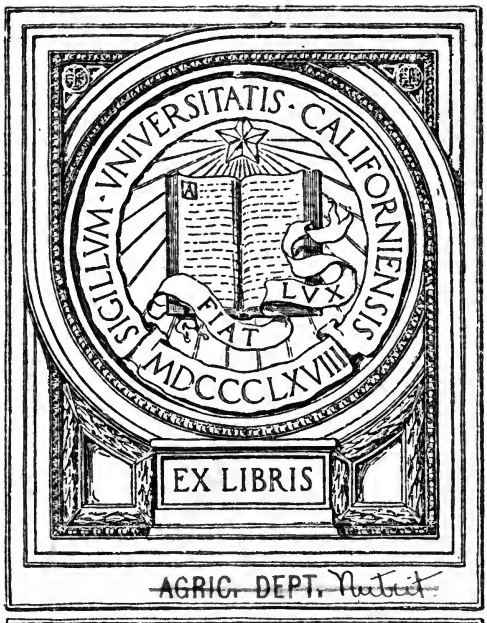
THE SIMPLER NATURAL BASES

BY
GEORGE BARGER, M.A., D.Sc.



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Nutrition

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MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

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THE SIMPLER NATURAL BASES

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TO
H. H. D.

AGRIC. DEPT.

PREFACE.

In the following pages I have endeavoured to give an account of those basic substances of animals and plants which are of general biological interest, either because of their wide distribution, or on account of their close relationship to the proteins and phosphatides. In contradistinction to the typical vegetable alkaloids, these bases have a simple chemical constitution.

By a more or less arbitrary delimitation of the subject matter, involving for instance the total exclusion of purine bases, I have aimed at giving, in the space at my disposal, a somewhat detailed account of the chemistry of the bases dealt with, and of their derivatives. Some, like the amines and adrenaline, are remarkable on account of their physiological action, and in each case, therefore, a brief description of this action has been added. In this way I have endeavoured to make the monograph also of interest to those who are concerned with the biological rather than with the chemical aspect of the subject.

A brief chapter on the practical methods used in the isolation of the simple bases has been added, and special attention has been given to the bibliography which extends to the autumn of 1913.

It is a pleasant duty to express my great indebtedness to Dr. H. H. Dale, without whose advice and criticism much of the pharmacological sections would have remained unwritten.

G. B.

Englefield Green,
Surrey,
November, 1913.

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CONTENTS.

CALIFORNIA

INTRODUCTION AND SCOPE.

THE substances described in this monograph do not constitute a homogeneous group, like the proteins or carbohydrates, and the choice of a title was therefore difficult. Many are derived in various ways from the amino-acids of protein, a few are constituents of phosphatides; some are of bio-chemical interest on account of their wide distribution in animals and in plants, others are important because of their physiological action.

It is common to nearly all the simpler natural bases, however, that they are insoluble in ether and chloroform and readily soluble in water, so that their isolation is generally more difficult than that of the complex vegetable alkaloids, which can be extracted by making the aqueous solutions of their salts alkaline and then shaking with a solvent immiscible with water. The separation of the simpler bases from each other and from non-basic substances like peptones must be carried out by means of suitable precipitants and crystalline derivatives. The special technique required for this purpose constitutes the chief bond between the bases with which we are here concerned. This technique was first elaborated in a systematic manner by Brieger, who employed mercuric chloride in the isolation of putrefaction bases. The introduction of phosphotungstic acid, by Drechsel, as a general precipitant for basic substances and its use for preparative purposes marked a great advance; later Kossel added the silver method for the separation of imino-bases, such as arginine and histidine. Since then the details of technique have been chiefly elaborated in three centres.

Schulze at Zürich, in a long series of researches on plant bases, discovered phenylalanine and arginine and more lately extended our knowledge of betaines. Kutscher and his pupils, in Germany, have isolated bases from a variety of sources, and Gulewitsch, at Moscow, has studied exhaustively the bases in meat-extract.

The history of the simpler natural bases has been greatly influenced by the need of special methods for their isolation. Another influence, adverse to their study, was the presence of alkaloids in drugs and stimulants, which directed attention to these complex bases having obvious physiological actions rather than to simpler bases of more

general biological importance. Thus the basic nature of morphine was recognised as long ago as 1806, and in 1820 quite half a dozen of the most important vegetable alkaloids were known, but our knowledge of animal bases is of a much later date. Pettenkofer prepared creatinine from urine in 1844 and Strecker first obtained choline from pig's bile in 1849, but for a long time hardly any other animal bases were known, and betaine, which is now known to occur in many plants and some animals, was not discovered until 1863. The more volatile amines, trimethylamine and amylamine, were obtained as putrefaction products in 1855 and 1857 respectively, and about the year 1866 it became generally recognised that bases are formed in putrefaction, but for a long time these bases were regarded as similar to the vegetable alkaloids, and their isolation was attempted by similar methods. For this there were two reasons. In the first place the poisonous properties of putrid material were considered analogous to those of plant alkaloids, and secondly the medico-legal examination of corpses in murder trials revealed the presence of bases (called ptomaines by Selmi) which gave reactions like those of coniine, nicotine, atropine, etc. In no single instance did these early investigations result in the preparation of a pure substance, so that they do not concern us further.

The chemistry of putrefaction bases may be said to begin in 1876 when Nencki correctly analysed a base $C_8H_{11}N$, obtained from putrid gelatin; he afterwards identified it as phenylethylamine. It seems highly probable that this amine, perhaps mixed with diamines, was the "animal coniine" of earlier investigators.

The next great advance was due to Brieger who, breaking away from the methods used for plant alkaloids, and relying chiefly on mercuric chloride, platinic chloride and similar reagents, discovered putrescine, cadaverine, and many putrefaction bases which had been overlooked by his predecessors. Gradually it became evident that ptomaines, or putrefaction bases, are the products of bacterial action on protein and phosphatides, and since then our knowledge of these bases has become more and more intimately associated with what we know of the amino-acids from which protein is built up. Two examples of this association may be given. The discovery of phenylalanine by Schulze and Barbieri in 1881 enabled Nencki to surmise the constitution of his base $C_8H_{11}N$ referred to above; it is derived from the amino-acid by loss of carbon dioxide. Later Ellinger proved that Brieger's diamines were similarly derived from the amino-acids ornithine and lysine.

Since then the amines corresponding to nearly all the known

amino-acids have been found to occur as putrefaction products. These amines are described in Chapter I and include substances with interesting physiological actions. Another group of bases, likewise derived from protein, is described in Chapter II. The members of this group still retain a carboxyl group of the amino-acid, so that they are but feebly basic, and without marked physiological action. They include the ω-amino-acids, formed by putrefaction, and urocanic and kynurenic acids, two substances occurring in dog's urine and derived from histidine and tryptophane respectively. A third group of simple bases related to the amino-acids of protein is dealt with in Chapter III, namely that of the betaines, derived from amino-acids by methylation. Several new examples of this class have been discovered during the last few years, both in animals and in plants.

The first three chapters deal therefore with bases which are derived by slight modifications from the constituent units of protein. These modifications are irreversible. As long as protein is not broken down beyond the amino-acid stage, its fragments are still available for synthesis. Thus when an amino-acid is set free in the germinating seed by the action of proteoclastic enzymes, it may re-enter a protein molecule in a cell of the growing point. If the degradation of protein proceeds farther, if the amino-acid is de-aminized or decarboxylated and also probably if it is methylated, it is no longer available for protein synthesis in animals and in the higher plants; it no longer constitutes a food, except for bacteria and some fungi. To these degradation products of protein which have passed out of the metabolic circulation, Ackermann and Kutscher [1910, 2] have applied the term *aporrhegmata*. They include under this denomination not only bases, but also acidic products, such as succinic acid, which is derived from aspartic acid by the loss of an amino-group during putrefaction.

In addition to the proteins, lecithin and other phosphatides constitute a source of bases in the organism. Here there is less variety, for only two primary fission products of basic character are known with certainty, choline and amino-ethyl alcohol. Neurine and trimethylamine are secondary decomposition products of choline and there are also a few closely related bases, like muscarine. All these bases are described in Chapter IV (with the exception of trimethylamine, which is included in Chapter I as it may also be formed from sources other than choline).

Of the bases dealt with in the first four chapters some are found in animals, some in plants, and many in both; the remaining chapters

are devoted entirely to animal bases, Chapter V dealing with creatine, creatinine, and other guanidine derivatives, and Chapter VI with adrenaline, one of the most interesting of simple bases.

Twenty years ago it could hardly have been imagined that the suprarenal gland constantly secretes into the blood minute quantities of a base having an intense physiological action, and that this base has a simple chemical constitution and can be synthesised. At first adrenaline stood entirely by itself; later some of the putrefactive amines of Chapter I were found to have considerable physiological activity, and one of them, p-hydroxy-phenyl-ethylamine, which resembles adrenaline chemically, was found to have an essentially similar, although weaker, action on the animal organism. There are moreover indications that other internal secretions owe their activity to bases of comparatively small molecular weight. This appears to be the case with the highly active principle of the pituitary body which is possibly a histidine derivative, and shows some analogies to β-iminazolyl-ethylamine described in Chapter I. Unfortunately hardly anything is known with regard to the chemistry of the pituitary active principle, so that it is only included in Chapter VII (bases of unknown constitution) on account of its physiological importance. Secretine, the substance which when introduced into the blood stream, causes secretion of pancreatic juice, is probably also a base—and like the active principles of the adrenal gland and of the pituitary body, it is moderately stable in boiling aqueous solution.

The case of the bacterial toxins and antitoxins, which are rapidly destroyed below the temperature of boiling water, is very different. After working on the products of putrefactive bacteria, Brieger investigated the bases produced in cultures of pathogenic organisms, such as the typhoid and the tetanus bacillus, but the simple bases which he obtained could not be regarded as the principal cause of disease, and his further work on tetanus toxin showed this substance to be extremely active and apparently also extremely complex. We may say "apparently" for the following reason. When a minute quantity of an active principle accompanies large quantities of proteins and other colloids it may remain adsorbed on these in such a way as to make a separation impossible, even when the active principle has a comparatively small molecular weight. The difficulties are particularly great when the active principle is very soluble in water but hardly at all in alcohol, as is often the case with bases of the animal body. A good deal of optimism is required for the belief that our present methods will ever suffice for the isolation of bacterial toxins in a state

of purity, and here we are likely to learn more from colloidal than from organic chemistry. Recent work on anaphylaxis seems to indicate that this phenomenon is primarily concerned with a basic part of the protein molecule which is resolved by hydrolysis into diamino-acids.

We are almost as ignorant of the more interesting toxic products of putrefaction as we are of pathogenic toxins. Very little is known about the poisonous substances in food, popularly called ptomaines. Many cases of so-called ptomaine poisoning are in reality bacterial infections, but others are purely chemical intoxications. Perhaps the best known of these is due to *Bacillus botulinus* which, without obvious signs of putrefaction, produces in meat or even in vegetable nitrogenous substances (beans) an excessively poisonous toxin, readily destroyed at 80° and capable of yielding an antitoxin (Van Ermengem [1907, 1912; Ch. I]; Ornstein [1913; Ch. I]). The poisonous properties occasionally exhibited by boiled mussels are on the other hand due to a thermostable base [Brieger, 1886, 1, p. 65; Ch. I]. The physiological actions of the most active amines described in Chapter I do not account satisfactorily for such intoxications; other substances must be present, and one of these is sepsine, a base of simple constitution obtained by Faust from putrid yeast. The experimental difficulties of the subject are illustrated by the fact that 100 kilos. of yeast did not yield enough of the pure substance for quite satisfactory analysis. Against this difficulty, that many of the bases described in the following chapters are only obtainable in minute quantity from natural sources, we may, however, set the advantage of a simple constitution, so that when the latter has once been fully established, a synthesis on a large scale may be possible, which in some cases has greatly increased our knowledge of the chemical and physiological properties of the base. Without an exact knowledge of the properties, the identification is often very difficult and for this reason detailed descriptions have as far as possible been given in the appendix. Many bases which have been insufficiently characterised have not been mentioned, except where it was possible to suggest identity with better known ones.

In conclusion we may discuss the meaning of the following terms.

Base.—Many substances of physiological importance are at the same time acids and bases; those in which the basic character predominates have been included in this monograph; others, like the *a*-amino-acids of protein are not generally regarded as bases, although glycine, for instance, yields a hydrochloride. The predominance of the basic character may be deduced from a comparison of the (basic and acidic) affinity constants (see the beginning of Chapter II). For our purposes a better

practical definition is to describe a base as a substance which is precipitated by phosphotungstic acid. Adopting this criterion we consider creatinine to be a base but creatine not.

Alkaloid.—Some writers have used this term to include all natural bases, but the objections to this are evident from what has been said above, and the word is best restricted to complex vegetable heterocyclic bases derived from pyridine, quinoline, etc.[1] There is no doubt as to what is generally meant by an alkaloid, but nevertheless a rigid definition is almost impossible. On the one hand narceine, for instance, is a typical alkaloid from opium, but the nitrogen atom does not form part of a ring; narceine is an amine. On the other hand histidine and its derivatives are not classed as alkaloids, although they contain the heterocyclic glyoxaline ring, which is also present in pilocarpine. The latter substance is an undoubted alkaloid. In a few cases the inclusion of bases in this monograph is arbitrary; thus hordenine, which is usually called an alkaloid, has been included on account of its relationship to tyrosine; ephedrine, which is isomeric with hordenine, has been excluded. All betaines have been included, for no typical alkaloid shows a betaine structure. One further point should be noted. The typical alkaloids are generally found only in one or a few closely related species, but the simpler natural bases, in accordance with their close connection with proteins and phosphatides, have generally a much wider distribution.

Ptomaine was originally applied by Selmi to bases from corpses and afterwards became identical with putrefaction base (Brieger). Some writers have restricted the term to poisonous bases. Lately it has fallen into disuse.

Leucomaine was a term used by Gautier for animal bases such as creatinine, which are not formed by putrefaction; this term is now quite obsolete.

Toxins are poisonous bacterial products which when injected cause the production of anti-bodies, neutralising their poisonous properties; an example is diphtheria toxin. Gautier has applied the word, in a different sense, to simple poisonous putrefaction bases.

[1] Winterstein and Trier define plant alkaloids as nitrogenous substances which can no longer be utilised for building up protein. Thus they would call betaine an alkaloid.

CHAPTER I.

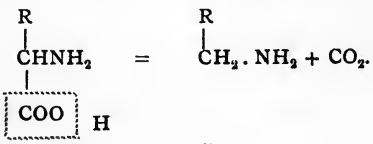
AMINES DERIVED FROM PROTEIN.

The Putrefactive Decomposition of Amino-acids.

BOTH animals and plants decompose proteins into their constituent amino-acids; the hydrolysis by trypsin and by erepsin in animals is similar to the formation of amino-acids in germinating seeds, which has been studied especially by Schulze and his pupils. The hydrolysis of proteins into their constituent amino-acids is also the first stage of putrefaction, but bacteria (and other fungi) are peculiar in being able to break down the amino-acids themselves into bases and acids which in general have not been demonstrated as products of the metabolism of animals and the higher plants.

This degradation may take place in two ways: either an amino-group may be eliminated (deaminization) or a carboxyl-group may be removed (decarboxylation); various modifications and combinations of these two processes are possible. Little is known about the conditions determining which process takes place; generally the two go on simultaneously and deaminization preponderates. Ackermann who has carried out a number of experiments on the bacterial decarboxylation of pure amino-acids, finds that this process is favoured by the addition of peptone which serves as a source of nitrogen and in this way lessens deaminization. An organism which decarboxylates histidine has been isolated by Mellanby and Twort [1912]. Berthelot and Bertrand [1912, 1, 2; 1913, 1, 2; Bertrand and Berthelot, 1913] have described a similar organism from the human intestine, *Bacillus aminophilus intestinalis*, which decarboxylates histidine, tyrosine, tryptophane, etc.

The various amines dealt with in the present chapter are all derivable from monobasic amino-acids by decarboxylation, and it is therefore with this process that we are more particularly concerned. Decarboxylation may take place by the simple removal of carbon dioxide:



or the carboxyl-group may be eliminated as formic acid, in which case reduction must take place:—

$$\begin{array}{l} R \\ | \\ CHNH_2 + H \\ | \\ COOH \quad H \end{array} \quad = \quad \begin{array}{l} R \\ | \\ CH_2 . NH_2 + H . COOH. \end{array}$$

Neubauer [1911] considers that decarboxylation generally takes place in both these ways, since carbon dioxide and formic acid are among the regular products of putrefaction. In either case a primary amine results.

The same process, applied to dibasic monamino-acids, results in the formation of ω-amino-acids, which are feebly basic putrefaction products and are described in the next chapter. ω-Amino-acids are also formed by the deaminization of diamino-acids; the deaminization of monamino-acid yields non-nitrogenous acids such as isocaproic (from leucine) and succinic (from aspartic acid). Deaminization is accompanied by reduction, since hydroxy-acids and unsaturated acids apparently do not occur in putrefaction:—

$$\begin{array}{l} R \\ | \\ CHNH_2 + 2H \\ | \\ COOH \end{array} \quad = \quad \begin{array}{l} R \\ | \\ CH_2 \\ | \\ COOH \end{array} + NH_3.$$

By a combination of the two processes of decarboxylation and deaminization, methane may be formed from glycine and *n*-butyric acid from glutamic acid (Neuberg and Rosenberg [1907]). A putrefactive process involving only reduction is the conversion of proline into δ-aminovaleric acid.

The importance of reduction in the above bacterial actions is expressed by the fact that they chiefly take place under anærobic conditions. Bienstock [1899, 1901], one of the chief workers in this field on the bacteriological side, concludes that putrefaction, in the ordinary sense, cannot take place without an obligate anærobe, such as *Bacillus putrificus*. *B. coli* hinders the action of *B. putrificus* and *B. tetani* has no action on fibrin. Rettger [1906, 1907; Rettger and Newell, 1912] shares the view that putrefaction is the work of strict anærobes.

The access of oxygen induces further changes; p-hydroxy-phenyl-propionic acid (formed by the deaminization of tyrosine) is oxidised, according to Baumann and Nencki, to p-hydroxy-phenyl-acetic acid, which is successively converted into p-cresol and phenol, and similarly indole-propionic acid (from tryptophane) yields indole-acetic acid, skatole, and indole. Oxidation also accounts for the shortening of

the carbon chain in the production of succinic acid from glutamic acid by putrefaction.

Some putrefaction bases are formed from substances other than proteins; thus lecithin is broken down to choline, neurine, trimethylamine, monomethylamine, and ammonia; creatine yields monomethylguanidine and perhaps also dimethylguanidine; the trimethylamine of stale urine is derived from more complex betaines; purine and pyrimidine bases probably also contribute to the formation of putrefaction bases.

When an entire tissue or organ, and to a less extent when a single protein is putrefied, as in the experiments of Nencki, Gautier, Brieger, Salkowski, Emmerling, Barger and Walpole, and the earlier experiments of Ackermann, a complex mixture of bases is obtained from various parent substances. A better insight into the chemistry of putrefaction is possible when a simple substance, such as a single amino-acid, is subjected to bacterial action. This method depends on a knowledge of the constituents of protein, and was first applied to the study of bases by Ellinger, who showed that putrescine and cadaverine are derived from ornithine and lysine respectively. Further work in this direction has been carried out principally by Ackermann and by Neuberg. (The products of the action of bacteria on indole-propionic acid (Nencki) and of yeast on proteins (F. Ehrlich) are not bases, and they are therefore not included in this monograph.) It is generally much more difficult to grow bacteria in a solution of a pure amino-acid than on protein, and Ackermann therefore adds 0·25 per cent. Witte peptone to the solution, together with 0·5 per cent. glucose and a few drops of sodium phosphate and magnesium sulphate; calcium carbonate is sometimes added to prevent the solution becoming acid, but a faint alkaline reaction is secured more certainly by adding sodium carbonate from time to time. Although Neuberg [1911, 1] has pointed out the theoretical objections to the addition of peptone he yet agrees with Ackermann that in many cases this addition is desirable. For the decomposition of histidine Mellanby and Twort [1912] used a culture medium containing only ammonium tartrate and inorganic salts (see p. 133). A similar medium was used by Berthelot and Bertrand [1912, 1].

Of late years nearly all the putrefaction products, which might be expected to result from the known amino-acids, have been obtained by bacterial action. Exceptions are ε-amino-caproic acid which might be formed from lysine, guanidino-valeric acid (from arginine), pyrrolidine (from proline), oxypyrrolidine (from oxyproline) and the amines from cystine and serine.

The decarboxylation of amino-acids is not necessarily accompanied by any obvious sign of bacterial action such as putrefactive odour; some of these amines occur in cheese and they have repeatedly been obtained in fermentation experiments supposed to be sterile (Langstein, Emerson, Lawrow; see the section on putrescine and cadaverine). The difficulties of ensuring sterility, particularly in autolysis, have often been underestimated and have been emphasised by Schumm [1905-6], Rothmann [1908], Kikkoji [1909], Salkowski [1909], Ohta [1910], Harden and Maclean [1911], Beker [1913]. Chloroform should not be used in conjunction with toluene, which dissolves the chloroform from the aqueous layer. It is best, according to Schumm and Kikkoji, to use water saturated with chloroform, or chloroform in excess and to ensure continued saturation by means of stoppered bottles and frequent shaking. Sterility tests should be made by smear.

In the absence of bacteria, decarboxylation of amino-acids does not occur; at least the corresponding primary amines are not found. (Kutscher and Lohmann [1905], Schumm [1905-6], Bissegger and Stegmann [1908], Schulze [1906], Kiesel [1911].) The occurrence of methylated bases such as tetramethyl putrescine and hordenine in the higher plants perhaps implies the intermediate formation of primary amines. Apart from putrefaction, putrescine and cadaverine occur in cystinuric urine, agmatine in herring spawn and p-hydroxy-phenyl-ethylamine in the salivary gland of Cephalopoda. It has further been established that fresh fungi may contain amines resulting from the decarboxylation of amino-acids or at any rate these amines are formed by autolysis independently of bacterial action. The close relationship between the fungi proper and bacteria makes this less surprising.

Ergot, which has been examined more thoroughly than any other fungus, contains p-hydroxy-phenyl-ethylamine, β-iminazolyl-ethyl-amine, putrescine, cadaverine, agmatine, and probably isoamylamine, and owes much of its physiological action to the first two of these bases. It is almost certain that they are to some extent present in fresh ergot, but the amount is increased after death, probably by autolysis. Reuter [1912] recently found putrescine in fresh specimens of *Boletus edulis* and when this fungus was autolysed under sterile conditions, isoamyl-amine, phenyl-ethylamine, probably p-hydroxy-phenyl-ethylamine and possibly iminazolyl-ethylamine were formed in addition. Schenck [1905, 1] had previously obtained putrescine from autolysed yeast. Reuter's experiments are of particular interest; sterility tests showed that bacteria were absent, and he concludes that fungi possess ferments capable of decarboxylating amino-acids.

Methylamine, Ethylamine, Dimethylamine.

Methylamine occurs according to Trier [1912, 3; p. 8] in species of *Mercurialis* and the root of *Acorus Calamus* and has been frequently met with as a product of bacterial action (see P. Rona, *Biochemisches Handlexicon*, Band IV, p. 801). It is perhaps formed from glycine, by decarboxylation, but so far it has not been possible to demonstrate this experimentally. The source of methylamine is in most cases more probably trimethylamine (from choline). Thus Hasebroek [1887] obtained this amine along with ammonia by the anærobic putrefaction of choline, and Mörner [1896] found amines present in a peculiar Swedish food ("surfisk"). This fish is pickled with a little salt and allowed to ferment anærobically; it probably contains monomethylamine, and certainly dimethylamine and choline, but not putrescine or cadaverine. Ackermann and Schütze [1910, 1911] also found that a little methylamine, together with trimethylamine, is formed by the action of *Bacterium prodigiosum* on choline. Emmerling [1897] obtained mono- and trimethylamine by the action of *Streptococci* on fibrin, but here also the amines appear to be derived from admixed lecithin.

Ethylamine was said more than fifty years ago to be produced in the putrefaction of yeast and of wheat flour, but these observations require confirmation. It might result by the decarboxylation of alanine, from which it is indeed formed on destructive distillation.

Dimethylamine was stated by Bocklisch [1885] and by Mörner [1896] to occur in putrid fish, and by Ehrenberg [1887] in cultures from a bacillus isolated from poisonous sausages. In the latter case at least a confusion with putrescine was not unlikely, since the platinichlorides of the two bases have nearly the same composition. If dimethylamine is formed at all it would be most probably derived from choline and trimethylamine, although it could also result from the decarboxylation of sarcosine (from creatine).

Trimethylamine, $N(CH_3)_3$.

Trimethylamine occurs in the leaves of *Chenopodium Vulvaria* (the Stinking Goosefoot) where it is readily detected by the odour on bruising the leaves; it is also present in hawthorn flowers (*Cratægus Oxyacantha*) and in ergot. Unlike the other amines dealt with in this chapter, trimethylamine is not formed from an amino-acid, but is a decomposition product of choline and allied quaternary bases; it is therefore of common occurrence in putrefaction. Thus it is present in herring brine, the first natural source to be discovered by Winckler in

1855. On an industrial scale it is formed by the destructive distillation of beet sugar molasses; here the parent substance is betaine.

Examples of the production of trimethylamine by pure cultures are the action of *Proteus vulgaris* on wheat gluten and on meat, of *Bacillus liquefaciens* on commercial gelatin and of *Bacterium prodigiosum* on choline and on lecithin. Ackermann and Schutze [1910, 1911] found that the last-named organism does not produce trimethylamine from betaine, and that *B. vulgatus* does not decompose choline.

The alleged occurrence of trimethylamine in urine has been the subject of several investigations. Long ago Dessaignes [1856] obtained it by distillation of urine with caustic soda (3·7 grm. of the free base from 65 litres of human urine). He, however, left open the question whether trimethylamine is present as such or is formed by the decomposition of some other compound by the alkali. This question was likewise left unanswered by de Filippi [1906] who worked out a process for the estimation of urinary trimethylamine (see appendix).

Takeda [1909] used magnesium oxide instead of caustic soda, and distilled under reduced pressure; he found no trimethylamine in the urine of horses and of dogs and only doubtful traces in human urine; it is however formed in putrefaction. Kinoshita [1910, 1], using Herzig and Meyer's method for the estimation of N-methyl groups, found only traces, and Erdmann [1910] has also arrived at the conclusion that "fresh normal urine does not contain trimethylamine". According to Kutscher the trimethylamine in urine is formed from such bases as novaine and reducto-novaine.

Isobutylamine, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH\,.\,CH_2\,.\,NH_2$.

This base was obtained by the putrefaction of racemic *a*-amino-isovaleric acid (*d.l.* valine) by Neuberg and Karczag [1909]. A solution of 10 grams of the amino-acid in 450 c.c. of water, with a little KCl, Na_2HPO_4 and $MgSO_4$ was rendered alkaline with sodium carbonate and yielded after inoculation and four weeks' incubation at 37° 0·424 grm. of a *platinichloride* $(C_4H_{11}N)_2H_2PtCl_6$, mp. 226-227°, in all probability that of isobutylamine.

A butylamine has also been obtained by Gautier from cod liver oil prepared by the old putrefactive process.

In *Fagara xanthoxyloides* isobutylamine occurs in combination with piperonylacrylic acid as an amide, fagaramide (Thoms and Thumen, [1911]).

Isobutylamine is the lowest amine causing any appreciable rise of blood pressure when injected intravenously.

Isoamylamine, $\begin{matrix}CH_3\\ \\ CH_3\end{matrix}\!\!\rangle CH \cdot CH_2 \cdot CH_2 \cdot NH_2.$

An amylamine has been obtained from putrid yeast (Muller [1857]), from cod liver oil (Gautier and Mourgues [1888]), from putrid horse meat (Barger and Walpole [1909, **1**]), putrid placenta (Rosenheim [1909]), from *Boletus edulis* on sterile autolysis (Reuter [1912]), and probably from fresh ergot (Barger and Dale [1909]).

In all these cases isoamylamine (derived from leucine) was probably mixed with the isomeride 2-methylamino-butane (derived from isoleucine), and possibly with normal amylamine from norleucine. Isoamylamine is further formed from leucine on rapid heating, and in the dry distillation of bones and horn. Ciamician and Ravenna (quoted by Trier [1912, **3**]) found isoamylamine in tobacco. The oxalate of isoamylamine was obtained in an impure form from putrid meat by Abelous, Ribaut, Soulié and Toujan [1906, **1, 2**]; Abelous and Ribaut [1908] deduced the erroneous formula $C_6H_{11}ON$ for the base, and were the first to observe its power of raising the blood pressure when injected intravenously. Extracts of putrid meat were shown by Barger and Walpole to owe their pressor action principally to isoamylamine and to p-hydroxy-phenyl-ethylamine.

Pyrrolidine, C_4H_9N.

This base should result from the amino-acid proline by decarboxylation, but has never been isolated as a putrefaction product, probably because putrefactive bacteria rupture the pyrrolidine ring by reduction (see Chapter V).

Pyrrolidine has, however, been isolated in minute quantity from carrot leaves (*Daucus Carota*) by Pictet and Court [1907]. They also found pyrrolidine and N-methylpyrroline in minute quantities in tobacco, and have termed these bases proto-alkaloids.

Amino-ethyl Disulphide, $S_2(CH_2 \cdot CH_2 \cdot NH_2)_2$.

Neuberg and Ascher [1907] obtained this amine in small quantity by the dry distillation of cystine, from which it is derived by loss of carbon dioxide. The *picrate* melts at 197°. The amine has no pronounced physiological activity, and has so far not been obtained by bacterial action.

Putrescine and Cadaverine, $C_4H_{12}N_2$ and $C_5H_{14}N_2$.

These two homologous diamines have similar properties and generally accompany each other, so that they may be most conveniently considered together. They were discovered by Brieger [1885, **1**, 2] by his new method of investigating putrefaction bases; cadaverine was soon afterwards shown by Ladenburg [1886] to be identical with the pentamethylene-diamine previously obtained by reduction of trimethylene dicyanide, and later Udranszky and Baumann [1888, **2**] proved the identity of putrescine with tetramethylene-diamine.

Putrescine and cadaverine are among the commonest of all putrefaction bases. They probably escaped the notice of earlier investigators on account of their sparing solubility in ether and in chloroform, but Brieger obtained them repeatedly from various sources and they have been isolated many times since. The possibility of the formation of cadaverine from lysine by loss of CO_2 was already considered by Udranszky and Baumann and the origin of both amines was definitely established by Ellinger [1900] who obtained putrescine by the action of putrefactive bacteria on ornithine:

$$NH_2 . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH = NH_2 . CH_2 . CH_2 . CH_2 . CH_2 . NH_2 + CO_2;$$

and similarly cadaverine from lysine:

$$NH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH = NH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . NH_2 + CO_2.$$

These important results furnished the first examples of the bacterial decarboxylation of amino-acids. With access of air Ellinger obtained a 12 per cent. yield of putrescine and under anærobic conditions a 50-60 per cent. yield (three days at 37°); with cadaverine the yield was 36 per cent. Ackermann [1909, **1**], who more recently repeated Ellinger's experiments, was at first unable to obtain putrescine and cadaverine from the pure amino-acids but succeeded in the case of the products of the hydrolysis of caseinogen by acids. He showed that putrescine but not cadaverine is formed in the putrefaction of gliadin [1909, **2**], which does not contain lysine, and ultimately he [1910, **3**] found that the addition of 0·25 per cent. Witte peptone and 0·5 per cent. glucose to the culture medium greatly facilitated decarboxylation. In the earlier experiments only traces of inorganic salts had been added. When once formed, cadaverine and putrescine are apparently very resistant to the action of micro-organisms, for Brieger and others isolated the bases in considerable quantity after putrefaction had been going on for months.

Apart from such bacterial formation of putrescine and cadaverine,

both bases have been isolated from ergot by Rieländer [1908] and putrescine has been found in autolysed yeast by Schenck [1905, 1], in fresh specimens of *Boletus edulis* by Reuter [1912] and in *Datura* (a Phanerogam) by Ciamician and Ravenna (Trier [1912, 3]).

The diamines further occur in some cases of cystinuria (Udranszky and Baumann [1889], Cammidge and Garrod [1900], Loewy and Neuberg [1904], Garrod and Hurtley [1906]; the last-named paper should be consulted for the literature of other cases). In some cases of cystinuria the diamines are only excreted occasionally, or not at all, in Loewy and Neuberg's case only when arginine and lysine were given by the mouth. On the other hand the diamines do not pass into the urine when given by the mouth to a normal animal (Udranszky and Baumann [1890]). Garrod's impression [1909] is "that the likelihood that diamines will be detected in any given specimen of cystin urine is comparatively small, but that if in any case the examination be continued over sufficiently long periods they are likely to be found eventually". Lately Ackermann and Kutscher [1911] have found a minute quantity of lysine in cystinuric urine. The excretion of diamines in the urine indicates a peculiarity of metabolism, probably not intimately connected with the excretion of cystine.

Cadaverine was also found by Roos [1892] in the urine in two cases of malaria, but this may have been the result of bacterial action. Other cases of the alleged fermentative formation of the two diamines may safely be ascribed to this cause. Thus Lawrow [1901] obtained both bases in the autolysis of pig's stomach, Langstein [1901, 1902] isolated cadaverine after digesting egg white with pepsin for more than a year, Steyrer (referred to by Emerson [1901]) obtained the same base from a pancreatic digest and Werigo [1892] from pancreas macerated with chloroform water. In some of Werigo's experiments incipient putrefaction was indeed noticed, and we may well attach more weight to the experiments of Kutscher and Lohmann [1905] and of Schumm [1905-6], who could not isolate either putrescine or cadaverine when pancreas was autolysed under sterile conditions, and to those of Bissegger and Stegmann [1908] who likewise could not obtain the diamines by the tryptic or peptic digestion of caseinogen. Schulze showed [1906] that putrescine and cadaverine, unlike their parent substances, are absent from germinating seedlings.

Among the cases where putrescine and cadaverine are formed by bacterial action we may further mention that both bases have been obtained from putrid Soy beans (Yoshimura [1910]) and from

Emmenthaler cheese (Winterstein and Thöny [1902]). Van Slyke and Hart [1903] found a little putrescine in ordinary Cheddar cheese, but none in a sterile chloroform cheese.

According to Garcia [1892-3, **2, 3**] the yield of the diamines from putrid horse meat and from pancreas is diminished by the addition of carbohydrates (compare p. 25); four-fifths is already formed in the first twenty-four hours of incubation and the maximum is reached after three days. Once formed, putrescine and cadaverine appear to be very resistant to bacterial action. Gulewitsch [1894] obtained cadaverine from horse meat kept four months at 15°.

Hyoscyamus muticus contains tetramethyl-putrescine (see appendix).

Agmatine, $C_5H_{14}N_4$.

Agmatine, or guanidino-butylamine, was obtained by Kossel [1910, **1**] from herring spawn after heating with dilute sulphuric acid (5 per cent. by volume) in an autoclave at 4 atmospheres pressure. The base differs from arginine by CO_2, the chief amino-acid in herring spawn, so that it may be considered as being derived from arginine by decarboxylation :—

$NH_2 . C(:NH) . NH . CH_2 . CH_2 . CH_2 . CH_2 . NH_2$ agmatine.
$NH_2 . C(:NH) . NH . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH$ arginine.

Agmatine has also been isolated from ergot by Engeland and Kutscher [1910, **1, 2**] who obtained from their base on oxidation guanidine and guanidino-butyric acid,

$$NH_2 . C(:NH) . NH . CH_2 . CH_2 . CH_2 . COOH.$$

Kossel [1910, **2**] synthesised agmatine from cyanamide and tetramethylene diamine,

$$NH_2 . CN + NH_2(CH_2)_4NH_2 = NH_2 . C(:NH) . NH . (CH_2)_4 . NH_2 .$$

Phenyl-ethylamine, $C_6H_5 . CH_2 . CH_2 . NH_2$.

β-Phenyl-ethylamine is of some interest, since it was the first putrefaction base of which the composition was determined. Nencki [1876] obtained the base from a mixture of 200 grams of ox pancreas and 600 grams of gelatin dissolved in 10 litres of water, which was putrefied at 40° for five days.

Nencki, like Selmi and other early investigators of putrefaction bases, was most impressed by their analogy to vegetable alkaloids such as coniine and nicotine, and he at first considered his base to be a pyridine homologue, dimethylpyridine or collidine. Finding later that his hydrochloride, unlike that of collidine, yielded on destructive distilla-

tion a substance resembling xylene in odour and other properties, he concluded [1882] that the base obtained from gelatin was an aromatic amine, probably α-phenyl-ethylamine, $C_6H_5.CH(NH_2).CH_3$. Still later he regarded phenylalanine, which Schulze and Barbieri had discovered in etiolated lupin seedlings, as the parent substance of his putrefaction base, which he [1889] therefore considered to be β-phenyl-ethylamine, formed according to the equation :—

$$C_6H_5.CH_2.CH(NH_2).COOH = C_6H_5.CH_2.CH_2.NH_2 + CO_2.$$

Nencki was thus also the first to invoke the decarboxylation of an amino-acid in explanation of the origin of a putrefaction base.

Nencki's "collidine" was further obtained from putrefied egg white by his pupil Jeanneret [1877]. The identity of the base from putrid gelatin with β-phenyl-ethylamine was first rendered absolutely certain by Spiro [1901]. Putrefaction bases of the formula $C_8H_{11}N$ or of a similar formula, with properties somewhat resembling those of phenyl-ethylamine, have at various times been obtained by other investigators and one is tempted to regard all these bases as identical with that first isolated by Nencki. In some cases this is indeed almost or quite certain. Thus by the action of a *Streptococcus* on fibrin, Emmerling [1897] obtained a base of the formula $C_8H_{11}N$ of which the picrate melted at the same temperature as that of synthetic β-phenyl-ethylamine; the only discrepancy is that the platinichloride is described as readily soluble in water. Similarly a base obtained from putrid horse meat by Barger and Walpole [1909, I], and having the boiling point and physiological properties of β-phenyl-ethylamine, was doubtless identical with this amine.

It is much more difficult to draw the same conclusion with regard to certain bases described as pyridine derivatives and isolated by Gautier and Etard [1882, 1883] and by Oechsner de Coninck [1886-91]. The former investigators obtained from putrid mackerel a base, boiling at 210°, $d_0 = 1{\cdot}0296$, which was analysed as platinichloride. The formula deduced was $C_8H_{13}N$ and the base was named dihydrocollidine, but the analyses are in better, although not good, agreement with the formula $C_8H_{11}N$. No evidence of its being a pyridine derivative was adduced and Nencki [1882] at first regarded Gautier and Etard's hydrocollidine as identical with phenyl-ethylamine, but subsequently [1889], after a visit to Gautier, he gave up this view. Oechsner de Coninck obtained a base of the formula $C_8H_{11}N$ from putrid cuttle-fish; on oxidation it yielded nicotinic acid; it was examined much more closely than Gautier and Etard's "hydrocollidine" and in this

case at least, a confusion with phenyl-ethylamine seems completely excluded. Compare further the section on p. 48.

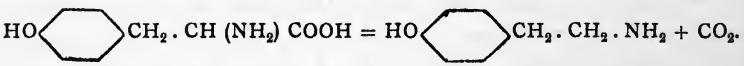
Phenyl-ethylamine does not accompany phenyl-alanine in seedlings (Schulze [1906]), but with regard to the higher plants it should be mentioned that Le Prince [1907] has isolated a volatile base $C_8H_{11}N$ from the European mistletoe (*Viscum album*) and that Crawford [1911] attributes the pressor action of the U.S.P. extract of the American mistletoe (*Phoradendron flavescens*) to the presence of a base, $C_7H_{11}N$ or $C_8H_{11}N$, which he thinks is perhaps identical with phenyl-ethylamine. This base requires further investigation; the presence of phenyl-ethylamine may possibly depend on the fact that the mistletoe is a semi-parasite. Although phenyl-ethylamine has not been found in any fresh fungus, Reuter [1912] obtained it from *Boletus edulis* by aseptic autolysis. Derivatives of phenyl-ethylamine have been found in various essential oils; thus phenyl-ethyl-alcohol

$$C_6H_5 . CH_2 . CH_2OH$$

occurs in rose oil and is also produced from phenyl-ethylamine by yeast (Ehrlich); phenyl-acetonitrile, $C_6H_5 . CH_2 . CN$, was found by Hofmann [1874] in the essential oil of *Nasturtium officinale*, and phenyl-ethyl-*iso*-thiocyanate is present in the oil from the root of *Reseda* according to Bertram and Walbaum [1894], and yields phenyl-ethylamine on hydrolysis. Possibly phenyl-ethylamine is an intermediate stage in the formation of all three substances from phenyl-alanine.

p-Hydroxy-phenyl-ethylamine, $OH . C_6H_4 . CH_2 . CH_2 . NH_2$.

This amine was first obtained by Schmitt and Nasse [1865] by heating tyrosine, when the following change occurs:—



p-Hydroxy-phenyl-ethylamine was subsequently isolated from autolysed pancreas by Emerson [1901] and from a prolonged peptic digestion of egg-albumin by Langstein [1901, 1902]. It seems pretty certain that in these experiments bacterial action was not completely excluded (see p. 10). Gautier and Mourgues [1888] isolated the base from the mother liquors obtained in the putrefaction of cod-livers (in the old process of making cod-liver oil). Gautier also obtained in small quantity a lower homologue C_7H_7NO and a higher one $C_9H_{11}NO$ and named the three bases "tyrosamines". The last two do not, however, appear to have been sufficiently well characterised.

p-Hydroxy-phenyl-ethylamine is fairly abundant in various kinds of cheese. It was found by Van Slyke and Hart [1903] in Cheddar

cheese prepared in the usual manner, but not in a cheese prepared with chloroform milk, so as to ensure sterility. The normal cheese was found to give off considerable quantities of carbon dioxide during ripening and Van Slyke and Hart consider that the carbon dioxide arose from the decarboxylation of amino-acids. The chloroformed cheese produced only traces of carbon dioxide and when finally analysed yielded a considerable quantity of arginine, while the normal cheese contained only traces of arginine, but instead of it guanidine and putrescine were present. The cavities in Emmenthaler ("Gruyère") cheese are mostly filled with carbon dioxide, and p-hydroxy-phenyl-ethylamine was isolated from this kind of cheese by Winterstein and Küng [1909].

It is further almost certain that one of Brieger's ptomaines, *mydine* [1886, 1, p. 26], was identical with p-hydroxy-phenyl-ethylamine. The base had the composition $C_8H_{11}NO$, yielded a soluble platinichloride, and a picrate crystallising in broad prisms melting at 190°. It was obtained from putrid human viscera, and was non-poisonous; ferric and gold salts were reduced by it. (The picrate of the synthetic amine crystallises in "short prisms" melting at 200°; the other properties are identical with those described for mydine by Brieger.)

The physiological action of p-hydroxy-phenyl-ethylamine was first brought to light by its identification, by Barger and Walpole [1909, 1], as the chief pressor constituent in extracts of putrid meat. The blood pressure raising property of such extracts had already been observed by Abelous, Ribaut, Soulié, and Toujan [1906, 1, 2]. Dixon and Taylor [1907] had also noticed that extracts of human placenta raised the blood pressure on intravenous injection and caused, in addition, contraction of the pregnant uterus. Rosenheim [1909] showed that this effect was not produced by extracts of perfectly fresh placenta, and after Barger and Walpole's identification of the pressor constituent of putrid meat, he was further able to show that the active constituent in Dixon and Taylor's placental extracts was also p-hydroxy-phenyl-ethylamine. Finally this amine is the chief pressor constituent of certain extracts of ergot, as shown by Barger and Dale [1909]. A certain quantity is apparently present in perfectly fresh ergot, where it has also been found by Engeland and Kutscher [1910, 2] and by Burmann [1912]. p-Hydroxy-phenyl-ethylamine is probably also present in autolysed *Boletus edulis* (Reuter [1912]). That tyrosine is indeed the parent substance of p-hydroxy-phenyl-ethylamine was shown by Barger and Walpole [1909, 1]; the yield in putrefaction was minute (less than 1 per cent. of the tyrosine present). Ackermann

[1909, 1] also isolated the base after putrefying the mixture of amino-acids obtained by boiling caseinogen with sulphuric acid.

Henze [1913] has made the most interesting observation that p-hydroxyphenyl-ethylamine occurs in the salivary gland of Cephalopoda and has a paralysing action on crabs, which are the chief food of these Molluscs.

Syntheses.

Larger quantities of p-hydroxy-phenyl-ethylamine are obtained by synthesis, most conveniently by the reduction of p-hydroxy-phenyl-acetonitrile with sodium and alcohol (Barger [1909, 1]), according to the equation :—

$$OH.C_6H_4.CH_2.CN + 4H = OH.C_6H_4.CH_2.CH_2.NH_2.$$

Two other syntheses of this amine were described by Barger and Walpole [1909, 2]; according to one of these benzoyl-phenyl-ethylamine is nitrated and the p-nitro-derivative is reduced, diazotised, and hydrolysed :—

$$C_6H_5.CH_2.CH_2.NH.CO.C_6H_5 \rightarrow NO_2.C_6H_4.CH_2.CH_2.NH.CO.C_6H_5$$
$$\rightarrow NH_2.C_6H_4.CH_2.CH_2.NH.CO.C_6H_5 \rightarrow OH.C_6H_4.CH_2.CH_2.NH.CO.C_6H_5$$
$$\rightarrow OH.C_6H_4.CH_2.CH_2.NH_2.$$

The other synthesis starts from anisaldehyde which is successively converted into p-methoxy-phenyl-acrylic acid, p-methoxy-phenyl-propionic acid, and its amide, p-methoxy-phenyl-ethylamine and p-hydroxy-phenyl-ethylamine :—

$$CH_3O.C_6H_4.CHO \rightarrow CH_3O.C_6H_4.CH:CH.COOH \rightarrow CH_3O.C_6H_4.CH_2.CH_2.COOH$$
$$\rightarrow CH_3O.C_6H_4.CH_2.CH_2.CO.NH_2 \rightarrow CH_3O.C_6H_4.CH_2.CH_2.NH_2$$
$$\rightarrow OH.C_6H_4.CH_2.CH_2.NH_2.$$

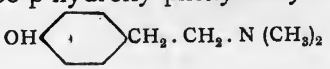
The yield by the last synthesis is poor; the p-methoxy-phenyl-ethylamine is better prepared by Rosenmund's method [1909], by the reduction of the condensation product of anisaldehyde with nitro-methane :—

$$CH_3O.C_6H_4.CHO + CH_3.NO_2 = CH_3O.C_6H_4.CH:CH.NO_2$$
$$\rightarrow CH_3O.C_6H_4.CH_2.CH:NOH \rightarrow CH_3O.C_6H_4.CH_2.CH_2.NH_2.$$

Rosenmund then boils the latter compound with colourless hydriodic acid and obtains p-hydroxy-phenyl-ethylamine.

Hordenine, $OH.C_6H_4.CH_2.CH_2.N(CH_3)_2$.

An infusion of barley germs, a by-product obtained in the preparation of malt, had been employed in the South of France against dysentery. This led to the isolation by Léger [1906, 1] of an "alkaloid" from barley germs, which he named hordenine. The base was found by Léger [1906, 2, 3; 1907] and independently also by Gaebel [1906] to be p-hydroxy-phenyl-ethyl-dimethylamine



The constitution of hordenine was deduced by Léger from the oxidation of acetyl-hordenine to acetyl-p-hydroxy-benzoic acid and the distillation of the ammonium base from hordenine methiodide methyl-ether, which yielded trimethylamine and p-vinylanisole,

$$CH_3O.C_6H_4.CH:CH_2.$$

Gaebel, on methylating and oxidising, obtained anisic acid from hordenine.

The *synthesis* of hordenine was first carried out by Barger [1909, **2**] from phenyl-ethyl-alcohol, a commercial product, as follows:—

$$C_6H_5.CH_2.CH_2.OH \rightarrow C_6H_5.CH_2.CH_2.Cl \rightarrow C_6H_5.CH_2.CH_2.N(CH_3)_2$$

$$\downarrow$$

$$HO.C_6H_4.CH_2.CH_2.N(CH_3)_2 \leftarrow NH_2.C_6H_4.CH_2.CH_2.N(CH_3)_2 \leftarrow NO_2.C_6H_4.CH_2.CH_2.N(CH_3)_2$$

Closely related to this is the synthesis from tyrosol, by Ehrlich [1912]:—

$$OH.C_6H_4.CH_2.CH_2OH \rightarrow OH.C_6H_4.CH_2.CH_2Cl \rightarrow OH.C_6H_4.CH_2.CH_2.N(CH_3)_2$$

The attempted conversion of p-hydroxy-phenyl-ethylamine into hordenine by methyl-iodide resulted only in the formation of the quaternary iodide, but Rosenmund [1910] has succeeded in methylating p-methoxy-phenyl-ethylamine to the tertiary base, hordenine methyl-ether, from which hordenine was obtained by boiling with hydriodic acid. Other syntheses are by reduction of p-hydroxy-phenyl-dimethyl-amino-methyl-ketone

$$HO.C_6H_4.CO.CH_2.N(CH_3)_2$$

(Voswinckel [1912]) and by distillation in a vacuum of the quaternary hordenine methiodide (prepared from p-hydroxy-phenyl-ethylamine) according to D.R.P. 233069 of Farbenfabriken vorm. F. Bayer & Co.:—

$$OH.C_6H_4.CH_2.CH_2.N(CH_3)_3I = OH.C_6H_4.CH_2.CH_2.N(CH_3)_2 + CH_3I.$$

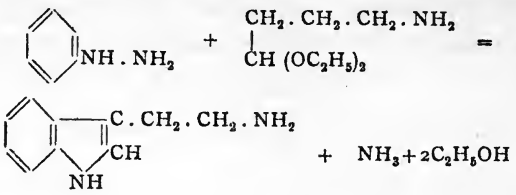
Hordenine has only a transitory existence during the germination of barley. According to Torquato Torquati [1910] it is not present in the ungerminated seed and is most abundant after four days, when the rootlets contain 0·4 – 0·45 per cent. It then gradually diminishes and has disappeared after twenty-five days. It is absent in germinating wheat, peas and lupins.

Indolethylamine (3-β-Amino-ethylindole), $C_{10}H_{12}N_2$.

3-β-Amino-ethylindole is the amine derived from tryptophane by decarboxylation. It was obtained by Ewins and Laidlaw [1910, **2**] both synthetically and by the action of putrefactive bacteria on the amino-acid.

The synthesis, subsequently described by Ewins [1911], is the

most convenient method for obtaining the base in quantity ; γ-amino-butyrylacetal is heated with phenyl-hydrazine and zinc chloride.



From the concentrated solution of the crude hydrochloride (obtained by washing the reaction mixture with ether and removing the zinc as sulphide) the free base is precipitated by sodium hydroxide as an oil, which on keeping crystallises to a mass of fine needles.

Laidlaw [1911] dissolved 0·5 grm. tryptophane in 250 c.c. of tap water, together with 0·5 grm. peptone, 2 grm. glucose, traces of sodium phosphate and magnesium sulphate and added 5 grm. of calcium carbonate ; this is the culture medium employed by Ackermann in the decarboxylation of histidine (p. 132). After infection with a subculture from putrid pancreas and incubation for a fortnight the mixture was boiled with charcoal and concentrated. Picric acid then precipitated the deep orange red picrate of indolethylamine. Yield after purification = 0·14 grm. = 14 per cent. of the theoretical.

The decarboxylation of tryptophane cannot be effected by heat. The author's experiments in this direction were carried out under a pressure of 1 mm. ; the only substance which could be isolated from the sublimate was a small quantity of unchanged tryptophane.

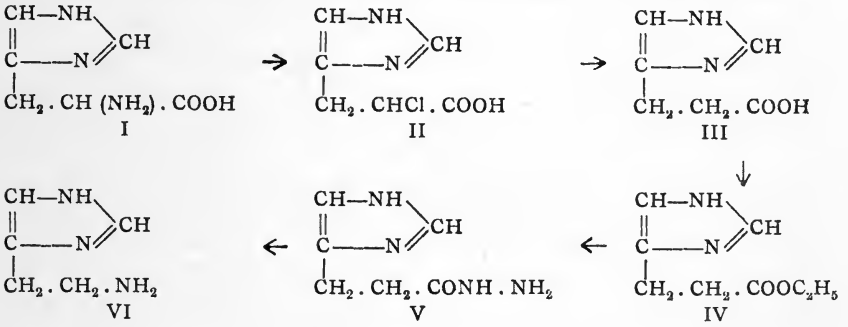
β-Iminazolyl-ethylamine, $C_5H_9N_3$.

β-Iminazolyl-ethylamine (4-β-amino-ethyl-glyoxaline) is the amine derived from histidine by decarboxylation ; it is of considerable interest on account of its great physiological activity. The base was first obtained by Windaus and Vogt [1907] who prepared it by Curtius's method from iminazolyl-propionic acid, which can be made by synthesis as well as from histidine. A few years later Ackermann [1910, 1] submitted pure histidine hydrochloride to the action of putrefactive bacteria and obtained a relatively large yield of iminazolyl-ethylamine (together with a small quantity of iminazolyl-propionic acid). The physiological activity of the amine, however, remained unknown until the latter was identified as one of the active principles of ergot by Barger and Dale [1910, 2-4]. The same active principle was simultaneously isolated from ergot by Kutscher [1910, 1] who at first regarded it as closely related to iminazolyl-ethylamine, but

not identical with it, on account of a supposed difference in the physiological action of the two bases. Iminazolyl-ethylamine has also been obtained from the intestinal mucosa by Barger and Dale [1911]; it is therefore present in crude solutions of secretine, to which it gives a depressent action. Its formation in the intestinal wall is probably due to bacilli, isolated by Mellanby and Twort [1912] and by Berthelot and Bertrand [1912, **1**, **2**]. The base has further been isolated from putrid Soy beans by Yoshimura [1] [1910]; it probably also occurs in commercial extracts of meat, of yeast, etc.

The yield from almost all the above sources is very small; larger quantities may be prepared from histidine, as well as by direct synthesis. The decarboxylation of histidine has been carried out indirectly by Windaus and Vogt [1907] as mentioned above.

The reactions involved are the transformation of histidine (I)



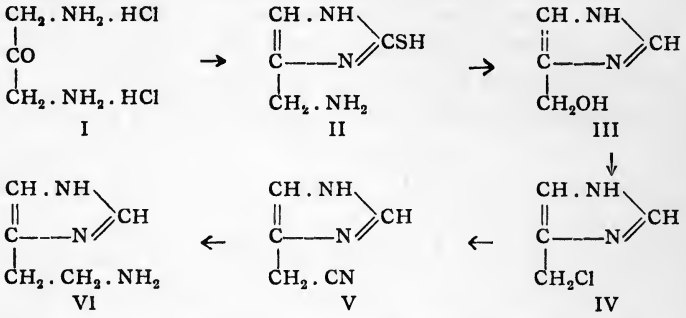
into α-chloro-β-iminazolyl-propionic acid (II) (by sodium nitrite and hydrochloric acid); the reduction of this substance to β-iminazolyl-propionic acid (III), which can also be synthesised from glyoxyl-propionic acid; the successive conversion of this acid into the ester (IV) and the hydrazide (V); finally the conversion of the latter into the azide and urethane (in alcoholic solution by amylnitrite and hydrogen chloride) and the hydrolysis of the urethane by concentrated hydrochloric acid, which gives the hydrochloride of the desired amine (VI).

The direct decarboxylation of histidine can be carried out more conveniently by bacterial action and is applied industrially, according to patents by Hoffmann, La Roche & Co. [1912], and by Farbenfabriken vorm. F. Bayer & Co. (D.R.P. 250110). Details of the method are given in the appendix.

An attempt to decarboxylate histidine by heat alone results only

[1] Yoshimura [1909] probably obtained iminazolylethylamine by putrefaction before Ackermann, but he did not identify it. He found that the Japanese beverage Tamari-Shoyu, prepared from Soy beans, contains per litre 0·7 grm. of a base $C_6H_9N_3$, which he surmised was derived from histidine.

in the formation of traces of the amine, and Ackermann, by heating histidine with lime, could only obtain glyoxaline. Ewins and Pyman [1911], however, obtained a 10-20 per cent. yield by heating benzoyl histidine in a vacuum to 240° and subsequent hydrolysis, and a 24 per cent. yield by heating histidine hydrochloride with 20 per cent. sulphuric acid to 265-270°. The most convenient method of preparing iminazolyl-ethylamine is, however, by the synthetical method of Pyman [1911]. Diaminoacetone dihydrochloride (I) (obtained from citric acid) is heated with one molecular proportion of potassium sulphocyanide; the thiolglyoxaline (II), thus formed by



Gabriel's general method, is oxidised with nitric acid; the nitrous acid formed in the reaction further attacks the amino-group so that a glyoxaline alcohol (III) results. This is successively converted into the chloro-compound (IV) and the cyano-compound (V); the latter yields on reduction the desired amine (VI).

The lower homologue, iminazolylmethylamine, has been prepared by Windaus and Opitz [1911].

PHYSIOLOGICAL PROPERTIES OF THE AMINES DERIVED FROM AMINO-ACIDS.

The chief interest attached to the amines described in this chapter is due to their physiological action and to the possibility of their formation in the organism, wherever proteins or amino-acids are exposed to bacterial action as, for instance, in the intestine. By far the most active amines are those containing a ring, namely those derived from phenyl-alanine, tyrosine, tryptophane, and histidine. Their formation does not take place in acid solution, and would, therefore, appear to be prevented or lessened by the sour-milk treatment recommended by Metchnikoff. Berthelot and Bertrand [1913, 1] find, however, that their *Bacillus aminophilus* even produces β-iminazolylethylamine in 0·3 per cent. lactic acid, unless much glucose is present, when the sugar alone is attacked. The same investigators [1913, 2] find that rats, fed on a milk diet, are not affected by either *Proteus vulgaris* or *B. aminophilus intestinalis* when given separately, but that if the two organisms are given simultaneously, the rats may develop a fatal diarrhœa in from 4-8 days. Normally these putrefactive amines appear to be destroyed in the liver; Ewins and Laidlaw [1910, 3; 1913] have shown that p-hydroxy-phenyl-ethylamine and indole-ethylamine are transformed by perfusion through a surviving liver into p-hydroxy-phenyl-acetic acid and indole-acetic acid respectively. Oehme [1913] states that 0·6 mg. may kill a rabbit when given intravenously, but that the lethal dose is much higher when injected into the portal circulation. Rabbits will even stand 0·5 grm. by the mouth. Nevertheless the amines may perhaps play a part in certain diseases; thus p-hydroxy-phenyl-ethylamine may be connected with a persistent high blood pressure, and Mellanby [1911] has attempted to connect β-iminazolyl-ethyl-amine with cyclic vomiting. Pharmacologically these bases are important on account of their presence in ergot.

Ehrlich and Pistschimuka [1912] have shown that they are transformed by yeast into the corresponding alcohols, and according to Czapek [1903] the amines with 3-7 carbon atoms are a good source of nitrogen for *Aspergillus*.

The action of many synthetic amines has been examined; it seems

that the most active are cyclic ones with a side chain of two carbon atoms like the last four naturally occurring ones described in this chapter. This conclusion with regard to the side chain was deduced for aromatic amines by Barger and Dale [1910, 1]; it is further supported by toxicity determinations of several iminazole derivatives by Friedberger and Moreschi [1912]. Von Braun and Deutsch [1912] have found, however, that when the side chain of hordenine is lengthened the pressor action is diminished and the toxicity is increased. With four and five carbon atoms in the side chain the toxicity is ten times as great as with three carbon atoms.

The natural amines described in this chapter may be arranged in two groups, of monamines and of diamines, and physiological action is more or less of the same type within each group. The monamines (see p. 29) produce effects similar to those caused by stimulation of the sympathetic nervous system. They may be termed sympathomimetic (see p. 98). The most powerful sympathomimetic base is adrenaline (see Chapter VI). Of the bases already described the most powerful is p-hydroxy-phenyl-ethylamine: the others in descending order of activity are phenyl-ethylamine, isoamylamine, isobutylamine.

One of the most marked of sympathomimetic actions is the raising of the blood pressure on intravenous injection and *isobutylamine* is the lowest amine which has any marked pressor action. 10-20 mg. of *isoamylamine*, injected intravenously as the hydrochloride, produce a marked rise of blood pressure in the cat (Dale and Dixon [1909]). The effect of other aliphatic monamines is very similar. Normal amylamine has a slightly greater activity than its isomeride, and hexylamine is still more active, but in ascending the series beyond this point the activity again declines, heptylamine being less active than hexylamine and octylamine much less so (Barger and Dale [1910, 1]).

The introduction of a benzene ring in *phenyl-ethylamine* greatly increases the activity and this base is at least five times as active as any aliphatic amine. Thus 2 mg. of the base may raise the blood pressure of a cat from 30 to 180 mm. Phenyl-ethylamine has the same carbon skeleton as adrenaline.

p-Hydroxy-phenyl-ethylamine has an activity something like $\frac{1}{20}$ of that of adrenaline, and has been studied by Dale and Dixon [1909].

Doses of 1-2 mg., injected intravenously, cause a sudden and pronounced rise of arterial blood pressure, which is somewhat less transitory than that caused by adrenaline. As with the latter sub-

stance, the output of the heart is increased, the non-pregnant cat's uterus relaxes, the pregnant cat's uterus contracts, the salivary gland is stimulated to secretion.

p-Hydroxy-phenyl-ethylamine differs from adrenaline in causing little vaso-constriction when applied locally to a mucous surface, and in being hardly toxic. Thus 100 mg. given hypodermically to a cat, produced all the symptoms of intense stimulation of sympathetic nerves, but no after-effects and no glycosuria.

Since p-hydroxy-phenyl-ethylamine is formed from tyrosine by the action of fæcal bacteria, it doubtless occurs in the alimentary canal and might therefore perhaps play a part in certain pathological states in which a high blood-pressure is the most prominent symptom. A pressor substance has been found in the urine by Abelous and termed urohypertensine (perhaps identical with isoamylamine) and Bain [1909, 1910] obtained from normal urine a pressor base, giving Millon's reaction; the latter base was not isolated in a state of purity and its identity with p-hydroxyphenylethylamine, suggested by Bain, is very doubtful. Bain found that the amount of this base was diminished in the urine from gouty patients and particularly in that from patients with a high blood pressure; on the other hand it did not disappear from normal urine during a milk diet or when medicinal doses of antiseptics were administered.

On account of the possible clinical significance of p-hydroxy-phenyl-ethylamine, as indicated above, Ewins and Laidlaw [1910, 3] have investigated the fate of this amine in the organism. They found that when given by the mouth to dogs, something like one-half the amount is excreted in the urine as p-hydroxy-phenylacetic acid; the other half remains unaccounted for. The conversion of the amine into the acid readily takes place in the perfused rabbit's liver, and also to some extent in the perfused isolated uterus, but in the isolated heart the amine, when perfused, was completely destroyed and no p-hydroxy-phenylacetic acid could be isolated.

Other papers of clinical interest are those by Harvey [1911], who induced renal disease and vascular sclerosis in rabbits by prolonged intravenous and oral administration of p-hydroxy-phenyl-ethylamine, by Clark [1910] and by Findlay [1911] who examined the effect of this amine on man. Clark found that large doses (30-200 mg.) given by the mouth generally gave a slight rise of blood pressure lasting for several hours, and that 20-60 mg., given subcutaneously, produced in the healthy subject a considerable rise of blood pressure, lasting for about twenty minutes. The suggestion by

Burmann [1912] and Heimann [1912] that p-hydroxy-phenyl-ethylamine can replace ergot, or even that it is the most important constituent of this drug, is erroneous (see especially a paper by Guggenheim [1912]). The action of the base has also been studied lately by Fröhlich and Pick [1912], by Handovsky and Pick [1913] and by Bickel and Pawlow [1912].

According to Engel [1912] p-hydroxy-phenyl-ethylamine has no necrotising effect on tumours, although this effect is produced by phenyl-ethylamine, which has only one-fifth of the pressor activity of the first-named base. The effect is also shown by hordenine and by adrenaline.

p-Hydroxyphenyl-ethylamine has a paralytic action on Crustacea and occurs in the salivary gland of Cephalopoda which feed on crabs [Henze, 1913].

Hordenine, which is the N-dimethyl-derivative of the last-named base, has a much weaker action, and has been studied by Camus [1906]. The minimal lethal dose of the sulphate is 0·3 grm. per kilo. for dogs, injected intravenously, and 2 grm. per kilo. for guinea-pigs injected subcutaneously, so that the toxicity is very slight. The base has a feeble pressor action. Its *methiodide*, however, causes a very rapid and evanescent rise of blood pressure in cats, when injected intravenously in doses of 1 mg. The effect superficially resembles that of adrenaline but is in reality of the nicotine type (Barger and Dale [1910, 1]). Von Braun and Deutsch [1912] have prepared homologues of hordenine, having the formula

$$OH \cdot C_6H_4 \cdot (CH_2)_n \cdot N(CH_3)_2$$

with $n = 3$, 4 and 5. In these the pressor action of hordenine is diminished. The lethal dose for rabbits is respectively 0·1 grm., 0·01 grm., 0·02 grm., as compared with 0·3 grm. for hordenine. Comp. von Braun, Ber. deutsch. chem. Ges., 1914, **47**, 492.

The physiological action of *indolethylamine* has been studied by Laidlaw [1911]. Doses of 10-20 mg. of the hydrochloride given intravenously to rabbits and cats, produce a transient stimulant effect upon the central nervous system, causing clonic and tonic convulsions, tremors of limbs, and vaso-constriction. In the spinal cat 2 mg. causes a large rise of blood pressure due to vaso-constriction and increased cardiac activity. In this respect the amine resembles p-hydroxy-phenyl-ethylamine. Indolethylamine has further a direct stimulant action on plain muscle, which is most marked in the arterioles, the iris, and the uterus. This action of the amine from tryptophane is on the whole much less than that of the amine from histidine. Speaking

very broadly, indolethylamine (with two nitrogen atoms of which only one is basic) has a physiological action intermediate between that of the sympathomimetic monamines such as p-hydroxy-phenyl-ethylamine, and the diamines, like iminazolyl-ethylamine.

Ewins and Laidlaw [1913] have more recently studied the fate of indolethylamine in the organism; in the perfused liver the base is converted into indole-acetic acid, a change quite comparable to the transformation of p-hydroxy-phenyl-ethylamine into p-hydroxy-phenyl-acetic acid (see p. 27). In dogs the indole-acetic acid is however excreted in the urine in combination with glycine as *indole-aceturic* acid $C_8H_6N . CH_2 . CO . NH . CH_2 . COOH$, mp. 94°, forming an orange red picrate which melts at 145°.

Among diamines β-iminazolyl-ethylamine is the only one having a cyclic structure, and it is by far the most active. *Putrescine* and *cadaverine* have at most a very slight toxicity; on intravenous injection in the cat they lower the blood pressure. *Agmatine* has according to Engeland and Kutscher [1910, 1] a powerful action on the isolated uterus, causing contraction, but Dale and Laidlaw [1911, p. 194] state that agmatine does not make any significant contribution to the activity of ergot and is only feebly active as compared with β-iminazolyl-ethylamine, also present in ergot. Thus 5 mgs. of agmatine produced a much smaller effect on the cat's uterus than 0·1 mg. of the latter base.

The physiological action of *β-iminazolyl-ethylamine* has been investigated by Ackermann and Kutscher [1910, 1] and more fully by Dale and Laidlaw [1910, 1911].[1] According to the latter authors the fundamental and characteristic feature of the action is a direct stimulant effect on plain muscle, producing exaggerated rhythm or tonic contraction, according to the dose. The most sensitive plain muscle is the non-pregnant uterus of some species and it is this reaction which led to the identification of the base in ergot. A marked contraction of the isolated uterus is produced by adding to the bath of Ringer's solution sufficient of the base to give a concentration of 1 : 25,000,000 and the effect of 1 : 250,000,000 is often quite definite (compare also Fröhlich and Pick [1912] and Sugimoto [1913]). The muscular coats of the bronchioles are also highly sensitive to the action of β-iminazolyl-ethylamine, especially in rodents, but not in the ox (Trendelenburg [1912]). Baehr and Pick [1913, 1] have studied the effect on the musculature of the surviving guinea-pig's lung. Here

[1] Many scattered observations on its action occur in the pharmacological literature of the last few years.

the contraction due to β-iminazolylethylamine is permanently abolished by adrenaline, which is not so in the intact animal. Large guinea-pigs are killed in a few minutes by an intravenous injection of 0·5 mg., owing to asphyxia resulting from the constriction of the bronchioles; post-mortem the lungs are found to be permanently distended. This corresponds closely to the effects of poisoning by Witte's peptone and the toxic effects of serum or other protein in the sensitised guinea-pig, known as anaphylactic shock. Unlike peptone, iminazolyl-ethylamine does not, however, possess in any marked degree the power of rendering the blood incoagulable. According to Popielski the physiological effect of peptone is produced by a hypothetical substance "vasodilatin," and he [1910, **2**] has suggested that iminazolyl-ethylamine acts by liberation of vasodilatin, when injected intravenously, a supposition rejected by Dale and Laidlaw [1911]. Attention may also be drawn to a possible connection between iminazolyl-ethylamine and the "depressor substances" of various observers, such as the urohypotensine of Abelous and Bardier [1909]; the depressent action of Bayliss and Starling's secretine is indeed explained by the isolation from it of iminazolyl-ethylamine by Barger and Dale [1911].

The resemblance of the symptoms of poisoning with iminazolyl-ethylamine to those of anaphylactic shock is indeed very striking (Dale and Laidlaw [1910, 1911], Pfeiffer [1911], Biedl and Kraus [1912], Schittenhelm and Weichardt [1912], Aronson [1912], Friedberger and Moreschi [1912]); not only does it extend to the bronchial constriction in guinea-pigs, mentioned above, but also to a fall of body temperature, which is one of the characteristics of the milder degree of the "shock". Thus the intraperitoneal injection of 3 mgs. of iminazolyl-ethylamine was found by Dale and Laidlaw to lower the rectal temperature of a guinea-pig gradually from 38·5° to 28·5° in the course of two hours; next day it was again 38°. Extremely minute doses of serum may, on the other hand, cause a rise of body temperature in an anaphylactic animal, and the same applies to iminazolyl-ethylamine when given in sufficiently small doses to a (normal) guinea-pig, as has been shown by Pfeiffer [1911]. The correspondence is also illustrated by the relatively great resistance of dogs, both to anaphylactic shock and to the amine. In this connection we may refer to a paper by Engeland [1908, **3**] in which evidence is adduced that histidine derivatives are more readily broken down by carnivora than by herbivora. No data are available to fix the lethal dose of β-iminazolyl-ethylamine in man, but a *Macacus* monkey of 1·25 kilo. was killed by

an intravenous injection of 0·065 grm. of the hydrochloride [Berthelot and Bertrand, 1912, 3].

Lately the close similarity between the symptoms of poisoning by β-iminazolyl-ethylamine and those of anaphylactic shock have been emphasised anew by Oehme [1913]. He and Loewit [1913; Ch. V, methyl guanidine] both criticise the conclusion of Heyde [1912; Ch. V, methylguanidine] that methylguanidine rather than iminazolyl-ethylamine is of importance in this respect.

The supposed connection between β-iminazolyl-ethylamine and anaphylactic shock has even led to the statement (by Aronson [1912]) that the amine is formed by incubating histidine with normal guinea-pigs' serum, but this has been disproved by Friedberger and Moreschi [1912] and Modrakowski [1912] denies that the amine is the cause of anaphylactic shock since it does not render the blood incoagulable.

In recording the fact, "as a point of interest and possible significance," that the immediate symptoms with which an animal responds to an injection of a normally inert protein, to which it has been previously sensitised, are to a large extent those of poisoning by β-iminazolyl-ethylamine, Dale and Laidlaw consider that "the correspondence cannot yet be regarded as sufficient basis for theoretical speculation". Pfeiffer thinks that β-iminazolyl-ethylamine will certainly be of significance for the solution of the problem of anaphylaxis.

The effect of iminazolyl-ethylamine on the vascular system is complex and varies in different species, as well as in the same species under different conditions. In rodents a rise of blood-pressure occurs, owing to constriction of the arterioles, but may be masked by embarrassed respiration. It was the different behaviour of rabbits to the base from histidine and that from ergot, which led Kutscher [1910, 1] to regard the two bases as different. Barger and Dale [1910, 3] have however shown that both kinds of physiological effect are obtainable with the base from either source, so that the identity cannot be doubted. In carnivora, in the fowl, in the monkey (and probably therefore in man) iminazolyl-ethylamine causes vasodilatation and a fall of systemic blood pressure. The following table (Barbour [1913]) gives the effects of the amine, compared with those of adrenaline and p-hydroxy-phenyl-ethylamine :—

	Blood Pressure.	Peripheral Vessels.	Coronary Vessels (Ox).	Non-pregnant Uterus.
Epinephrin (adrenaline)	+	+	−	−
Tyramin (p-hydroxy-phenyl-ethylamine)	+	+	+	−
Histamin (β-iminazolyl-ethylamine) .	−	+	+	+

+ means rise of blood pressure or constriction, − the opposite; the last-named amine may have a pressor effect in some animals.

The pulmonary arterioles, however, are constricted and the pulmonary blood pressure is raised. This combination of a vasodilator fall of systemic blood pressure with a vasoconstrictor rise of pulmonary pressure has been described as characteristic of the action of ergot (Bradford and Dean [1894]), and is doubtless due to the iminazolyl-ethylamine present in the drug. For the effect of the base on the pulmonary vessels consult Baehr and Pick [1913, **2**], and on the frog's blood vessels, Handovsky and Pick [1913].

Finally it should be mentioned that iminazolyl-ethylamine has a weak stimulant action on the salivary glands and on the pancreas, qualitatively resembling that of pilocarpine, which alkaloid also contains a glyoxaline ring. The action on the pancreas is not at all like that of secretine, being abolished by a small dose of atropine.

CHAPTER II.

ω-AMINO-ACIDS AND OTHER BASES DERIVED FROM PROTEIN CONTAINING A CARBOXYL-GROUP (UROCANIC AND KYNURENIC ACIDS).

IN the monamino-acids, formed by the hydrolysis of proteins, the acidic properties of the carboxyl-group are neutralised more or less completely by an adjoining amino-group in the α-position, and only the diamino-acids histidine, lysine, and arginine are bases. When the amino-group is not in the α-position the basic character is more pronounced, and the so-called ω-amino-acids are feeble bases, being precipitated by phosphotungstic acid; several of them are formed from protein fission products by putrefaction, and these are described in this chapter.

The influence of the position of the amino-group on the acid dissociation constant K_a and on the basic dissociation constant K_b is evident from the following table (Ley [1909, p. 358]):—

	K_a	K_b
Glycine	180×10^{-12}	$2{\cdot}7 \times 10^{-12}$
α-amino-propionic acid . .	230×10^{-12}	$3{\cdot}1 \times 10^{-12}$
β-amino-propionic acid . .	71×10^{-12}	51×10^{-12}
γ-amino-butyric acid . . .	37×10^{-12}	170×10^{-12}

An amino-acid may also be rendered basic by complete methylation of the nitrogen atom, as in the betaines described in Chapter III.

ω-Amino-acids are produced by putrefaction in three ways:—

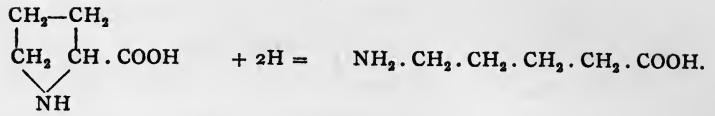
1. By partial deaminization of a diamino-acid, as in the formation of δ-amino-valeric acid from ornithine:—

$$NH_2 . CH_2CH_2CH_2CH(NH_2)COOH + 2H = NH_2 . CH_2CH_2CH_2CH_2COOH + NH_3.$$

2. By the partial decarboxylation of a dibasic amino-acid, e.g. the production of γ-amino-butyric from glutamic acid:—

$$COOH . CH(NH_2) . CH_2 . CH_2 . COOH = NH_2 . CH_2 . CH_2 . CH_2 . COOH + CO_2.$$

3. By the reduction of a cyclic amino-acid. Ackermann [1911, 2] and Neuberg [1911, 1] have recently shown that α-pyrrolidine carboxylic acid (proline) yields δ-amino-valeric acid in putrefaction:—



The ω-amino-acids differ from α-amino-acids in being precipitated by phosphotungstic acid, even in dilute solutions; they yield platinichlorides soluble in alcohol (Ackermann). The γ-, δ-, and ε-amino-acids are so weakly acidic that they do not form blue copper salts on boiling with cupric oxide, or on addition of cupric acetate, this property belonging only to α- and β-amino-acids (Fischer and Zemplen [1909, p. 4883]). On heating γ-amino-butyric and δ-amino-valeric acids are transformed into their anhydrides, pyrrolidone and piperidone.

β-Alanine, β-amino-propionic Acid, $NH_2 . CH_2 . CH_2 . COOH$.

This substance, long known synthetically, was first isolated from Liebig's extract of meat by Engeland [1908, 1]; Micko [1905] had previously obtained an alanine from the same source and assumed that it was the α-amino-acid.

β-Alanine is formed from the meat base carnosine by hydrolysis (see next section), and since Engeland's process of isolation involved evaporation in hydrochloric acid solution, Gulewitsch [1911; see under carnosine] questions whether β-alanine is present as such in muscle.

It was to be expected that β-alanine could also be formed from aspartic acid by putrefaction, according to the second general method given in the preceding section, and after some failures Ackermann [1911, 1] has succeeded in demonstrating this.

One hundred grm. of aspartic acid in a culture medium similar to that used for preparing β-iminazolyl-ethylamine yielded 2 grm. of β-alanine hydrochloride.

β-Alanine is broken down to urea in the dog (Abderhalden and Schittenhelm [1907]).

γ-Amino-n-butyric Acid, $NH_2 . CH_2 . CH_2 . CH_2 . COOH$.

This acid is formed in putrefaction from glutamic acid by the second general process (p. 33).

Ackermann [1910, 3] obtained 2·1 grm. of γ-amino-butyric acid aurichloride from 50 grm. of glutamic acid. Abderhalden and Kautzsch [1912] lately failed to repeat Ackermann's experiment, but afterwards Abderhalden, Fromme and Hirsch [1913] obtained 0·3 grm. of the platinichloride of γ-amino-butyric acid from 25 grm. of glutamic acid.

δ-Amino-n-valeric Acid, $NH_2 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$.

This, the first known example of a natural ω-amino-acid, was obtained by E. and H. Salkowski [1883] from putrefied fibrin and muscle, and later by H. Salkowski [1898] from putrefied gelatin. Ackermann [1907, **2**] isolated it from putrid pancreas (and at first called it putridine, because he failed to identify it). The substance was prepared synthetically by Schotten [1884] by the oxidation of benzoyl-piperidine with potassium permanganate.

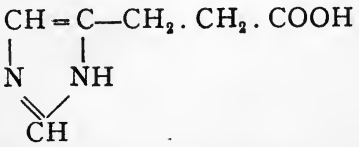
δ-Amino-valeric acid is derived in putrefaction from both arginine (ornithine) and proline. Ackermann [1910, **3**] submitted 56 grm. of arginine carbonate to putrefaction in the same way as aspartic acid and glutamic acid (preceding sections), and obtained putrescine, ornithine, δ-amino-valeric acid (about 15 grm. of the aurichloride) but not agmatine. The arginine is no doubt first broken down to ornithine, and the latter by the first general process (p. 33) yields δ-amino-valeric acid.

The putrefactive formation of δ-amino-valeric acid from proline (α-pyrrolidine carboxylic acid) has been observed more recently by both Ackermann and Neuberg; two hydrogen atoms are added and the ring is opened.

ε-Amino-caproic Acid, $NH_2 . (CH_2)_5 . COOH$.

This substance should be obtainable from lysine by putrefactive deaminization; an attempt to prove this was made by Ackermann [1910, **3**] with 98 grm. of lysine chloride. He obtained a large quantity of cadaverine and a small quantity of a platinichloride fairly readily soluble in alcohol and in water; the analysis of this salt did not agree with the composition required for the platinichloride of the desired amino-caproic acid.

β-Iminazolyl-propionic Acid,



This acid was first obtained from histidine by chemical means and was also prepared synthetically by Knoop and Windaus [1906] (see Plimmer's "Chemical Constitution of the Proteins," Part I, p. 126). Ackermann [1910, **1**] then showed that it is also formed by putrefaction from pure histidine hydrochloride; the principal product was imin-

azolyl-ethylamine (described in Chapter I, p. 22), but in addition a small quantity of iminazolyl-propionic acid was obtained.

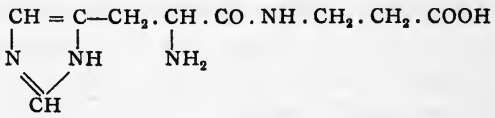
Carnosine (Ignotine), $C_9H_{14}O_3N_4$.

This substance is described in this chapter as it is a derivative of β-alanine. Carnosine is, after creatine, the most abundant base in meat extract. It was discovered by Gulewitsch and Amiradžibi [1900, 1, 2]; Krimberg [1906, 1] obtained 0·13 per cent. from fresh ox meat. Ignotine, subsequently isolated by Kutscher [1905] from meat extract and regarded by him as an isomeride, was shown by Gulewitsch [1906], by direct comparison, to be identical with carnosine, and the identity has been admitted by Kutscher after prolonged controversy. Carnosine has also been obtained from horse meat, to the extent of 1·82 grm. per kilo. (Smorodinzew [1913]) and from fish, crabs, oysters and wild rabbits.

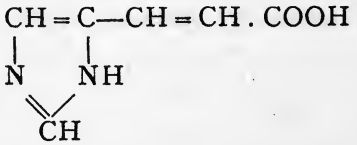
On heating with baryta to 140°, carnosine is hydrolysed to histidine and β-alanine in equimolecular proportions (Gulewitsch [1907, 1911]) according to the equation :—

$$C_9H_{14}O_3N_4 + H_2O = C_6H_9O_2N_3 + C_3H_7O_2N.$$

It is, therefore, similar to a dipeptide and must be either histidyl-β-alanine or β-alanyl-histidine; it gives the red coloration with sodium p-diazobenzene sulphonate, characteristic of histidine, and yields on boiling with cupric carbonate a copper salt similar to that of β-alanine. Perhaps, therefore, histidyl-β-alanine is the more likely constitution :—



Urocanic Acid, Iminazolyl-acrylic Acid,

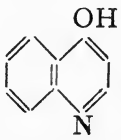


This acid contains two hydrogen atoms less than iminazolyl-propionic acid described above and may be considered to be derived from histidine by loss of ammonia, without reduction. It was discovered by Jaffé [1874, 1875] in the urine of a dog; after a few days the dog ran away, and, to Jaffé's great disappointment, it was never recaptured. The substance was not observed again until Siegfried

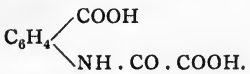
[1898] found it once more in dog's urine. In both cases the substance was constantly present; no other case of its occurrence in urine has been observed and it would appear that the two dogs presented a rare anomaly of metabolism. Recently Hunter [1912], although unable to find a dog secreting urocanic acid, obtained the same substance by prolonged tryptic digestion of caseinogen and was able to identify it by comparison with a specimen of iminazolylacrylic acid which Barger and Ewins [1911] had obtained as a degradation product of ergothioneine and had also synthesised.

Among closely related substances from human urine we may mention *histidine* itself, a *base* yielding a picrolonate $C_5H_7O_2N_3$, $C_{10}H_8O_5N_4$ melting at 244°, and a *base* giving an aurichloride $C_{15}H_{36}O_{13}N_8$, $HAuCl_4$ very soluble in water and blackening at 100°. These bases were obtained by Engeland [1908, 3] who regards the second as *amino-iminazolylacetic acid*, a lower homologue of histidine, and the third as probably a *polypeptide of histidine*. According to Engeland histidine is broken down more readily by carnivora than by herbivora; the urine of rabbits and horses gives a stronger reaction with p-diazobenzene sulphonic acid than that of the cat or dog.

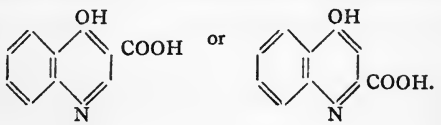
Kynurenic Acid,



Long ago Liebig [1853] discovered an acid which occasionally separated from dog's urine in minute quantity. The substance was further investigated by Schmiedeberg and Schultzen [1872] and by Kretschy [1881-84] who showed that the product formed by heating the acid above its melting point, the so-called kynurine, C_9H_7ON, was an oxyquinoline, and that kynurenic acid was therefore an oxyquinoline carboxylic acid. Heated with zinc dust kynurine was reduced to quinoline, and on oxidation of kynurenic Kretschy obtained oxalylanthranilic acid,



Hence, when Wenzel [1894] had shown by synthesis that kynurine is 4-hydroxy-quinoline, kynurenic acid was found to be either 4-hydroxy-3-quinoline carboxylic acid, or 4-hydroxy-2-quinoline carboxylic acid.



Camps [1901, **1, 2**] prepared both acids and wrongly concluded that the former was identical with the acid from dog's urine, but Miss Homer [1913] has shown, by the mixed melting point, that kynurenic acid has the latter constitution.

Liebig [1853], Kretschy [1881] and others had already found that kynurenic acid only makes its appearance, or is most abundant, in the urine of dogs fed on large quantities of meat. Many fruitless investigations were undertaken to find the precursor of the acid, until finally its formation was shown to depend on a product of tryptic digestion of protein (Glaessner and Langstein [1902]). This Ellinger [1904, **1, 2**] identified as tryptophane (see Plimmer's "Chemical Constitution of the Proteins," Part I, p. 137). Abderhalden, London, and Pincussohn [1909] have shown that the transformation of tryptophane into kynurenic acid does not take place in the liver.

Kynurenic acid, taken by the mouth, is not excreted in the urine in man and in the rabbit (Hauser [1895], Solonin [1897]); the reason is probably that the acid is an intermediate product of metabolism which is not destroyed so rapidly in the dog as in man.

CHAPTER III.

BETAINES.

THE betaines are amino-acids in which the nitrogen atom is completely methylated. In addition to trimethyl-glycine, which has been known for a long time and occurs both in plants and in animals, fully methylated derivatives of proline, oxyproline, histidine, and tryptophane have so far been obtained from plants, and corresponding derivatives of γ-amino-butyric and of γ-amino-hydroxy-butyric acid from animals. Except in the case of trigonelline, which occurs in many plants but is not related to any known decomposition product of protein, the betaine grouping does not occur in the typical vegetable alkaloids; the two cases of its alleged occurrence, in damascenine and in chrysanthemine, have lately been disproved (respectively by Ewins [1912] and Yoshimura and Trier [1912, section on stachydrine]).

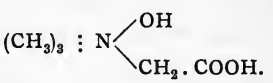
The betaines therefore form a fairly natural group comprising feeble bases of simple constitution; the α-betaines are devoid of marked physiological activity, but the two γ-betaines (being presumably stronger bases) have a distinct action. A comprehensive study of the chemical behaviour of betaines has been made by Willstätter [1902, 1] whose nomenclature is here employed. He points out that α-betaines and the isomeric esters of dimethyl-amino-acids are interconvertible:—

In the case of the betaines of β-and-γ-amino-acids the above change only proceeds from left to right, but not in the reverse direction. From the methyl ester of β-dimethyl-amino-propionic acid β-propio-betaine is thus obtainable; when γ-dimethyl-amino-butyrate is heated, the γ-butyro-betaine which no doubt first results, is unstable and yields trimethylamine and γ-butyro-lactone. Further details concerning the interconversion in the case of trimethyl-glycine are given in the next section.

The α-betaines differ greatly in the ease with which they split off

trimethylamine. Some are so unstable that they cannot be formed by the ordinary process of methylation. Thus aspartic acid, when treated with methyl iodide and alkali, breaks up into trimethylamine and fumaric acid. The same applies to tyrosine and it is noteworthy that the betaines of tyrosine and of phenylalanine have never been found in nature, whereas the corresponding unsaturated acids (p-cumaric and cinnamic acids) are often met with in plants. The betaine of tryptophane is somewhat more stable, and ergothioneine requires heating with concentrated alkali to decompose it into trimethylamine and the unsaturated acid.

The free betaines when dried above 100° have a composition corresponding to a cyclic anhydride (the second of the above formulæ). Salts are formed by direct addition of an acid, when the ring is broken down. Most betaines crystallise with one molecule of water and in this condition their constitution is probably illustrated by the formula :—



The main physiological interest of betaines is derived from the question whether they may re-enter the metabolism of plants or whether they are merely waste products ; this question is further discussed in the next section. Pharmacologically the *α*-betaines are inert, but γ-butyrobetaine is toxic to higher animals.

Betaine, Trimethylglycine, $(CH_3)_3 : N\begin{<}O\\CH_2\end{>}CO.$

While searching for alkaloids in *Solanaceæ*, Husemann and Marmé [1863, 1864] isolated a base from *Lycium barbarum*, which was found to have the composition $C_5H_{11}O_2N$ and was named by them *lycine*. Three years later Scheibler [1866] obtained from the sap of the sugar beet (*Beta vulgaris*) and from beet molasses a "soluble alkaloid" which he described in detail later [1869] and called *betaine*. Soon afterwards Scheibler [1870] and Liebreich [1870] showed the identity of betaine with *oxyneurine*, a base prepared by Liebreich [1869, **2**] by the oxidation of "bilineurine" (= choline) and also synthetically by the action of trimethylamine on mono-chloracetic acid. Griess [1875] prepared betaine according to his general method, by methylating glycine and Husemann [1875] proved the identity of lycine with betaine ; the second (and later) name for this base has, however, passed into general use.

Betaine is of rather widespread occurrence in plants and has also been found repeatedly in animals, but it is by no means so common as choline. Staněk and Domin [1910] have given a list of plants containing betaine; it was found in all species of *Chenopodiaceæ* examined; this natural order includes the sugar beet and also *Chenopodium Vulvaria* which gives off trimethylamine during life. In the closely related order of *Amarantaceæ* betaine was found by Staněk and Domin in some genera only; in other orders it only occurs sporadically and in small amount. The dry leaves of *Atriplex canescens* (N.O. *Chenopodiaceae*) contain as much as 3·78 per cent. of betaine, but in rye the amount is only 0·3 per cent. of the dry weight. Young sugar beets contain 2·5 per cent., old ones 1 per cent. of betaine (Scheibler).[1]

Various authors have at different times expressed the view that betaine may replace choline in lecithin. According to Trier [1912, **3**, p. 83; Ch. IV, choline] they were misled on account of the difficulty of purifying the phosphatide.

In the manufacture of beet sugar most of the betaine remains in the molasses, but crude beet sugar may contain 0·375 per cent. of betaine (Waller and Plimmer [1903]). When the molasses are desaccharified by means of strontium, the final liquor ("Schlempe") is very rich in betaine (115 grm. per kilo., Andrlik [1903-4]).

Syntheses of betaine by Liebreich [1869, **2**] and by Griess [1875] have been referred to above; it is also formed by isomeric change from the methyl ester of dimethylamino-acetic acid in sealed tubes at 200° (see below). The estimation of betaine and its separation from choline by Schulze's method [1909; Ch. IV, choline] and by Staněk's method [1906, **1, 2**; Ch. IV, choline] are described on pp. 150-152.

[1] Other sources of betaine are: *Lycium barbarum* (Husemann and Marmé [1863]), the press cake of cotton seeds (Ritthausen and Weger [1884]), malt and wheat germs (Schulze and Frankfurt [1893; Ch. IV, choline]); (Yoshimura [1910, Ch. IV, choline] recently found 0·06 per cent. of betaine in air dry malt germs); sunflower seeds (Schulze and Castoro [1904]), tubers of *Helianthus tuberosus* (Schulze [1910]), seeds of *Avena sativa* (Schulze and Pfenninger [1911; Ch. IV, choline]), Kola nuts (Polstorff [1909, **2**; Ch. IV, choline]), bamboo shoots (Totani [1910, **2**; Ch. IV, choline]), green tobacco leaves (Deleano and Trier [1912]), ergot (Kraft [1906, Ch. IV, choline], Rieländer [1908, Ch. I]) and commercial mushroom extract (Kutscher [1910, **4**; Ch. IV, choline]).

For a long time the only recorded instance of the occurrence of betaine in animals was Brieger's discovery of the base in mussels (*Mytilus edulis;* [1886, **1**, pp. 77-79; Ch. I)]. Later a number of other animal sources have become known: in commercial shrimp extract (Ackermann and Kutscher [1907, **3**]), in the muscles of *Acanthias vulgaris*, 2 per cent. in embryos, 0·07 per cent. in adults (Suwa [1909, **1**], Kutscher [1910, **3**]), in the crayfish, *Astacus fluviatilis* (Kutscher [1910, **2**]), in a cuttle-fish (*Octopus*) (Henze [1910]). A substance from the Japanese cuttle-fish *Ommastrephes* identified by Suzuki and Yoshimura [1909] as δ-amino-valeric acid is, according to Kutscher [1909], betaine. Betaine is also present in mammalia; Bebeschin [1911] isolated 0·05 per cent. of betaine from ox-kidneys.

Physiological Properties and Importance of Betaine.

The question as to whether betaine can be utilised by the animal organism as a source of nitrogen is of some interest on account of the increasing use of molasses as a cattle food. In the dog after intravenous injection nearly the whole of the betaine is rapidly excreted in the urine, but when given by the mouth only about one quarter is so excreted (Andrlik, Velich and Staněk [1902-3], Völtz [1907]). Ruminants are more able to decompose betaine; a cow accustomed to molasses excreted no betaine in its urine, and a sheep only during the first few days of feeding on molasses. Nevertheless, according to Völtz, the whole of the betaine nitrogen is excreted in sheep even when there is a deficiency of nitrogen in the food, and the organism only retains the non-nitrogenous part of the betaine.

Although betaine is therefore not a food, it appears to be quite harmless. Andrlik, Velich and Staněk for instance gave a rat intravenously betaine representing 0·24 per cent. of its body weight without any appreciable effect.

Riesser [1913; Ch. V, creatine] injected betaine into rabbits and thereby increased their muscular creatine content by 6·3-11·3 per cent. He thinks that betaine may condense with an equimolecular proportion of urea to form creatine and methyl alcohol. When betaine chloride is melted with an excess of urea, methyl alcohol is given off. See also pp. 77-78.

Waller and Sowton [1903; Ch. IV, choline] have described a toxic action of betaine in the excised frog's heart and on isolated nerves, and Waller and Plimmer [1903] on intravenous injection. According to Velich [1904-5] the effects observed were due to hydrochloric acid, owing to insufficient neutralisation of the betaine chloride injected. Further experiments (unpublished) by Waller and Plimmer showed that the injection of the betaine produced a slight lowering of the blood pressure, which allowed some of the magnesium sulphate solution, contained in the cannulæ, to enter the circulation and exert a toxic action. A slight effect on the frog's heart has also been noted by Kohlrausch [1909, 1911].

With regard to the physiological importance of betaine in plants, Staněk [1911, 1] has recently attempted to prove that the base is not a waste product. He has shown that more betaine is present in the leaves than in the seeds from which the plant has been grown; the sugar beet may contain as much as 1·2 per cent. of its dry weight as betaine. Schulze and Trier [1912, 1] have similarly found that betaine

is formed during germination in *Vicia sativa* and trigonelline in *Pisum sativum*. In a later paper Staněk [1911, **2**] has concluded that there is more betaine in the dry substance of the young leaves than in that of the old, that betaine is formed during the germination of the seeds and that it travels from the roots to the leaves during the sprouting; the base collects in the etiolated leaves and on ripening of the organs it disappears, probably because it travels back into the root. This latter conclusion is not shared by Schulze and Trier [1910, **1**] who consider betaine to be a waste product which no longer takes part in metabolism (see also Trier [1912, **3**, pp. 83-7; Ch. IV, choline]). These authors point out that yeast cannot utilise betaine as a source of nitrogen (Staněk and Miskovsky [1907]) and that betaines pass unchanged through the animal organism. Some other fungi do utilise betaine, however. Ehrlich and Lange [1913] have shown that, in contradistinction to ordinary cultivated yeasts, some wild yeasts like *Willia anomala* transform betaine to glycollic acid :—

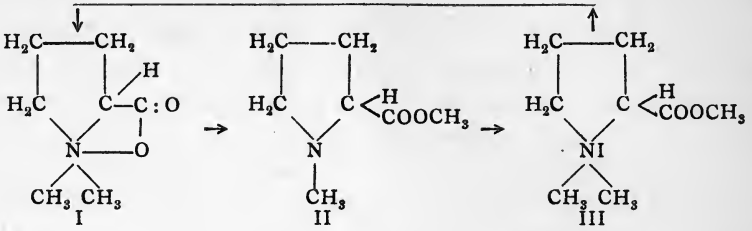
$$(CH_3)_3N \cdot CH_2 \cdot COO + H_2O = CH_2(OH) \cdot COOH + N(CH_3)_3$$

This is analogous to the change of primary amines, described on page 25. In any case it seems justifiable to draw the conclusion from Staněk's experiments that betaine occurs most abundantly in those parts of the plant where the vegetative processes are most active, and Schulze and Trier consider that betaines collect in young leaves because they are formed there. Young orange leaves also contain a greater proportion of stachydrine than the old ones.

Stachydrine, $C_7H_{13}O_2N$.

Von Planta [1890] discovered a base in the edible tubers of *Stachys tuberifera*. The base closely resembled betaine but yielded an aurichloride with a smaller gold content; it was further investigated by von Planta and Schulze [1893, **1**, **2**] who found it had the composition $C_7H_{13}O_2N$, and Jahns [1896] isolated the same base from the leaves of the orange tree (*Citrus vulgaris*) and proved the presence of a carboxyl-group. Stachydrine is also present in the flowers of *Chrysanthemum cinerariæfolium* and in *Galeopsis ochroleuca* (Yoshimura and Trier [1912]) and (with betonicine) in *Betonica officinalis* (Schulze and Trier [1912, **1**, section on betaine]). Stachydrine gives off dimethylamine on heating with potassium hydroxide, and since it contains two hydrogen atoms less than is required for a homologue of betaine, Jahns considered it to be dimethylamino-angelic acid. The base is, however, stable to potassium permanganate, and the deficiency of two

hydrogen atoms is not due to unsaturation but to ring formation; on heating, vapours are formed which give the pyrrole reaction with pine wood, and these facts led Schulze and Trier [1909, **2**; 1910, **2**] to regard the base as a derivative of *α*-pyrrolidine carboxylic acid (proline) which had meanwhile been recognised as a common fission product of proteins. They suggested for stachydrine the formula I, which was



I II III

soon afterwards also adopted by Engeland [1909, **3**] after comparing the properties of the methylation product of proline with those of stachydrine as given in the literature. Finally Trier [1910] converted stachydrine by distillation into the isomeric methyl ester of hygric acid [1] (formula II) and obtained stachydrine by hydrolysis of the methiodide of this ester (formula III); the methiodide had already been synthesised by Willstätter and Ettlinger [1903] starting from trimethylene dibromide and ethyl malonate.

Stachydrine, as obtained from most sources, is optically inactive, but Yoshimura and Trier [1912] have recently obtained the lævorotatory variety from *Galeopsis ochroleuca;* the base prepared by Engeland by methylating proline (from caseinogen) is optically active. Stachydrine has an unpleasant sweetish taste and is without marked physiological action; taken by the mouth it is excreted unchanged in the urine. The isomeric methyl ester of hygric acid on the other hand has a convulsant action (Trier [1910]).

Betonicine and Turicine, $C_7H_{13}O_3N$.

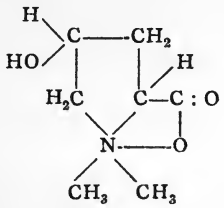
Schulze and Trier [1912, **1**, section on betaine] have found that in *Betonica officinalis*, a Labiate closely related to *Stachys*, stachydrine is accompanied by a base containing an additional oxygen atom, which base they have named *betonicine.* It is the N-dimethyl derivative of oxypyrrolidine-carboxylic acid (oxyproline), which occurs as a constituent of proteins.

The aurichloride $C_7H_{13}O_3N$, $HAuCl_4$ accompanies stachydrine auri-

[1] Hygric acid, or N-methyl α-pyrrolidine carboxylic acid, is an oxidation product of hygrine, an alkaloid accompanying cocaïne in *Coca* leaves.

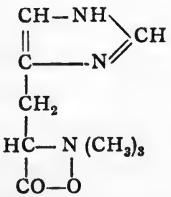
chloride, from which it is separated by its greater solubility in water; yield 5·5 grm. per kilo. of air dry *Herba Betonicæ*.

In a later paper Schulze and Trier [1912, **2**, section on betaine] state that another substance of the formula $C_7H_{13}O_3N$ is also present. Küng and Trier [1913] have shown that the latter base is dextro-rotatory and have named it *turicine*. It is the enantiomorph of betonicine, which is lævo-rotatory. Küng [1913] obtained both these bases by methylation of oxyproline from gelatin, so that they have the following constitution :—



Trimethylhistidine, $C_9H_{15}O_2N_3$.

A base of the above composition was isolated by Kutscher [1910, **4**] from the lysine fraction of a commercial mushroom extract and afterwards named hercynine. The base gave an intense red coloration with sodium p-diazobenzene sulphonate, but neither Millon's reaction nor any reaction for tryptophane. Only the aurichloride was prepared and of this the melting point was not given. Kutscher considered that the base was probably a trimethylhistidine and later Engeland and Kutscher [1912, **1**] showed its identity with the synthetic betaine obtained from α-chloro-β-iminazolyl-propionic acid and trimethylamine. The constitution is therefore

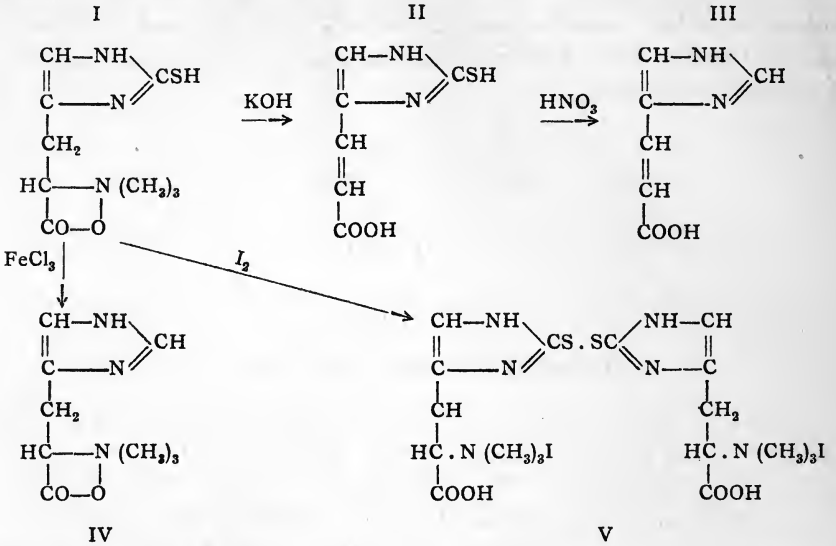


The same base was obtained more recently by Reuter [1912, Ch. I] from the arginine fraction of *Boletus edulis* (8 grm. of the monopicrate from 2½ kilos. of the dried fungus), and Barger and Ewins [1913] have shown that it is also formed by the oxidation of ergothioneine (see next section). The direct methylation of histidine with dimethylsulphate leads to the formation of a pentamethyl derivative, since the imino-group of the glyoxaline ring is also attacked (Engeland and Kutscher [1912, **2**]).

Ergothioneine, Thiolhistidine-betaine, $C_9H_{15}O_2N_3S$.

Tanret [1909] isolated from ergot a base of the composition $C_9H_{15}O_2N_3S$ and named it ergothioneine.

Barger and Ewins [1911] have shown it to be the betaine of thiolhistidine, as follows:—



On heating ergothioneine (I) with concentrated potassium hydroxide solution, trimethylamine was given off almost quantitatively and a yellow unsaturated acid (II) resulted, which still contained sulphur and was almost insoluble in water. On boiling this acid with dilute nitric acid, the sulphur was removed and iminazolylacrylic acid (III) was formed and identified by comparison with a synthetic specimen. This substance was subsequently shown by Hunter [1912; Ch. II, urocanic acid] to be identical with urocanic acid from dog's urine (see p. 36). On boiling ergothioneine with ferric chloride the betaine of histidine itself is formed (IV) (see previous section). On adding iodine in alcoholic solution two molecules combine to form the quaternary iodide (V) which is much less soluble than the salts of ergothioneine, to which it bears the same relationship as cystine does to cysteine. By reduction with hydrogen sulphide this iodide is reconverted into ergothioneine. The crystals of the dimeric iodide have the remarkable property of taking up excess of iodine from an aqueous solution and becoming steel grey or blue, like narceine and other substances.

The biochemical interest of ergothioneine is chiefly due to the sulphur atom contained in the glyoxaline ring. Oddly enough the

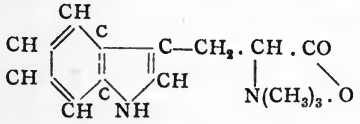
thiolglyoxalines are intermediate products in the chief method for synthesising glyoxalines, due to Gabriel. The sulphur of ergothioneine behaves very differently from that in cystine; it is not removed by alkalies and ergothioneine, therefore does not blacken lead hydroxide solution on boiling. On the other hand the sulphur atom is much more readily attacked by weak oxidising agents such as ferric chloride.

Bearing this in mind we may perhaps hope to isolate ergothioneine or similar sulphur compounds from sources other than ergot.

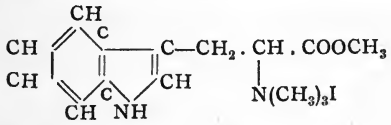
The physiological activity of ergothioneine is slight and it does not make any significant contribution to the action of ergot.

Hypaphorine, Trimethyltryptophane, $C_{14}H_{18}O_2N_2$.

Hypaphorine is the betaine of tryptophane and has the constitution



It was discovered by Greshoff [1898] in the seeds of *Erythrina Hypaphorus*, Boerl., a tree grown for the sake of its shade in the coffee plantations of Eastern Java, and known locally as "dadap minjak". The constitution of hypaphorine has been investigated by Van Romburgh [1911]. On heating with concentrated aqueous potassium hydroxide indole and trimethylamine result. The constitution was, however, determined by the synthesis from tryptophane (Van Romburgh and Barger [1911]). On heating tryptophane in methyl alcoholic solution with sodium hydroxide and methyl iodide the quaternary iodide of methyl-α-trimethylamino-β-indolepropionate is formed



and this, on warming with dilute alkali, yields a substance identical with the naturally occurring hypaphorine.

Physiological Action of Hypaphorine.—The substance has hardly any action on rodents and pigeons; thus intravenous doses of 0·5-1 grm. do not affect rabbits and the unchanged substance is rapidly secreted in the urine. In frogs, however, doses of 12-15 mg. produce increased reflex irritability and tetanus, lasting for days in non-fatal cases.

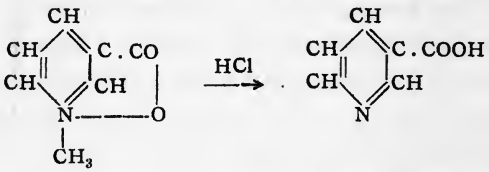
Trigonelline, $C_7H_7O_2N$.

This substance, the betaine of nicotinic acid, is not derived from a protein fission product; it contains a pyridine nucleus and is there-

fore to some extent more akin to the alkaloids. As it is however very similar to stachydrine, and as it has moreover been found in a number of species belonging to widely different natural orders, its inclusion here may be justified. Trigonelline was discovered by Jahns [1885] in the seeds of *Trigonella foenum graecum* (the Fenu greek). It has also been obtained from the seeds and seedlings of *Pisum sativum* (Schulze and Winterstein [1910]); from the seeds of *Phaseolus vulgaris*, *Cannabis sativa*, *Avena sativa* (Schulze [1896; Ch. IV, choline], *Strophanthus hispidus*, and *S. Kombé*, Thoms [1898, **1, 2**; Ch. IV, choline] and *Coffea arabica*, Polstorff [1909, **2**; Ch. IV, choline]); from the tubers of *Stachys tuberifera* and from potatoes (Schulze [1904; Ch. IV, choline]); from the roots of *Scorzonera hispanica* and the tubers of *Dahlia* (Schulze and Trier [1912, **1**; section on betaine]). It is generally present in very small quantity and will doubtless be found to occur in many more species.

Nicotinic acid, from which trigonelline is formed by methylation, occurs in rice polishings (Suzuki, Shimamura and Odake [1912], Funk [1913]; both references in Ch. VII, vitamine, oryzanin). When this acid is given to dogs, trigonelline appears in the urine (Ackermann [1912, **1**]).

The constitution of trigonelline was established by Jahns [1887].



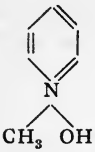
On heating with concentrated hydrochloric acid to 270° nicotinic (β-pyridine-carboxylic) acid was formed and trigonelline was shown to be identical with the "methylbetain" of nicotinic acid, previously synthesised by Hantzsch [1886].

Trigonelline is physiologically inert; given subcutaneously, 0·12 grm. had no effect on frogs, nor 0·5 grm. on rabbits (Jahns [1887]; compare also Kohlrausch [1909, 1911; section on betaine]). The methylation of nicotinic acid to trigonelline in the dog, discovered by Ackermann [1912, **1**], is similar to the methylation of pyridine to methylpyridinium hydroxide (see the next section).

Other Pyridine Bases.

Although they are not betaines, other derivatives of pyridine may be referred to here. For pyridine derivatives formed in putrefaction see Chapter I, p. 17.

His [1887] showed that when pyridine acetate is given by the mouth to dogs, about one quarter may be recovered from the urine as the quaternary base, methyl-pyridinium hydroxide.



The isolation was carried out by means of potassium mercuric iodide, and conversion into the gold and platinum salts. Kutscher and Lohmann [1906, **4**; section on butyro-betaine] obtained the same base from normal human urine (at first [1906, **3**] they mistook it for neurine). They [1907] consider that it is derived from the pyridine of tobacco smoke and of roasted coffee; 10 litres of men's urine yielded 0·17 grm. of the aurichloride, and 100 litres of women's urine 2·6 grm.; the greater content of women's urine they ascribe to the "bekannte Vorliebe der Frauen für pyridinhaltigen Kaffee". Roasted coffee beans contain 0·02 per cent. of pyridine [Bertrand and Weisweiller, 1913]. Methyl-pyridinium chloride has also been obtained from a commercial shrimp extract (Ackermann and Kutscher [1907, **4**; under betaine]). The physiological action was investigated by Kohlrausch [1909, 1911; under betaine]. The platinichloride $(C_6H_8N)_2PtCl_6$ forms large orange coloured plates, mp. 205-207°, little soluble in cold water, readily in hot, and the aurichloride $C_6H_8NAuCl_4$ yellow needles, mp. 252-253°, very little soluble in cold water.

Achelis and Kutscher [1907] obtained 0·7 grm. of γ-picoline aurichloride mp. 201° from 10 litres of horse urine. This salt has the same composition as the preceding and is said to be derived from pyridine derivatives of the fodder.

γ-n-Butyro-betaine, $(CH_3)_3 : N\begin{<}O\text{——}CO\\CH_2\text{—}CH_2\end{>}CH_2$

Among the ptomaines isolated by Brieger [1886, **1**, p. 27; Ch. I] from horse meat which had putrefied for four months, was a base $C_7H_{17}O_2N$. The chemical and physiological properties, as described by Brieger, correspond very closely with those of a betaine $C_7H_{15}O_2N$ obtained a few years ago by Takeda [1910] from the urine of dogs poisoned with phosphorus; Engeland and Kutscher [1910, 3] obtained Takeda's base by methylating γ-amino-butyric acid, so that there is no doubt as to its constitution; the identity with Brieger's base is almost equally certain, in which case his formula should contain two hydrogen atoms less.[1]

γ-Butyro-betaine was first synthesised by Willstätter [1902, **1**; under betaine] and was also obtained by Krimberg [1907, **2**] by the reduction of carnitine (see next section). Brieger isolated it from that part of the precipitate with mercuric chloride, which was the more soluble in water. After removal of the mercury, the base was precipitated as aurichloride.

The physiological action was studied in some detail by Brieger. On frogs it has a curare action, in accordance with the fact that it is a quaternary base and a γ-betaine. In the *α*-betaines so far described the

[1] Brieger's ptomaine and γ-butyro-betaine have a very similar composition, a gold salt of identical melting point, a soluble picrate and similar reactions to alkaloidal reagents: both arrest the frog's heart in diastole.

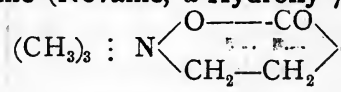
basic properties are more completely neutralised by the carboxyl-group, which is probably the reason for their physiological inertness (compare also the section on ω-amino-acids, p. 33). Brieger found that 10 mg. of his hydrochloride arrested the heart of a frog in diastole. In rabbits 0·05-0·3 grm. produced mydriasis, salivation, clonic convulsions, often violent lowering of body temperature, dyspnœa, paralysis and ultimately (after several hours) death with the heart in diastole (Brieger [1886, **1**, pp. 29-31 ; Ch. I]).

Brieger obtained two other bases of the composition $C_7H_{17}O_2N$. One of these is *gadinine*, obtained from putrid cod fish (Bocklisch [1885, Ch. I], Brieger [1885, **1**, p. 49 ; Ch. I]) and isolated as platinichloride. It "appeared" to be physiologically inert and the solution of the hydrochloride yielded a precipitate with picric acid, but not with gold chloride. Against these differences we may set the fact that the hydrochloride, like that of γ-butyro-betaine and of betaine itself, was insoluble in absolute alcohol.

The other base $C_7H_{17}O_2N$ is *typhotoxine*, obtained from cultures of typhoid bacilli (Brieger [1886, **1**, p. 86 ; Ch. I]). The melting point of the aurichloride was identical with that of the ptomaine from putrid horse meat (176°). Typhotoxine, however, yielded a sparingly soluble picrate, a yellow coloration with diazobenzene sulphonic acid, and amorphous precipitates with potassium tri-iodide, potassium mercuric iodide and potassium cadmium iodide. The physiological action of typhotoxine was also somewhat different from that of the ptomaine from putrid horse meat.

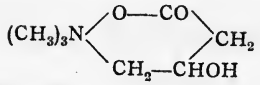
It does not seem wholly impossible, however, that all three bases were identical with γ-butyro-betaine.

Carnitine (Novaine, α-Hydroxy-γ-butyro-betaine),

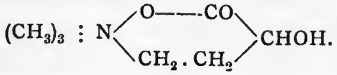


Carnitine, $C_7H_{15}O_3N$, is a hydroxy-derivative of the base described in the previous section and was discovered in extract of muscle by Gulewitsch and Krimberg [1905]. A few months later Kutscher [1905] obtained from Liebig's extract of meat a base "novain" which Krimberg [1908, **1**] proved to be identical with carnitine ; the identity has been admitted by Kutscher's pupils, if not explicitly by Kutscher himself. According to Kutscher a base $C_7H_{15}O_2N$, isolated by Dombrowski [1902] from normal human urine, was identical with novaine ; Kutscher thinks that in most cases (except in the dog) novaine passes into the urine as its reduction product reducto-novaine. Both carnitine and novaine were found by their discoverers to yield trimethylamine and crotonic acid (or an isomeride) on heating with baryta. By boiling with phosphorus and hydriodic acid Krimberg [1907, **2**] reduced carnitine to γ-butyro-betaine.

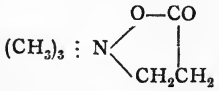
The only doubt now remaining was with regard to the position of the hydroxyl group in carnitine. Krimberg at first favoured the β-position, but β-hydroxy-γ-butyro-betaine



has been synthesised by Rollett [1910] and by Engeland [1910 **2**] and was found to differ from carnitine, which is therefore most likely *a*-hydroxy-γ-butyrobetaine



The *a*-position of the hydroxyl group seems also to result from the oxidation of carnitine by calcium permanganate (Engeland [1909, **1**]) to β-homobetaine

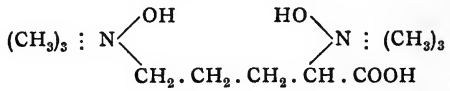


Racemic carnitine has probably been obtained by Fischer and Göddertz [1910] from γ-phthalimido-*a*-bromobutyric acid; the melting point of the platinichloride agrees with that of natural carnitine, but the aurichloride has a much higher melting point. Carnitine may be prepared from meat extract by Gulewitsch and Krimberg's method, or by that of Kutscher; the former method, in which the filtrate from carnosine is precipitated with potassium bismuth iodide, gives apparently the better yield (1·3 per cent. of the Liebig's extract employed). Smorodinzew [1913; Ch. II, carnosine] obtained 0·02 per cent. of carnitine from fresh horse meat. Carnitine probably passes unchanged into the urine, for Kutscher and Lohmann [1906, **2**] could isolate novaine (= carnitine) from the urine of a dog fed on meat extract but not from normal dog's urine. In the rabbit carnitine is, perhaps, reduced to butyrobetaine, according to Engeland [1908, **1**]. The physiological action of novaine (= carnitine) has been studied by Kutscher and Lohmann [1906, **1**]. One gram, given hypodermically to a cat, produced serious disturbance of the digestive tract; given intravenously novaine has a slight depressor action. *Oblitine*, a base obtained by Kutscher from meat extract, is according to Krimberg merely carnitine ethyl ester formed from carnitine during Kutscher's process of extraction (see appendix).

Reductonovaine $C_7H_{15}ON$ was isolated as the *aurichloride* $C_7H_{16}ONCl$, $AuCl_3$, mp. 155-180°, from women's urine by Kutscher [1907, **2**] who regards it as formed by loss of water from novaine to which it stands in the same relation as neurine to choline.

Myokynine (l-Hexamethylornithine ?), $C_{11}H_{28}O_4N_2$.

Working with Kutscher's method, Ackermann [1912, **2**] has isolated from the lysine fraction of an extract of dog's muscle a platinichloride $C_{11}H_{30}O_4N_2PtCl_6$, insoluble in ethyl alcohol, mp. 233-234°. The corresponding base was lævo-rotatory and gave off two molecular proportions of trimethylamine on heating with baryta. The composition of the platinichloride agrees with that of a platinum salt of hexamethylornithine with $2H_2O$. Hexamethylornithine was, therefore, prepared by methylating ornithine, and was found to be dextro-rotatory and to yield a platinichloride with $1H_2O$ melting at 232-233°. It is not unlikely, therefore, that myokynine is the enantiomorph of the synthetic base, having the constitution :—



Later Ackermann [1913, **1**] obtained 3 grm. of the same platinichloride from 30 kilos. of fresh *horse* meat. The base contains one carboxyl group. Unlike the natural base, synthetic hexamethylornithine gives a pyrrole reaction when heated with zinc dust. Ackermann points out that ornithine to some extent resembles glycine (compare the formation of ornithuric and hippuric acids); trimethyl-glycine or betaine has already been isolated from the muscles of a number of animals.

CHAPTER IV.

CHOLINE AND ALLIED SUBSTANCES.

THE previous chapters have dealt with basic substances derived from the amino-acid units of proteins by various modifications. We must next consider two bases which enter into the composition of the phosphatides; they are units or "Bausteine" of these compounds, and are analogous to the amino-acids (described in Plimmer's "Chemical Constitution of the Proteins"). One of these units, choline, is apparently present (in a combined form) in every living cell; the other, amino-ethyl alcohol, is probably the precursor of choline.

Allied to choline there are two bases, neurine and muscarine, which are derived from choline by dehydration and probably by esterification respectively. These bases do not enter into the composition of phosphatides; their physiological behaviour is different from that of choline; they are *modified* units and are therefore comparable to the modified amino-acids with which we have been concerned so far.

In this chapter are also included two other bases with pentavalent nitrogen and without a carboxyl-group; they are trimethylamine oxide and neosine; the latter is perhaps a homologue of choline.

Betaine is generally grouped with choline on account of a more or less accidental chemical connection, for it can be obtained in the laboratory by oxidising choline. There is, however, a considerable physiological difference between the two substances, for choline is a structural unit of phosphatides, but betaine plays no such part either in the phosphatide or in the protein molecule. Nor is a genetic relationship between the two substances apparent in the organism. It has been suggested that betaine is formed by the oxidation of choline, but recent work has made the conclusion almost inevitable that betaine is not formed in this way, but by the methylation of glycine (glycocoll), like the other betaines described in Chapter III. Choline and the substances derived from it further differ from the betaines in being strong bases, having a marked physiological action. To emphasise all these points of difference the two groups of substances are described in separate chapters.

Choline, Trimethyl-β-hydroxy-ethyl-ammonium Hydroxide,

$$(CH_3)_3 : N\begin{matrix}OH \\ CH_2 . CH_2OH.\end{matrix}$$

Strecker [1849] obtained from pig's bile the platinichloride of a base, of which he later [1862] published the formula and a further description, and which he then named *choline.* Meanwhile von Babo and Hirschbrunn [1852], by hydrolysis of the alkaloid sinapin from white mustard seeds, had prepared a strong base which was well characterised by its platinichloride and was named *sinkalin* (from *Sinapis* and alkali). The identity of the base from mustard with that from bile was established by Claus and Keesé [1867], but nevertheless Strecker's (later) name has passed into general use. Confusion was introduced when Liebreich [1865] obtained a base by the hydrolysis of the brain substance protagon, and termed it *neurin.* The analysis of an impure platinichloride led Liebreich to the erroneous formula $C_5H_{12}ON$, corresponding to vinyl-trimethyl-ammonium hydroxide, and to this substance the name neurine has become definitely attached. The identity of Liebreich's protagon base with choliné was established by Dybkowsky [1867] and for some years neurine was used as a synonym for choline, to which the name *bilineurine* was at one time also applied. The true formula of Liebreich's "neurin" was determined by Baeyer [1866, under neurine] who also converted it into the vinyl base [1869, under neurine], and "névrine" (= choline) was first synthesised by Wurtz [1867].

Since choline is a constituent of lecithin, it occurs probably in all living cells. It has been isolated by Schulze and his collaborators from every plant extract examined by them for its presence [Schulze and Trier, 1912, 3]. Choline has been found in the following tissues:—

In the brain: as phosphatide, Liebreich [1865], Gulewitsch [1908, 1], Vincent and Cramer [1904], Cramer [1904], Coriat [1904], Thudichum [1884, 1901; under amino-ethyl-alcohol]; it is not present in the free state, Kauffmann [1911]. In the cerebro-spinal fluid in disease (Mott and Halliburton [1899]; see below for an account of the controversy on this point). In many viscera (Kinoshita [1910, 2]), in the adrenal gland (Hunt [1899-1900], Lohmann [1907, 1911]), in the thymus, thyroid and lymphatic glands, and in the spleen (Schwarz and Lederer [1908]), in blood and in serum (Letsche [1907; Ch. IV, creatine], Gautrelet and Thomas [1909]), in ox testes (Totani [1910, 1]), in semen (Florence [1897]), in egg-yolk, the most convenient natural source (Diakonow [1868]), in autolysed pancreas (Kutscher and Lohmann [1903]), in meat extract (Kutscher [1906, 1; Ch. V, creatine]), in putrid horse meat (Gulewitsch [1884, Ch. I]), in human corpses (Brieger [1885, 2, p. 17; Ch. I]), in bile (Strecker [1849]), in secretine (von Fürth and Schwarz [1908]), in cheese (Winterstein [1904]), in herring brine (Bocklisch [1885, Ch. I]), in salted fish (Mörner

[1896, Ch. I]), in carnaubon, a glycerine free monophosphatide from ox kidney (Dunham and Jacobson [1910]), in sahidin (Fränkel and Linnert [1910]), from sinapin by hydrolysis (von Babo and Hirschbrunn [1852]), in seeds of *Vicia sativa* and *Pisum sativum* (Schulze [1890]), of *Strophanthus* (Thoms [1898, 1, 2]), of *Avena sativa* (Schulze and Pfenninger [1911]), in cotton seeds and beechnuts (Boehm [1885, 2]), in seeds of *Trigonella fœnum græcum* and of *Cannabis sativa* (Jahns [1885]), in seeds of *Artemisia cina* (Jahns [1893]), in etiolated seedlings of lupins and of *Cucurbita* (Schulze [1887]), in seedlings of *Soya hispida* (Schulze [1888]), in malt and wheat germs (Schulze and Frankfurt [1893]), in rice polishings (Funk [1911]), in potatoes and *Dahlia* tubers (Schulze [1904]), in tubers of *Stachys tuberifera* and in orange leaves (Schulze and Trier [1910, 2; Ch. III, stachydrine]), in beet molasses (von Lippmann [1887]), in roots of *Atropa Belladona*, *Hyoscyamus* and *Ipecacuanha* (Kunz [1885, 1887]), in bamboo shoots (Totani [1910, 2]), in the flowers of *Chrysanthemum cinerariæfolium* (Yoshimura and Trier [1912; Ch. III, stachydrine]), in *Areca* nuts, in pignuts (*Arachis hypogœa*) and in lentils (Jahns [1890]), in kola nuts (*Ilex paraguayensis*), Indian tea, and cocoa beans (Polstorff [1909, 2]), in hops and therefore in beer (Griess and Harrow [1885]), in grape juice and wine (Struve [1902]), in *Sesame*, *Cocos*, and palm seed press cake (Schulze [1896]), in the subterranean parts of *Brassica Napus*, *Helianthus tuberosus*, *Scorzonera hispanica*, *Cichorium Intybus*, *Apium graveolens*, *Daucus carota* and in the aerial parts of *Salvia pratensis* and *Betonica officinalis* (Schulze and Trier [1912, 3]), in ergot (Brieger [1886, 2; Ch. I], Kraft [1906], Rieländer [1908, Ch. I]), in *Amanita muscaria* (Harnack [1875; under muscarine]), in *Boletus luridus*, *Amanita pantherina* and *Helvella esculenta* (Boehm [1885, 1; under muscarine]), in *Cantharellus cibarius*, *Agaricus campestris*, and *Boletus edulis* (0·015-0·005 per cent.; Polstorff, [1909, 1]), in commercial mushroom extract (Kutscher [1910, 4]), in *Russula emetica* (Kobert [1892]) and in *Boletus satanas* (Utz [1905]).

The amount of choline obtainable from most sources is very small (in animal viscera and in seeds often of the order of 0·02 per cent.). Schulze considered that in seeds at least some of the choline is in the free state; he showed [1892, 1] that in *Vicia sativa* the choline content increases during germination from 0·017 per cent. in the seeds to 0·06 per cent. in the seedlings. The additional choline in the latter is derived from lecithin, of which the seeds contain 0·74 per cent., but four weeks' old seedlings only 0·19 per cent. We thus see that choline behaves in the same way as the amino-acids of protein, which are also formed by hydrolysis during germination. Betaine, which is also present in the seeds, on the other hand does not change in amount during germination, for it is not a unit or "Baustein".

The choline of the brain does not occur even partially in the free state. Liebreich [1865] obtained it by the hydrolysis of protagon; Gulewitsch [1899] found that at most one-fifteenth of the total amount is free choline, and Kauffmann [1911] has shown that if perfectly fresh ox brain is worked up rapidly, no free choline is obtainable. According to Coriat [1904] lecithin is not affected by trypsin or pepsin, but in autolysis choline is slowly split off by a ferment, which could not be isolated; during putrefaction choline is liberated more rapidly.

Mott and Halliburton [1899] found choline in the cerebro-

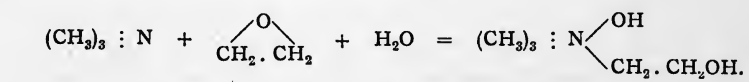
spinal fluid in certain degenerative nervous diseases, such as general paralysis of the insane, and they regard it as a break-down product of nerve substance. They used platinic chloride for the isolation, but since the amount of choline to be detected is at most very small, and since potassium and ammonium salts are also present, a good deal of controversy has taken place as to the identity of the platinichloride obtained.

Probably Mott and Halliburton's salt was contaminated with potassium, since even anhydrous alcohol, as employed by Donath [1905-1906], dissolves ammonium chloride. Donath has attempted to utilise the double refraction and chromatic polarisation of choline platinichloride which is not given by the isotropic crystals of the potassium and ammonium salts. The conclusions of Mott and Halliburton and of Donath have been criticised by Vincent and Cramer [1904], by Allen and French [1903] and by Mansfeld [1904]; Rosenheim [1905-6, 1907] and Allen [1904] have therefore attempted to find a more characteristic test in Florence's periodide reaction (see below) which may be applied to the platinichloride, or directly to the crude choline chloride.[1] According to Rosenheim and to Allen choline is indeed present in the cerebro-spinal fluid in certain diseases, but Donath's suggestion that choline is present in epilepsy and is the cause of the convulsions cannot be upheld (Allen [1904], Kajura [1908], and especially Handelsman [1908]). At most traces are present, wholly inadequate to account for the convulsions. Other authors, however, do not admit that choline has been demonstrated in the cerebro-spinal fluid even in diseases where there is a break-down of nervous tissue. Webster [1909] considers that no choline test hitherto employed is satisfactory. Kauffmann [1908, 1910] thinks that if traces of choline are present they are too small to be recognised with certainty. Kauffmann and Vorländer [1910] consider that the dimorphism of choline platinichloride (and conversion of the regular crystals into those of the monoclinic system, see below) affords a most characteristic test, and Kauffmann has concluded that an organic base is present in the cerebro-spinal fluid, which is not identical with choline. Stanford [1913] has recently arrived at the same conclusion, that the base present in disease gives alkaloidal reactions, but no trimethylamine. Handelsman [1908] has emphasised the fact that on igniting the platinichloride the odour of trimethylamine is never observed. It would appear that this controversy can only be ended by a satisfactory analysis of the platinum salt; the only published analysis (by Mott and Halliburton) is of little value (Pt found 34·8 per cent.; calculated 31·6 per cent.).

According to Mott and Halliburton the choline set free in nervous lesions passes into the blood, a conclusion shared by Allen [1904], criticised by Vincent and Cramer [1904] and particularly by Vincent's pupil Webster [1909], and maintained by Halliburton [1905].

Choline has been synthesised by several methods :—

1. By the action of trimethylamine on ethylene oxide in concentrated aqueous solution (Wurtz [1867]).



2. Trimethylamine combines with dry ethylene dibromide at 110-112° to yield trimethylamino-bromethylium bromide (Hofmann [1858, under neurine]).

[1] Possibly the very slight solubility of choline nitric acid ester perchlorate might be utilised with advantage.

$$(CH_3)_3 : N + Br.CH_2.CH_2.Br = (CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2.CH_2Br.\end{matrix}$$

By acting on the latter substance with silver oxide, Hofmann obtained the vinyl base instead of choline. Choline is however obtainable from it in two ways :—

(*a*) by boiling for eight days with silver nitrate (Bode [1892])

$$(CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2.CH_2.Br\end{matrix} + AgNO_3 + H_2O = (CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2.CH_2OH\end{matrix} + AgBr + HNO_3$$

(*b*) by heating with twenty-five parts of water to 160° for a few hours (Krüger and Bergell [1903])

$$(CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2.CH_2Br\end{matrix} + H_2O = (CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2.CH_2OH\end{matrix} + HBr.$$

3. Rather more than one equivalent of trimethylamine gas is passed into ethylene chlorohydrin cooled to − 12° to − 20° in a tube which is subsequently warmed to 80-90°; the yield is almost quantitative (Renshaw [1910]).

$$(CH_3)_3 : N + Cl.CH_2.CH_2OH = (CH_3)_3N\begin{matrix}\diagup Cl \\ \diagdown CH_2.CH_2OH.\end{matrix}$$

4. By the methylation of amino-ethyl alcohol (Trier [1912, **2**; under amino-ethyl-alcohol])

$$3(CH_3)I + 2NaOH + NH_2CH_2.CH_2OH = (CH_3)_3 : N\begin{matrix}\diagup I \\ \diagdown CH_2.CH_2OH\end{matrix} + 2NaI + 2H_2O.$$

The methods of Krüger and Bergell and of Renshaw appear to be the most convenient.

A method for the *estimation* of choline in animal tissues has been described by Kinoshita [1910, **2**]. For the isolation of choline from plant extracts, Jahns [1885] has employed potassium bismuth iodide (Kraut's reagent), Schulze has used phosphotungstic acid and mercuric chloride and Staněk utilises potassium tri-iodide. The two last named methods are more or less quantitative. Staněk's method [1905, 1906, **1, 2**] is the most convenient for the quantitative estimation of choline in the presence of betaine when other bases yielding periodides are absent (compare Kiesel [1907]). For a description of Staněk's and Schulze's methods see the appendix (Chapter VIII). The tests for, and chemical properties and salts of, choline are also described in the appendix (Chapter VIII).

Amino-ethyl Alcohol (Colamine) and the Origin of Choline; the Possible Presence of other Bases in Phosphatides.

By the hydrolysis of kephalin (a phosphatide from the brain) by means of baryta, Thudichum [1884, 1901] obtained long ago, in addition to choline, a base having the composition of "oxethylamin,"

$$NH_2 . CH_2 . CH_2 OH .$$

During the last few years Trier has isolated a base of the same composition from lecithin of various sources and has definitely identified it as hydroxy-ethylamine or amino-ethyl alcohol. By hydrolysis of the phosphatide from beans (*Phaseolus vulgaris*) Trier [1911] obtained a fraction, representing one-seventh of the nitrogen content of the phosphatide, which yielded an aurichloride $C_2H_5ON . HAuCl_4$, identical with that of a base previously synthesised by Knorr from ammonia and ethylene oxide:—

$$NH_3 + \begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix} \!\!>\!\! O = \begin{matrix} CH_2OH \\ | \\ CH_2NH_2. \end{matrix}$$

The same base was subsequently obtained from the lecithin of peas and oats and also from commercial ovolecithin of Merck (Trier [1912, 1]).

The amino-ethyl alcohol can be estimated in phosphatides by means of Van Slyke's method (see Plimmer's "Chemical Constitution of the Proteins," Part I, p. 69). Trier [1913, 2] concludes from this that the base is joined to the rest of the phosphatide molecule by means of its hydroxyl group. In one specimen of ovolecithin the amino-nitrogen was nearly half the total.

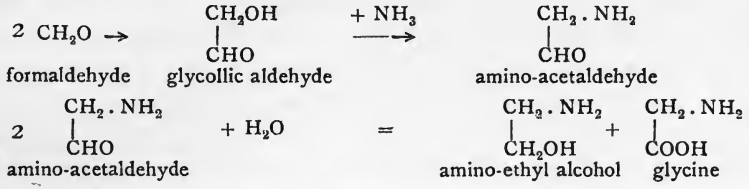
Baumann [1913] and Renall [1913] also used Van Slyke's method and showed that kephalin from human brain and from that of the sheep and ox contains as only base amino-ethyl alcohol and that here too the primary amino-group is free. They could not find choline and another base, which Thudichum believed to accompany the amino-ethyl alcohol.

Trier considers that choline is formed from amino-ethyl alcohol by the biologically common process of methylation, in the same way that the betaines are derived from amino-acids. Thus there would be no genetic relationship between choline and betaine.

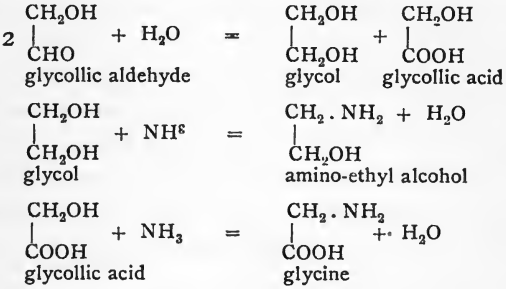
The question is then: How is amino-ethyl alcohol itself formed? Winterstein and Trier [1909, p. 311][1] put forward the hypothesis that formaldehyde is condensed to glycollic aldehyde and that the latter is converted by ammonia into amino-acetaldehyde. By simul-

[1] This and the subsequent references in this section will be found in the bibliography under choline.

taneous oxidation and reduction (Cannizzaro's reaction) amino-ethyl alcohol and amino-acetic acid (glycine) are then supposed to be formed from the aldehyde.



In his recent book on the simple plant bases Trier [1912, 3, p. 33] has modified the above hypothesis and imagines that glycollic aldehyde first undergoes Cannizzaro's reaction and that the two products of this reaction (glycol and glycollic acid) then condense with ammonia



Amino-ethyl alcohol and glycine are the simplest units for the formation of proteins and phosphatides respectively, and hence it becomes intelligible why, as Stoklasa has pointed out, protein and lecithin formation are two parallel processes. An argument for the biological significance of Cannizzaro's reaction is the occurrence of a number of alcohols as esters of the *corresponding* acid (e.g. benzyl benzoate and cinnamyl cinnamate in balsams ; cetyl-palmitate $C_{16}H_{31}O_2 . C_{16}H_{33}$ occurs in spermaceti and ceryl cerotinate $C_{27}H_{53}O_2 . C_{27}H_{55}$ in Chinese wax).

A ferment causing Cannizzaro's reaction ("aldehyde mutase") has been recently found in liver extracts by Parnas and by Batelli and Stern (see Dakin's "Oxidations and Reductions in the Animal Body," pp. 105, 106, in this series of monographs).

In addition to Thudichum, Trier, and Baumann, who isolated amino-ethyl alcohol, other investigators have suggested that phosphatides may contain bases similar to choline but containing fewer alkyl groups. These investigations however require careful scrutiny in the light of recent knowledge. Koch [1902] applied Herzig and Meyer's method for the estimation of N-methyl groups to kephalin and cerebrin and concluded that one N-methyl group is present in kephalin none in cerebrin, and three in lecithin. Fränkel and Neubauer, like

Koch, failed to isolate Thudichum's non-methylated "ox-ethylamin" from kephalin, and agreed with Koch that one N-methyl group is present. Fränkel and Linnert [1910] state that sahidin, from human brain, also contains a base with fewer methyl groups than choline. On the other hand Cousin [1907] could only obtain choline from kephalin. Koch, and Fränkel and Neubauer did not isolate their supposed monomethylated base and their results have been criticised by Baumann [1913]; he and Trier [1913, 5] find that amino-ethyl alcohol, when heated with hydriodic acid, gives off some ethyl iodide, thus simulating the presence of an N-methyl group. It should further be remembered that the accuracy of Herzig and Meyer's method for determining N-alkyl groups is not sufficiently great for the certain determination of their number in a molecule of the size of lecithin, and that its application becomes wholly illusory if more than one base is present.

Further mention of the presence in phosphatides of bases other than choline is to be found in papers by Erlandsen [1907] (on cuorin from ox hearts), by Baskoff [1908] (on the phosphatides of horse liver), by MacLean [1909], by Njegovan [1911], and in Trier's book on plant bases [1912, **3**, pp. 96-101]. According to Trier, Njegovan's base "vidine" was merely choline containing a little ammonia as impurity.

Neurine, Vinyltrimethyl-ammonium Hydroxide,

$$(CH_3)_3 : N\begin{matrix}\diagup OH \\ \diagdown CH : CH_2.\end{matrix}$$

Neurine was the name applied by Liebreich to a base obtained in the hydrolysis of protagon. Baeyer [1866] found that Liebreich's neurine yielded a mixture of platinichlorides, difficult to separate, but by means of the aurichlorides he subsequently [1869] showed that the principal base was identical with Strecker's choline. For the other base, which Baeyer obtained pure by the elimination of water from choline by chemical means, he reserved the name neurine, and Brieger [1885, **1**, p. 32] sharply differentiated the two bases; for a time much confusion was introduced by the continued use, by some authors, of neurine as a synonym for choline, but eventually the term neurine was restricted to the unsaturated base.

According to Gulewitsch [1899, under choline] protagon does not yield neurine at all, but only choline. It is very doubtful whether neurine occurs in the body or body fluids, and apart from the old confusion of nomenclature, statements concerning its presence should be

carefully scrutinised.[1] Neurine occurs as a product of putrefaction and was isolated by Brieger [1885, **1**, pp. 25-39] from putrid meat (horse, ox, human corpses). Brieger studied the physiological action of neurine in some detail and naturally assumed that the base was formed from choline by bacterial action. This assumption has never been proved rigidly, but the possibility should be taken into account with reference to Kutscher's alleged discovery [1905; Ch. V, creatine] of neurine in commercial meat extract. Krimberg [1906, **1**; Ch. V, methylguanidine] could not find neurine in an extract of perfectly fresh meat and concludes [1908, **2**; Ch. III, carnitine] that it is not present in muscle. Lohmann [1909] obtained neurine from the supra-renal gland, but here again it is not clear to what extent sterility was ensured. Brieger [1885, **1**, p. 61] obtained neurine from fresh human brain by hydrolysis with hydrochloric acid.

Neurine is most readily obtained synthetically and was first prepared by Hofmann [1858] nine years before the synthesis of choline by Wurtz. Hofmann treated the condensation product of trimethylamine and ethylene dibromide with moist silver oxide, which removes hydrobromic acid, and forms neurine bromide :—

$$(CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH_2 \cdot CH_2Br\end{matrix} + AgOH = (CH_3)_3 : N\begin{matrix}\diagup Br \\ \diagdown CH : CH_2\end{matrix} + AgBr + H_2O.$$

Baeyer [1869] prepared neurine from choline by heating the latter with concentrated hydriodic acid and then treating the resulting iodo-compound with silver oxide as in Hofmann's synthesis. Neurine is perhaps also formed from choline by boiling with concentrated baryta and this may have caused it to accompany choline in Liebreich's hydrolysis of protagon. According to Brieger [1885, **1**, pp. 33, 34] neurine appears to be formed from choline by long standing in aqueous solution.

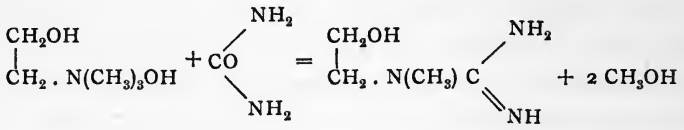
Physiological Action of Choline and of Neurine.

When given subcutaneously or by the mouth to rabbits in doses of 1 grm., choline produces no severe symptoms and is not excreted in the urine (von Hoesslin [1906]). Riesser [1913; Ch. V, creatine] found that rabbits often withstood a daily injection of 0·5-1 grm. choline. Similarly the urine of rabbits, fed on lecithin, does not contain choline, but only a little glycero-phosphoric and formic acids

[1] Thus Kutscher and Lohmann's statement [1906, **2**, under choline] that neurine occurs in human urine has passed into the literature ("Biochemisches Handlexicon"), although these authors subsequently [1906, **4**; Ch. V, methylguanidine] stated that their supposed gold salt of neurine was in reality methylpyridyl ammonium aurichloride.

(Franchini [1908]). Muscarine, neurine and betaine, on the other hand, are at least partially eliminated in the urine, and in this respect choline behaves like an amino-acid unit of protein. Whether choline is oxidised or whether it is synthesised into phosphatides is not known, but the latter alternative is in agreement with the conception of choline as a unit (Baustein) of phosphatides. The formation of choline in seedlings has been referred to above and its behaviour towards micro-organisms is mentioned in the appendix.

Riesser [1913; Ch. V, creatine] has recently carried out some experiments which suggest that choline, when injected subcutaneously, may be partially converted into creatine. In some rabbits he increased the muscular creatine content 10-15 per cent. by this means. Riesser supposes that choline condenses with urea according to the following equation :—



and that the alcoholic group of the condensation product is then oxidised to a carboxyl group, yielding creatine. The choline must therefore lose some of its methyl groups, and in support of this theory Riesser quotes an experiment in which choline chloride is carefully heated with sodium tellurite and sodium formate (the latter salt acting as a reducing agent); the garlick-like smell of methyl telluride is produced; see also p. 77.

The physiological action of *choline* has been studied by Gaehtgens, and by Boehm [1885, **2**] who observed salivation, myosis, and diastolic arrest of the heart; in frogs Boehm obtained general paralysis with 0·025-0·1 grm.; in mammals 0·01-0·02 grm. injected intravenously gave a rise of blood pressure. The action is somewhat analogous to that of pseudo-muscarine (synthetic "muscarine"). Brieger [1885, **1**, p. 38] found that the toxic action of choline is inhibited by atropine ("in präcisester Weise").

A detailed study of the action was made by Mott and Halliburton [1899], who found that small doses of choline injected intravenously cause a fall of blood pressure, but after a preliminary dose of atropine a rise occurs.

The antagonism between choline and atropine has been confirmed by all subsequent investigators, but a good deal of confusion and controversy has resulted from a statement by Modrakowski [1908] that pure choline always produces a rise of blood pressure and that the

depressent action observed by others was the result of an impurity. Popielski [1910, 1], in whose laboratory Modrakowski carried out his experiments, shares the latter's views, but Mott and Halliburton's statement that choline has primarily a depressent action has been confirmed by Busquet and Pachon [1909], Abderhalden and Müller [1910, 1911], Mendel and Underhill [1910], Pal [1910, 1911], Müller [1910], Lohmann [1907, 1908], and most recently by Mendel, Underhill and Renshaw [1912].

The general conclusion is that Modrakowski's and Popielski's aberrant results are not to be explained by impurities in the choline employed by others, but rather to differences in anæsthesia and dosage. With small doses up to 1 mg. per kilo. in dogs and cats under ether or urethane, a fall of blood pressure always results, which with somewhat large doses may be followed by a slight rise. Larger doses, especially when repeated, may at once exert a pressor action. With slight anæsthesia, or with the medulla oblongata cut, small doses may also produce a rise of blood pressure.

The depressent action is partly due to an effect on the heart and partly to vaso-dilatation in the limbs and splanchnic area. After atropine, perfusion of an isolated organ produces only vaso-constriction. According to Müller this vaso-motor reversal depends on a paralysis by atropine of the dilator elements of the vascular walls, and resembles the adrenaline vaso-motor reversal by ergotoxine (Dale [1906, Ch. VI]).

Choline has a stimulant effect on the isolated muscle of the intestine, uterus and iris, resembling in this respect physostigmine somewhat closely. It further stimulates the secretion of the lachrymal, salivary, and sweat glands. Salivation is one of the first symptoms of choline poisoning in an intact animal (Brieger). The physiological activity of choline is, however, slight, only about $\frac{1}{10}$–$\frac{1}{20}$ of that of neurine. The minimal lethal dose for rabbits of 1 kilo. is 0·5 grm. according to Brieger, but Mott and Halliburton were unable to kill an animal by choline injections. Compare also Riesser [1913; Ch. V, creatine].

The action of choline on isolated nerves and the excised heart of the frog has been studied by Waller and Sowton [1903].

Hunt and Taveau [1911] have studied the action of a large number of synthetic choline-like substances and their derivatives. In particular acetyl-choline

$$(CH_3)_3\,N(OH)\,.\,CH_2\,.\,CH_2\,.\,O\,.\,OC\,.\,CH_3$$

is remarkable in being 100,000 times as depressent as choline itself. According to Mr. A. J. Ewins [Bio-Chem. J., 1914, 8, 44] acetyl choline is present in small quantity in some ergot extracts. The lower homologue formocholine $(CH_3)_3\,N(OH)\,.\,CH_2OH$ is also more active than choline. The nitrous acid ester of choline is identical with Schmiedeberg and Harnack's *pseudo*-muscarine (see p. 68).

Other synthetic substances allied to choline have been described by Schmidt [1891, 1904, 1, 2], Malengreau and Lebailly [1910, under homocholine], Menge [1911], and Berlin [1910, 1, 1911, under homocholine] who gives further literature.

The action of *neurine* shows a general resemblance to that of choline and muscarine, and like these, it is antagonised by atropine. To rabbits it is 10-20 times as toxic as choline (Brieger [1885, 1, p. 39]); on subcutaneous injection the lethal dose is about 40 mg. per kilo. Cats are more susceptible and react violently to doses of a few milligrams. The effects are profuse salivation, dyspnœa, an initial acceleration and then a retardation of the heart beat and death in diastole; the intestine is stimulated to violent peristalsis; there is often myosis in rabbits and always in cats. Atropine is a powerful antidote. In frogs there is a curare-like paralysis and diastolic arrest of the heart's action, after injection of 1-2 mg. into the dorsal lymph sac.

Waller and Sowton [1903] studied the effect of neurine and other bases on isolated nerves and on the excised heart of the frog; neurine was the most toxic, rather more than muscarine, and very much more so than choline.

Lohmann [1911] finds that neurine in doses of 10 mg. first lowers the blood pressure of rabbits and then raises it. The general effect of neurine on the blood pressure is to produce a rise after a preliminary fall (Mott and Halliburton [1899]; Pal [1911]). Minute doses, of $\frac{1}{1000}$ mg., may be either pressor or depressor. The rise of blood pressure is due to constriction of the peripheral vessels (compare Samelson [1911] who found, by the Laewen-Trendelenburg method, that neurine acts on the frog's limb in a dilution of 1 : 800,000). The physiological action of synthetic bases allied to neurine has been described by Schmidt [1891, 1904, 1].

Natural and Synthetic Muscarines and their Physiological Action.[1]

Muscarine is the name given by Schmiedeberg and Koppe [1869] to an extremely poisonous base which they obtained from *Amanita muscaria* (the Fly Agaric). Very small amounts arrest the frog's heart in diastole and the action is antagonised by atropine.

Other bases of somewhat similar composition and similar physiological action have been obtained synthetically, and one of these was at one time considered to be identical with natural muscarine. It seems certain, however, that this is not so.

[1] Compare the important addendum on p. 68.

Schmiedeberg's base was isolated as the gold salt which Harnack [1875] found to be contaminated with choline ("amanitine") aurichloride; a separation was effected by crystallisation from hot water, the muscarine salt being the more soluble. Harnack found muscarine aurichloride to have the composition $C_5H_{14}O_2N \cdot AuCl_4$; the base therefore differs from choline in having an additional oxygen atom. Soon afterwards Schmiedeberg and Harnack [1877] obtained a base of this composition by heating dried choline chloride with concentrated nitric acid on the water bath; the new base was isolated as the platinichloride; the chloride, when left in a desiccator, sets to a crystalline mass and the base has according to Schmiedeberg and Harnack the constitution $(CH_3)_3 : NCl \cdot CH_2 \cdot CH(OH)_2$, being therefore a hydrated aldehyde like chloral hydrate (but compare addendum, p. 68).

This synthetic, artificial, or *pseudo*-muscarine is chemically very similar to the natural substance, and the physiological resemblance is sufficiently close to have induced Schmiedeberg and Harnack to believe in the identity of the two bases. Boehm [1885, **2**] was the first to point out the differences in the physiological action. He found that $\frac{1}{2}$ mg. of *pseudo*-muscarine (from choline) was required to stop the frog's heart in diastole, whereas the corresponding dose of natural muscarine is only $\frac{1}{20}$-$\frac{1}{30}$ mg., according to Schmiedeberg and Harnack. Recently this large difference in the activities of the two bases has been confirmed in Schmiedeberg's laboratory by Honda [1911], who again prepared natural muscarine and found it active on the frog's heart in doses of $\frac{1}{20}$-$\frac{1}{10}$ mg., according to the season of the year, whereas the same effect was only produced by $\frac{1}{2}$-$1\frac{1}{2}$ mg. of *pseudo*-muscarine from choline. Boehm further found that in larger doses (10 mg.) *pseudo*-muscarine produces a curare-effect in mammals, which is not given even by large doses of the natural base; moreover there is no complete antagonism between *pseudo*-muscarine and atropine: cats which have been poisoned by *pseudo*-muscarine cannot be kept alive by a subsequent dose of atropine. The curare-like action of *pseudo*-muscarine on frogs is according to Boehm fifty times as great as that of choline from which it is derived (the minimal paralytic doses being 0·1 and 50 mg.), and according to Honda [1911] *pseudo*-muscarine has one-fifth of the activity of pure curarine in this respect. According to H. Meyer (see below) *pseudo*-muscarine causes contraction of the pupil in birds, natural muscarine does not.

Another synthetic substance, much more distantly related to muscarine than the oxidation product of choline, is trimethylamino-

acetaldehyde, $(CH_3)_3 : N(OH) . CH_2 . CHO$, which was first prepared by Berlinerblau [1884] by the action of trimethylamine on monochloracetal and subsequent hydrolysis, and later by Fischer [1893] by the methylation of acetalamine. The platinichloride has the composition $[(CH_3)_3N . CH_2 . COH]_2PtCl_6 . 2H_2O$; the water of crystallisation is given off at 105°. The constitution of this base is quite certain, for Fischer [1894] oxidised it to betaine and accordingly suggested for it the name betaine aldehyde. In an abstract of a dissertation by Nothnagel [1893], E. Schmidt [1904, **1**, p. 47, under choline] quotes a report by Hans Meyer, who found that the anhydro-muscarine of Berlinerblau (= betaine aldehyde of Fischer) does not arrest the action of the frog's heart in doses of 10 mg., nor does it produce vagus inhibition in the mammalian heart in doses of several centigrams. It causes salivation and sweating, however, and kills by respiratory paralysis. Betaine aldehyde differs also chemically from muscarine, but on the other hand natural muscarine and Schmiedeberg and Harnack's *pseudo*-muscarine are chemically very similar, according to Schmidt and Nothnagel. The platinichlorides of both bases have the composition

$$[(CH_3)_3N . CH_2 . CH(OH)_2]_2PtCl_6 . 2H_2O$$

and do not lose water at 100°. The physiological differences observed by Boehm were however also found by Hans Meyer; *pseudo*-muscarine in doses of 0·1-0·05 mg. paralyses the intra-muscular nerve-endings of a frog; natural muscarine does not. The cardiac effect of the natural base, even in doses of 6 mg., is counteracted by atropine, but this is not so with *pseudo*-muscarine. Natural muscarine does not affect the pupil of birds, but maximal myosis is produced by a 1 per cent. solution of *pseudo*-muscarine.

Schmidt has suggested that the physiological differences may be due to stereo-isomerism, but in this case the relationship cannot be that between an optically active and a racemic modification, for then the one variety could not be 10-15 times as active as the other.

Further investigation of the chemical properties of natural muscarine is very desirable, but the base is unfortunately difficult to obtain in sufficient quantity. Schmiedeberg's process of isolation was a complicated one, and Harmsen [1903] calculates from physiological data that Schmiedeberg only isolated about 6 per cent. of the muscarine present in the fungus. According to Harmsen 100 grm. of fresh fungus (= 5 grm. of dried material) contain about 16 mg. of muscarine. The amount seems, however, to be very variable, as does also the amount of choline which accompanies the muscarine. The chief difficulty in isolating natural muscarine is the separation from choline.

Honda [1911] first separates a good deal of the latter base by means of its acid tartrate, which is less soluble than the muscarine salt. The discovery of a muscarine salt which is less soluble than the corresponding choline salt would greatly facilitate the preparation of pure muscarine.

The fate of *pseudo*-muscarine (from choline) in the animal organism has been investigated by Führer [1908, **1**; 1909]. The lethal dose for rabbits of 1·5 kilo. is 0·3-0·5 grm. by the mouth and 0·04-0·05 grm. subcutaneously; the drug is partly secreted in the urine unchanged (in the toad the whole is so excreted). In this respect *pseudo*-muscarine resembles betaine and differs from choline; it is not a "Baustein".

Harmsen has concluded that the muscarine content of *Amanita muscaria* is quite insufficient to account for the poisonous effects of eating this fungus and considers that the effect is mainly due to a complex toxin insoluble in alcohol and not counteracted by atropine. From an allied species *Amanita phalloides*, Abel and Ford [1906] have prepared a hæmolysin which they regard as a nitrogenous glucoside.

Muscarine occurs in small quantity in *Amanita pantherina* and in *Boletus luridus* (Boehm [1885, **1**, under choline]). Brieger [1885, **1**, p. 48, Ch. I] isolated from putrid codfish a platinichloride $(C_5H_{14}O_2N)_2\,PtCl_6$; the physiological action of the base was that of muscarine. The physiological action of synthetic bases allied to muscarine has been described by Schmidt [1891, 1904, **1**, under choline]; Brabant [1913] has recently synthesised β-homo-muscarine $(CH_3)_3N(OH)CH_2 . CH_2 . CHO$.

Trimethylamine Oxide, $(CH_3)_3NO$.

This base, the only member of its class known to occur naturally, was isolated by Suwa [1909, **1**, **2**] from the muscles of *Acanthias vulgaris*. One dozen of this fish, yielding 23 kilos. of muscle, gave 20 grm. of the hydrochloride of trimethylamine oxide, together with a quantity of betaine, but hardly any creatine, or creatinine.

The hydrochloride melts at 205-210°, the *picrate* forms thin needles, mp. 197°, sparingly soluble in ethyl alcohol and cold water; the *platinichloride* forms rhombic leaflets, mp. 214°; the *aurichloride* $C_3H_9ON . HAuCl_4$, mp. 250°, is sparingly soluble in hot water.

In concentrated aqueous solutions of the hydrochloride alcoholic solutions of mercury and cadmium chlorides precipitate $C_3H_{10}ONCl . 4HgCl_2 . H_2O$ and $C_3H_{10}ONCl . CdCl_2$ respectively.

By putrefaction and also (at least in part) in the organism of the rabbit, trimethylamine oxide is reduced to trimethylamine from which it can be produced by oxidation with hydrogen peroxide.

Neosine, $C_6H_{17}O_2N$.

There is still a good deal of doubt concerning the nature of this base, one of those obtained by Kutscher [1905, Ch. V, creatine] from extract of meat. Krimberg [1906, 1, Ch. V, methylguanidine] could not find neosine in fresh meat and doubted whether it is present in faultless meat extract. Ackermann and Kutscher [1907, 4, Ch. III, betaine] afterwards isolated the base from a commercial extract of shrimps which is the most abundant source. They [1908] found that trimethylamine is given off on heating and accordingly surmised that neosine is a homologue of choline, but various attempts to identify it with synthetic choline homologues have failed, including the most recent and thorough attempt of Berlin [1911] who found that Kutscher's neosine was contaminated with choline.

The uncertainty with regard to this base is shown by the various melting points ascribed to the *aurichloride*. Kutscher found 202-205°; Kutscher and Ackermann 205°; Engeland [1908, 1] for the base from meat extract 150-152°; Berlin, after freeing the crude neosine from choline, obtained a few grams of a gold salt melting at 244-245° from 6 kilos. of Liebig's extract of meat.

Berlin has also reinvestigated the synthetic homocholines of previous authors and concludes that Morley, Weiss, Partheil and more recently Malengreau and Lebailly [1910] obtained β-homocholine $(CH_3)_3N(OH) . CH_2 . CHOH . CH_3$ of which the aurichloride melts at 163-164°.

By the action of trimethylamine on trimethylene chlorohydrin $CH_2Cl . CH_2 . CH_2OH$ and (less readily) by the methylation of γ-amino-propylalcohol Berlin [1910, 2, 1911] prepared γ-homocholine $(CH_3)_3N(OH) . CH_2 . CH_2 . CH_2OH$ which yields an aurichloride crystallising in leaflets and melting at 193°, a mercurichloride $C_6H_{16}ONCl . 6HgCl_2$, mp. 208°, and a picrate exploding at 255°. The constitution of this base follows from its oxidation to homo-betaine $(CH_3)_3N(OH) . CH_2 . CH_2 . COOH$, and since it does not contain an asymmetric carbon atom, neosine, which is optically inactive, was at first regarded as identical with it. But the melting points of neosine aurichloride (244-245°) and of *neosine mercuric chloride* $C_6H_{16}ONCl . 6HgCl_2$ (252°) render this hypothesis untenable. The physiological action of γ-homocholine is similar to that of choline but slightly more intense (Berlin [1910, 1, 1911]).

Addendum to Muscarine.

While this book was in the press Dr. H. H. Dale and Mr. A. J. Ewins have, according to a private communication, established that the *pseudo*-muscarine of Schmiedeberg and Harnack and of Schmidt and Nothnagel is not an aldehyde at all, but the nitrous acid ester of choline. The platinichloride has the formula $[(CH_3)_3N . CH_2 . CH_2ONO]_2PtCl_6$, instead of $[(CH_3)_3N . CH_2 . CH(OH)_2]_2PtCl_6 . 2H_2O$. This explains why no water of crystallisation is given off at 100°; the loss of weight at 130° is due to decomposition. The percentage composition required by the two formulæ is very similar, except as regards nitrogen, the estimation of which presents difficulties here. This discovery further disposes of the inherent improbability that two hydroxyls should be attached to the same carbon atom; such an arrangement has so far only been observed in compounds in which the carbon atom is attached to negative groups, as in chloral hydrate, mesoxalic acid and triketohydrindene hydrate. An analogy for the great modification of the physiological action of choline by esterification is to be found in the case of acetyl choline, p. 63, and of the nitric acid ester, p. 153. In its action the latter, according to Dale and Ewins, resembles natural muscarine even more closely than does the nitrous acid ester. (Comp. Proc. Physiol. Soc., March 14, 1914.)

CHAPTER V.

CREATINE AND CREATININE, GLYCOCYAMINE AND GUANIDINES.

A. Creatine and Creatinine.

Creatine was described and named as long ago as 1835, by Chevreul [1835], in a report to the French Academy of Sciences on commercial meat extracts. Chevreul did not analyse the substance, but noticed its resemblance to asparagine. Berzelius later failed to prepare creatine, but Wöhler succeeded, and when Schlossberger [1844] obtained the same substance from the muscles of an alligator, its importance as a general constituent of muscle was recognised.

Our detailed knowledge of creatine dates from Liebig's classical investigation of the constituents of muscle juice [1847]. Liebig prepared creatine from the flesh of various animals, analysed it and converted it into its anhydride which he named creatinine and found to be identical with a substance isolated three years previously from urine by Pettenkofer [1844]. By boiling creatine with baryta, Liebig further obtained a new substance, sarcosine. Dessaignes [1854, 1855] showed that creatine is oxidised by mercuric oxide to methyl-guanidine ("methyl-uramine"). Sarcosine was synthesised by Volhard, who obtained creatine from it [1868].

Our physiological knowledge of creatine and creatinine did not advance so rapidly as the chemical, largely perhaps owing to the want of a convenient and accurate method of estimation. Such a method was, however, supplied by Folin in 1904, and this, together with his theory of metabolism, has led during recent years to many investigations on the physiology of creatine and creatinine.

Creatine was synthesised by Volhard [1868] by the action of cyanamide on sarcosine in alcoholic solution at 100°.

$$\begin{array}{l} CH_2 . NH . CH_3 \\ | \\ COOH \end{array} + CN . NH_2 = \begin{array}{l} CH_2 . N\!\begin{array}{l}\diagup CH_3 \\ \diagdown C(:NH)NH_2\end{array} \\ | \\ COOH \end{array}$$

Horbaczewski [1885] also obtained it by heating sarcosine with guanidine carbonate to 140-160°. The necessary sarcosine may be obtained by the hydrolysis of caffeine, but neither of these syntheses is so convenient as the preparation from natural sources.

Creatine and creatinine are interconvertible. The change from the former to the latter substance can be brought about quantitatively by heating with acid or even without a solvent (see appendix).

According to Gottlieb and Stangassinger [1907, 1908, 1] creatine is also converted into creatinine by autolytic ferments. The hydration of creatinine to creatine is brought about (partially) by alkalies; for instance by standing for a long time in solution in lime water (Liebig), ammonia (Dessaignes), or by boiling with lead hydroxide (Heintz [1849]).

Although fresh muscle contains at most only traces of creatinine (Grindley and Woods [1906], Mellanby [1908]), the evaporation of the extract in the presence of the natural acids of the muscle may cause a considerable anhydration to creatinine, so that the latter substance may be abundant in commercial meat extracts. According to Grindley and Woods [1906] beef contains 0·41 per cent., fish 0·31 per cent., chicken 0·24-0·29 per cent. of creatine; in beef extracts they found 0·55-4·79 per cent. of creatine and 0·83-5·27 per cent. of creatinine; the total creatine + creatinine in meat extract is however fairly constant, generally about 6 per cent. Baur and Barschall [1906] give as maximum 1·25 per cent. of creatine and 3 per cent. of creatinine.

Supposed existence of several creatinines. Johnson [1892] considered that the creatinine from urine was not identical with that obtainable from creatine, and Thesen [1898] obtained a yellow "iso-creatinine" from fish. The supposed differences in these cases are however due to insufficient purification, as shown by Poulsson [1904], Toppelius and Pommerehne [1896] and by Korndörfer [1904, 1]. Similarly the xantho-, chryso-, and amphicreatinine of Gautier [1896, Ch. I] were doubtless also impure, as already suggested by Brieger [1886, 1, p. 10, Ch. I]. Indeed, no one has apparently thought it worth while to re-investigate them.

The quantitative estimation of creatinine appears to have been attempted first by Heintz [1849]; Neubauer [1863] then worked out a method depending on the isolation of the base as zinc chloride compound. Salkowski showed that Neubauer's method gives results which are often much too low, and proposed modifications [1886, 1890]. Gregor [1900] attempted to utilise the copper reducing power and Edlefsen [1908] has suggested a method depending on the formation of creatinine salicylate, but all these methods have been displaced by Folin's colorimetric method, depending on the use of Jaffé's reaction (see appendix).

Since creatine can be quantitatively converted into creatinine the

former substance can also be estimated indirectly by Folin's method. A direct method for estimating creatine, due to Walpole [1911], is based on the colour reaction with diacetyl (see appendix).

Physiological.

Distribution.

Creatine is a constituent of all vertebrate muscle. It was found in the muscles of several animals by Liebig [1847], in the alligator and in man by Schlossberger [1844, 1848], in a whale by Price [1851], in a snake by Lyman [1908], in the cod and skate by Gregory [1848], in various fishes and in *Amphioxus* by Krukenberg [1881], and by Suzuki and co-workers [1912]. It is not present in invertebrate muscle [Krukenberg, 1881]. It is absent in the shrimp [Ackermann and Kutscher, 1907, 1-4; Chapter III, betaine], absent in the cuttle-fish [Henze, 1910; Chapter III, betaine], [Cabella, 1913], and absent or present only in traces in *Crustacea* and *Mollusca* [Okuda, 1912].

Recently Myers and Fine [1913, 1] have shown that for any particular species the muscle creatine is remarkably constant. They found 0·522 per cent. in the rabbit, 0·45 per cent. in the cat, 0·39 per cent. in man, 0·37 per cent. in the dog. Other recent observations are in close agreement with these determinations; thus in rabbit's muscle Riesser [1913] found 0·521 per cent. and Beker [1913] 0·523 per cent.; the latter found in dog's muscle 0·364 per cent.

Creatine is most abundant in voluntary muscle (Cabella [1913], Beker [1913]); there is less in cardiac and least in involuntary muscle. According to Cabella the pectoral muscle of birds contains more than that of the thighs; in voluntary mammalian muscle and in the bullock's heart the creatine nitrogen is 3-4 per cent. of the total; in birds' pectoral muscle 4-5 per cent.; in cardiac muscle of birds and in the muscle of the bullock's bladder 1 per cent.

The following table [Beker, 1913] gives the amount of creatinine in milligrams obtained from 100 grm. of various organs. The figures must be multiplied by 1·16 to give their content as creatine.

Voluntary muscle,	bullock	403	
,, ,,	rabbit	451	
,, ,,	pig	338	
,, ,,	dog	314	
Cardiac muscle,	bullock	215	
,, ,,	dog	243	
Uterus ,,	cow	38·18	
,, ,,	pig	30·05	
Testis,	bull	86·8	
Liver ,,		29·32	
,,	rabbit	20·05	
,,	pig	16·71	
Pancreas,	bullock	14·34	
Spleen ,,		14·67	
Blood ,,		2·179	

After two months' gestation, 100 grm. of voluntary muscle of the fœtal calf contained 22 mg., after nine months 250·4 mg. According to Mellanby [1908] creatine is not present in chick's muscle until the 12th day of incubation and the maximum content is only reached after hatching.

In the rabbit and in the fowl the percentage of muscle creatine increases during starvation [Mendel and Rose, 1911, **2**], probably owing to diminution of the non-creatine portion of the muscle. According to Myers and Fine [1913] it increases in the earlier part of starvation and afterwards diminishes. In malignant and some chronic diseases, but not in acute disease, the creatine content of muscle is diminished [Chisholm, 1912], apparently owing to diminished production.

Letsche [1907] found creatine in the blood serum.

Creatine is generally absent from mammalian urine, but it may be present in various conditions. It completely replaces creatinine in birds' urine [Paton, 1910] and occurs also normally in the urine of infants [Funaro, 1908] and of children [Rose, 1911 ; this paper should be consulted for further literature], [Folin and Denis, 1912], [Krause, 1913]. In women creatine occurs in the urine immediately after menstruation, also during and after pregnancy [Krause, 1911 ; Krause and Cramer, 1910]; its excretion is a concomitant of lactation [Mellanby, 1913].

In man creatine appears in the urine when no carbohydrates are taken as food, therefore in starvation [Cathcart, 1907 ; Benedict and Diefendorf, 1907 ; Mendel and Rose, 1911, **2**] and also on a diet of fats and proteins [Cathcart, 1909 ; Mendel and Rose, 1911, **1**]. Creatine further appears in the urine in diabetes [Krause and Cramer, 1910 ; Krause, 1910 ; M. R. Taylor, 1910], in phloridzin glycosuria [Cathcart and Taylor, 1910], in hepatic disease [Mellanby, 1908], in phosphorus poisoning [Forschbach, 1908], and in toxic fevers, mostly after the crisis [Myers and Volovic, 1913].

Creatinine is a normal constituent of mammalian urine [Pettenkofer, 1844 ; Fiebiger, 1903]. It is absent from muscle or present only in traces (for precautions to avoid its formation from creatine in extraction see Mellanby [1908] and Cabella [1913]). Small quantities have been found in cancer tumours [Saiki, 1909] and in egg-yolk [Salkowski, 1911], but the latter observation is contrary to that of Mellanby [1908].

Neither creatine nor creatinine occurs in the urine of fish [Denis, 1912] nor in that of cuttle-fish [von Fürth, 1900]. According to

Sullivan [1911] creatinine (and possibly also creatine) occurs in wheat, rye, clover and other crops, whence it finds its way into cultivated soils, from which it was isolated in the crystalline condition by Shorey [1912]. According to Skinner [1912] creatine and creatinine have a beneficial effect on plant growth.

Metabolism.

The close chemical relationship between creatine and creatinine already suggested to Liebig that the former substance is converted in the animal organism into the latter and is then excreted in the urine. This view as to a genetic relationship between the two substances was rejected by Folin, whose colorimetric estimation first made accurate investigation possible. He [1905, **1**] was the first to show that on a creatinine free diet the amount of creatinine excreted in the urine is remarkably constant for any given individual, and this important result was soon confirmed by various investigators, e.g. Koch [1905], van Hoogenhuyze and Verploegh [1905], Closson [1906], af Klercker [1907], Shaffer [1908], Levene and Kristeller [1909]. Various authors give slightly different limits for the daily output; thus Folin gave 1·3-1·7 grm. for a man of 70 kilos., i.e. 19-24 mg. per kilo. of body weight, Closson 15-19 mg. and Shaffer 19-30 mg. of creatinine per kilo. *per diem.*

On this constancy of the creatinine output in the individual Folin [1905, **2**] has based a theory of protein metabolism (see Cathcart's monograph in this series, "Physiology of Protein Metabolism," pp. 94, 95, 98), according to which theory the creatinine excreted is a result and measure of the "endogenous" catabolism of the tissues and is independent of the "exogenous" catabolism and of the protein of the diet. Creatinine given by the mouth is rapidly and almost quantitatively excreted in the urine as such and this exogenous creatinine of the food is thus super-imposed on the constant endogenous amount. Creatine, on the other hand, as Folin [1906] has shown, when given by the mouth in moderate quantity, does *not* appear in the urine, neither as such, nor as creatinine. This observation has also been made by many other investigators, e.g. Czernecki [1905] and Plimmer, Dick and Lieb [1909]; the latter authors found, for instance, that creatine appeared in the urine after a daily dose of 2·5 grm. but not after 2·0 grm. In children the power of assimilating creatine is much smaller and even of doses of 0·3 grm. some appears in the urine, super-imposed on that normally present [Krause, 1913].

In accordance with Folin's theory the amount of endogenous

creatinine is diminished when the tissue metabolism is decreased. New-born infants excrete per kilo. of body weight one-third of the creatinine excreted by adults [Amberg and Morrill, 1907; Funaro, 1908]. Old people excrete less than young adults, and women less than men [Benedict and Myers, 1907, 1]. In muscular dystrophy [Spriggs, 1907], in Basedow's disease [Forschbach, 1908], in hepatic disease [Mellanby, 1908], in diabetes [Krause, 1910], and in other pathological conditions [Shaffer, 1908] the creatinine output is diminished. On the other hand the more rapid metabolism of fevers causes an increased creatinine output [Leathes, 1907] and this applies also to artificial hyperthermia [Myers and Volovic, 1913]. The latter authors record an increase up to 36 per cent. As will be seen, however, the decrease in creatinine output is also in accordance with the theory which regards creatine as the precursor of creatinine; when the output of the latter substance falls off, the former may take its place in the urine, as in diabetes and in hepatic disease.

Folin's denial of a genetic relationship between creatine and creatinine has not met with general acceptance. It was endorsed by af Klercker [1907] and by Lefmann [1908], but, as has been pointed out by van Hoogenhuyze and Verploegh [1909], Lefmann's results hardly support his conclusion and rather indicate a partial conversion of injected creatine to creatinine. Most authors do not agree with Folin's sharp differentiation between muscular creatine and urinary creatinine; there is a good deal of evidence in support of the view that one of these substances is derived from the other. Mostly creatine has been regarded as the precursor of creatinine, but Mellanby [1908] takes the converse view. According to him creatinine is formed in the liver from substances brought there by the blood stream, and is subsequently rendered innocuous by hydration to creatine. In the young chick creatine is at first absent from the muscles and gradually increases until the saturation point is reached, and then the excess of creatinine is excreted as such in the urine. Other investigators agree with Mellanby in regarding the liver as the seat of transformation, but consider the change to be in the opposite direction, viz. a dehydration of muscular creatine to creatinine which is then excreted. When the activity of the liver is impaired, as in phosphorus poisoning and in hepatic disease, some creatine escapes dehydration and appears in the urine as such (see above).

A further argument for the view that creatine is converted into creatinine and then excreted, has recently been supplied by Myers and Fine [1913, 1], who find that the creatine content of muscle

varies from species to species, but is very constant in the individuals of the same species; those species with muscles richest in creatine show also the greatest output of creatinine in the urine. The constancy of content of muscle and of creatinine output would thus be the expression of a dynamic equilibrium.

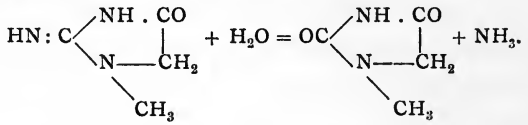
The question whether creatine is formed as the result of muscular work has been answered in the negative.

Liebig found ten times as much creatine in the muscles of a fox killed in the chase as in the captive animal, but Voit [1868] found no increase after work or after tetanising. Van Hoogenhuyze and Verploegh [1905; consult this paper for the earlier literature] only found an increase when muscular work was done during absolute fasting. Mellanby [1908], Scaffidi [1913] and others have also failed to change the creatine content of muscle by work; Brown and Cathcart [1909] observed a slight increase after stimulation, but only with isolated frog's muscles.

Although creatine formation is not a function of rapid muscular contractions, Shaffer [1908] regarded the creatinine output per kilo. as directly parallel to muscular development or strength ("muscular efficiency"), and Pekelharing and his pupils have during the last few years connected creatine formation and creatinine output with muscular tonus. Weber [1908] had already shown that the surviving pulsating heart, perfused with Ringer's solution, gave off creatine to the perfusion fluid, and this observation was confirmed by Howell and Duke [1908]. Weber also found that in the dog an increased creatinine excretion could be induced by cinchonine convulsions (which increase the tonus) but not by work; the creatine in the muscles decreased. Pekelharing and van Hoogenhuyze [1909, 1911] then developed a new theory as to the effect of tonus on creatine formation. They also observed a slight increase of the creatine content of muscle during rigor; the additional creatine is excreted in the urine as creatinine. Pekelharing [1911] showed that there is an increase of urinary creatinine after standing at attention for some hours in a military position, but not after a long march. During sleep van Hoogenhuyze and Verploegh [1905] had previously observed a decrease in the creatinine output, which may be connected with the diminished tonus. Beker [1913] has also supported this theory; he found that in pregnancy the creatine content of the uterus increases in the cow from 0·038 per cent. (calculated as creatinine) to 0·084 per cent. in the gravid and 0·060 per cent. in the non-gravid horn. For pregnant and non-pregnant human uteri the figures were 0·0766 and 0·0446 respec-

tively. This may be connected with the *post partum* excretion of creatine.

Attempts to increase the muscular creatine or urinary creatinine by giving creatine by the mouth have not been very successful, perhaps because of bacterial action in the intestine. Thus van Hoogenhuyze and Verploegh [1908] found only slightly more creatinine in the urine after taking 2 grm. of creatine. The destruction of creatine by bacteria has been studied by von Jaksch [1881], Vandevelde [1884], and particularly by Twort and Mellanby [1912]. Ackermann [1913] has shown that in putrefaction creatinine is not broken up like creatine, but is changed to N-methylhydantoin.



When creatine was administered subcutaneously or intravenously, however, a certain amount of direct evidence of its conversion to creatinine has been obtained in rabbits [Pekelharing and van Hoogenhuyze, 1910] and in dogs [Lefmann, 1908]. Recently Myers and Fine [1913, 3] have shown that of injected creatine 5 per cent. appeared in the muscles in rabbits; 25-80 per cent. appears in the urine as such, and 2-10 per cent. as creatinine. Injected creatinine also causes a slight increase of muscular creatine.

Assuming the conversion of creatine to creatinine, we may next inquire where this change takes place. Experiments on dogs, in which the liver was put out of action by an Eck's fistula, have not proved that the liver has any important function in creatinine metabolism [London and Boljarski, 1909; Foster and Fisher, 1911; Towles and Voegtlin, 1911]. The last-named authors found that creatine, given to dogs, increases the creatinine output, but that putting the liver out of action made very little difference. Paton and Mackie [1912], from experiments on birds, likewise consider that the liver plays no part in the conversion of creatine into creatinine. The appearance of creatine in the urine in hepatic disease may suggest incomplete dehydration to creatinine in the liver, but the formation of creatine might be increased through the disturbance of the carbohydrate metabolism, resulting from damage to the liver. When the supply of carbohydrates in the body is insufficient (in fasting, in diabetes mellitus and in phloridzin glycosuria) the necessary energy must be obtained from another source, and this latter process may be accompanied by increased formation of creatine.

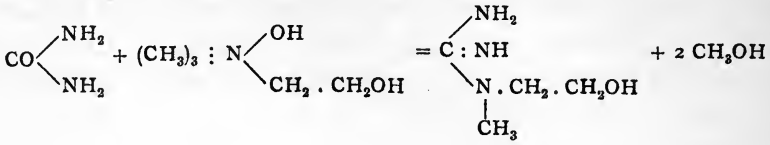
As bearing on the function of the liver in creatinine metabolism the experiments of Gottlieb and Stangassinger [1907; 1908, **1**, **2**] must be mentioned. They concluded that liver extract dehydrates creatine to creatinine and then decomposes it further; these changes may be brought about by autolysis, and creatinine is also formed by perfusing the surviving liver with creatine. Mellanby [1908] criticised the autolytic experiments and considered that in them the destruction of creatine was due to bacteria.

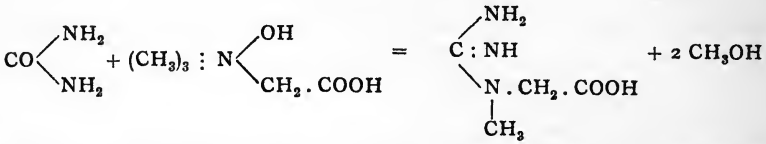
Rothmann [1908] and van Hoogenhuyze and Verploegh [1908] supported Gottlieb and Stangassinger, but Beker [1913] agrees with Mellanby that the destruction of creatine was due to bacteria. As pointed out on page 10 it is very difficult to ensure sterility in autolysis. Gottlieb and Stangassinger's perfusion experiments, on the other hand, are held by Beker to prove that the liver can dehydrate creatine to creatinine.

Possible Precursors of Creatine.

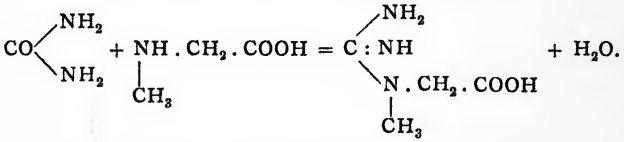
The oldest attempts to find a precursor of creatine were directed to showing that creatine can be formed in the organism from glycocyamine; Jaffé [1906] and Dorner [1907] adduced evidence in support of this, but since the transformation is a simple methylation, for which there are several examples in animal metabolism, and since glycocyamine does not occur in nature, the formation of creatine from this substance would hardly be a physiological process (see further the next section on glycocyamine). Suggestions as to the formation of creatine from muscle protein have been made by Seemann [1907] and by Urano [1907]. According to Antonoff [1906-7] certain bacteria (e.g. *B. coli*) can form from peptone a substance giving Weyl's reaction (creatinine?). The one known protein constituent containing a guanidine grouping is arginine, but neither van Hoogenhuyze and Verploegh [1905] nor Jaffé [1906] could obtain creatine from arginine in feeding experiments or by subcutaneous injection. The whole of the administered arginine was excreted in the urine. Dakin [1907] has shown that creatine is not affected by arginase from the liver. Lately, however, Inouye [1912] has observed a small formation of creatine from arginine by liver extract and when arginine is perfused through the isolated liver. Finally Riesser [1913], in a paper which contains a useful review of the whole problem, has described experiments in which creatine appears to be formed from choline and from betaine (see also pp. 62 and 42). By injecting these substances into rabbits, he increased the creatine content of the muscle, which is

normally very constant, by 10-15 per cent. in the case of choline, and by 6·3-11·3 per cent. in the case of betaine. Riesser considers that these two substances are partially demethylated and then condense with urea, according to the following equations :—





The two methyl groups would be eliminated as methyl alcohol; the condensation product from choline would undergo oxidation to creatine. Riesser also administered sarcosine and urea by the mouth and subcutaneously, and in half of the experiments obtained evidence of creatine formation, which would occur as follows :—



B. Glycocyamine and Glycocyamidine.

Although these bases do not occur naturally they may be briefly referred to on account of their relationship to creatine and creatinine respectively, from which they differ by having one methyl group less; glycocyamine is guanidino-acetic acid and glycocyamidine is the corresponding anhydride.

Glycocyamine was first obtained by Strecker, in 1861, by the addition of cyanamide to glycine. Nencki and Sieber [1878] heated glycine with guanidine carbonate at 140°, and Korndörfer [1905] found that heating in the water bath was sufficient and more convenient. H. Ramsay [1908] has described a convenient synthesis of glycocyamine, in which monochloracetic acid is heated with a concentrated aqueous solution of free guanidine (5 mols.) to 60° for two hours.

The physiological interest of glycocyamine and its anhydride chiefly depends on their supposed methylation in the organism to form creatine and creatinine. The question was first studied by Czernecki [1905] whose results were indecisive or negative; later Jaffé [1906] found (by Neubauer's method) that 4·5-14·3 per cent. of the glycocyamine,

given to rabbits by the mouth, appears in the urine as creatinine and as creatine in the muscles. His pupil Dorner [1907] confirmed these results, using Folin's method. Glycocyamidine given subcutaneously was also changed in rabbits to creatinine. Mellanby [1908] however failed to observe any effects of glycocyamine feeding.

C. Guanidine, $NH:C\begin{smallmatrix}NH_2\\NH_2\end{smallmatrix}$

Guanidine has been isolated from *Vicia* seedlings by Schulze [1892, **2**] (1 grm. of the nitrate from 3 kilos.) but it could not be obtained from the ungerminated seeds. A small quantity also occurs in the sap of sugar beets (Von Lippmann [1896]). It is further obtained in the autolysis of pancreas (Kutscher and Otori [1904]) and by oxidation of guanine and of various proteins with permanganates. Probably the "urea" obtained in the oxidation of egg white by Béchamp [1857] was in reality guanidine; its formation in this manner was first established by Lossen [1880]. Larger quantities were subsequently obtained from various proteins, gelatin, casein, pseudomucin, thymus nucleic acid by Kutscher and his collaborators [1903, 1904, 1905; Otori, 1904, **2**] by using calcium permanganate, and also in the case of pseudo-mucin by hydrolysis with acids (Otori [1904, **1**]).

The physiological action of guanidine was investigated by Gergens and Baumann [1876]. The base is a muscle poison affecting the nerve endings (Camis [1909]). The effect is due to the univalent guanidinium ion and resembles that of sodium salts (Führer [1908, **2**]).

D. Methylguanidine, $NH:C\begin{smallmatrix}NH.CH_3\\NH_2\end{smallmatrix}$

Methylguanidine is of greater physiological importance than guanidine itself, being a normal constituent of muscle. It is formed from creatine by boiling with mercuric oxide and dilute sulphuric acid (Dessaignes [1854, 1855, under creatine], Gulewitsch [1906]) and from creatinine and potassium permanganate (Neubauer [1861, **1**]). Brieger [1886, **1**, p. 34] obtained it from putrid horse meat. Kutscher [1905, under creatine] and Gulewitsch [1906] isolated it from commercial extract of meat (yield of the nitrate 0·38 per cent.; Gulewitsch). According to Krimberg [1906, **1**] methylguanidine occurs in fresh beef, where, however, Brieger [1886, **1**, p. 41] could not find it. Smorodinzew [1913, Ch. II, carnosine] obtained 0·083 per cent. of methylguanidine from fresh horse meat. Small quantities of methylguanidine also occur in normal human urine (Kutscher and Lohmann [1906, **3**], Engeland [1908, **3**]), in that of the dog after feeding on meat extract (Kutscher and Lohmann [1906, **4**]) and in that of the horse (Achelis [1906]). Smorodinzew [1912] recently obtained the base from liver. In the urine of parathyroidectomised dogs the amount of

methylguanidine is greatly increased (up to 1·9 gr. of the gold salt per litre; Koch [1912]) and fairly large quantities are also present in the urine of animals killed by anaphylactic shock or by burning (Heyde [1911, 1912]); normal urine only contains traces. The symptoms of anaphylactic shock cannot, however, be reproduced in any way by the administration of the base [Loewit, 1913].

Methylguanidine is distinctly poisonous; 0·2 grm. administered hypodermically killed a guinea-pig (Brieger [1886, **1**, p. 38]). The smallest dose producing a distinct effect in a frog (fibrillar twitchings of dorsal muscles) is 1 mg.; 50 mg. is fatal. The base acts peripherally on the nerve endings in the muscle; large doses produce tetanic convulsions (Gergens and Baumann). The action is similar to that of guanidine, *q.v.*

Methylguanidine in the organism is probably derived from creatine and the amount in the urine is increased after feeding with meat extracts (Achelis [1906]). The mechanism of this change is not clear, for the simple decarboxylation of creatine would yield dimethylguanidine, so that in addition to carbon dioxide a methyl group must be removed by oxidation. There is however some indirect evidence that bacteria can bring about the conversion of creatine into methylguanidine (Bocklisch [1887]).

E. as-Dimethylguanidine, $NH:C\begin{matrix}N(CH_3)_2\\NH_2\end{matrix}$.

This base appears to accompany the monomethyl derivative in normal urine; Engeland isolated 0·15 grm. of the aurichloride from 2 litres of dog's urine, and the picrolonate was probably obtained from human urine by Kutscher and Lohmann [1906, **3**, 4].

The formation of as-dimethylguanidine by bacterial decarboxylation of creatine has not yet been observed (cf. Twort and Mellanby [1912, under creatine]).

CHAPTER VI.

ADRENALINE (EPINEPHRIN, ADRENINE).

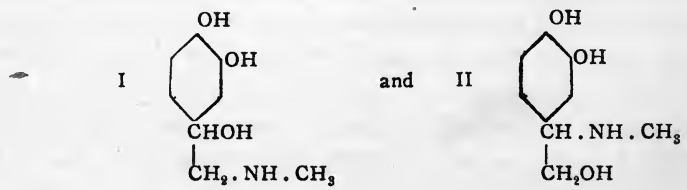
BOTH on account of its powerful physiological activity and its extensive therapeutic application, adrenaline is the most interesting of animal bases. The physiological importance of the supra-renal glands was first made clear by Addison [1849] who connected the disease, now named after him, with a pathological condition of these glands. Addison's work suggested an experimental investigation to Brown-Séquard [1856, **1**, **2**, 1857], who showed that extirpation of both supra-renals soon brings about the death of an animal; thus, on the average, rabbits only survived the operation for nine hours. About the same time Vulpian [1856, **1**, **2**] observed that the medulla of the supra-renal gland contains a specific substance, which in solution is coloured green by ferric chloride and rose-red by iodine; he also obtained the same reactions with blood from the supra-renal vein.

During the next forty years the "chromogen" was investigated by Virchow [1857] who confirmed Vulpian's results without adding fresh observations, by Arnold [1866], by Holm [1867], more fully by Krukenberg [1885], and lastly by Brunner [1892], but none of these authors were able to prepare the substance in anything like a pure condition. The physiological action of supra-renal extracts was the subject of papers by Pellacani [1879] and by Foà and Pellacani [1884], who, however, failed to observe the rise of blood pressure so highly characteristic of supra-renal extracts when injected intravenously. A full account of the earlier investigations on the gland, up to 1895, was given by Rolleston in his Goulstonian lectures [1895, under general references]. There is also an extensive bibliography in a paper by Szymonowicz, published in Pflüger's Archiv [1896], and in a dissertation by Langlois [1897, under general references].

In 1894 the subject entered upon a new phase and soon became of great physiological and biochemical interest. In that year Oliver and Schäfer [1894] observed the remarkable rise of blood pressure caused by supra-renal extracts on intravenous injection; they showed that the effect was due to vaso-constriction and also to a direct action on the heart. This pressor action was discovered independently and almost simultaneously by Szymonowicz [1895] who found that the low

blood pressure caused by extirpation of both supra-renals could be raised temporarily by an injection of an extract of the gland. Cybulski [1895], who continued the investigation, also obtained a pressor action with blood from the supra-renal vein. The isolation of the active principle was now attempted by several investigators. Moore [1895-97] working in Schäfer's laboratory, soon found that the physiological activity of extracts went parallel with the intensity with which they gave Vulpian's colour reactions and concluded that the chromogen was identical with the active principle. Attempts to isolate it were made by Fränkel, Mühlmann [1906], Gürber [1897], and especially by Abel and Crawford [1897], Abel [1898-1901], and by von Fürth [1898-1901], but these attempts were all unsuccessful. Von Fürth, indeed, obtained a highly active preparation of the substance, which he termed suprarenin, by precipitating it as iron compound by the addition of ferric chloride to a purified extract in methyl alcoholic solution, and Abel separated the active principle as benzoyl derivative, but he could not recover it in a pure state by subsequent hydrolysis. Abel's work, however, led to the crystallisation of the active principle by Takamine [1903, **1-3**] who named it "adrenalin," and very soon afterwards it was obtained independently by Aldrich [1901] who assigned to it the correct empirical formula $C_9H_{13}O_3N$.

The chemical constitution of adrenaline could now be investigated. On fusion with potash Takamine had already obtained from it two substances which he regarded as catechol and protocatechuic acid. Von Fürth confirmed the production of the latter substance and also showed that a methylamino-group and an alcoholic hydroxyl are present. Abel for a long time defended the erroneous formula $C_{10}H_{13}NO_3$, $\frac{1}{2}H_2O$ and termed the crystalline active principle "epinephrin hydrate". The substance obtained on complete hydrolysis of his benzoyl derivative he considered to have the composition $C_{10}H_{13}NO_3$, and this he called "epinephrin," but found later that it was chemically and physiologically different from the active principle of the gland. Abel's formula was disproved conclusively in favour of that of Aldrich by Pauly [1903], who analysed very carefully purified material and also showed that adrenaline contains an asymmetric carbon atom. Pauly reduced the number of possible constitutional formulæ to two, viz. :—



Jowett [1904] arrived at results similar to those of Pauly; on complete methylation and subsequent oxidation he obtained veratric acid and trimethylamine; of the above two formulæ he favoured the first, subsequently shown to be the correct one. Further investigations were carried out by Abderhalden and Bergell [1904] and by Bertrand [1904, **1, 2**]. In the meantime the problem was being attacked in a different way by Stolz whose results, although not published until 1904, had already led in August 1903 to a patent application of the Farbw. vorm. Meister, Lucius und Brüning [1904] describing the synthesis of a substance of the constitution 1 (above) which could not at first be obtained crystalline but seemed to be physiologically identical with adrenaline. Similar synthetic experiments were published somewhat later by Dakin [1905, **1-3**], but although the identity of the synthetic substance with adrenaline was rendered extremely probable, this identity could not be proved rigorously, until the former substance had been crystallised and finally resolved into its optically active components, one of which was found to be completely identical with natural adrenaline (Flächer [1908]). Before this, an independent proof of the constitution of adrenaline had been furnished by Friedmann [1904, 1906] who showed that von Fürth's tribenzenesulphonyl adrenaline, which is optically active, lost its activity on oxidation to the corresponding keto-derivative, which was crystallised. This proved that adrenaline is a secondary alcohol (formula I) and its constitution was further established by a comparison of the above-mentioned ketone with a synthetic specimen obtained from the amino-aceto-catechol of Stolz.

Nomenclature and Synonyms.

It is clear from the above that the active principle of the supra-renal gland has received different names from various investigators. The three principal ones are "epinephrin" (Abel), "suprarenin" (von Fürth) and "adrenalin" (Takamine), and these are the only ones in scientific use, together with "adrenine" which has lately been employed in the "Journal of Physiology". On grounds of scientific priority the name should be adopted, which was suggested by the chemist who first isolated the substance in a pure state; this was Takamine and we therefore use the name adrenalin(e) in the present monograph; this name also happens to be the one at present in most general use. The objection to adrenalin is that it is a proprietary trade-name. For this reason the English Chemical Society used for some time the name epinephrin, which has also been adopted more recently by the American Medical Association. Apart from the fact that Abel first applied this name to an amorphous and probably impure substance there is the additional confusion, that for a long time he designated by it a supposed artificial alkaloidal anhydride of the active principle, which latter he called epinephrin hydrate (=adrenalin) and some of his papers speak of epinephrin and adrenalin as two distinct substances. Later, when

the hydrate theory proved to be untenable, epinephrin was made synonymous with adrenalin.[1]

Preparation and Purification of Natural Adrenaline.

The various processes depend on the fact that the active principle is extracted from the glands by water, neutral or acidulated, that it is not precipitated from its concentrated aqueous solution by alcohol, nor by neutral lead acetate, and that it separates in a crystalline form from suitably purified and concentrated aqueous solutions on the addition of *concentrated* ammonia. On account of the readiness with which adrenaline undergoes oxidation various precautions have been suggested, such as preventing the access of air by means of a current of hydrogen or of carbon dioxide, and carrying out the final precipitation under a layer of petrol. For the same reason it is very convenient to extract with water containing sulphur dioxide.

Takamine [1901, 2] extracted the minced gland at 50-80° for five hours with water acidulated with acetic or hydrochloric acid, shaking at intervals. The extract was then raised to 90-95° for one hour to coagulate the proteins, using a layer of fat or current of carbon dioxide to avoid oxidation. The glands were extracted a second time and the mixed extracts were concentrated *in vacuo*, and then precipitated with 2-3 volumes of alcohol. After filtration, the filtrate was again evaporated to a small bulk and was then precipitated with excess of concentrated ammonia which caused the crude adrenaline to separate in sphæro-crystals.

Aldrich [1901] proceeded like Takamine, but before precipitating the concentrated solution with alcohol he added neutral lead acetate, centrifuged and removed the excess of lead from the solution by means of hydrogen sulphide. Then, after concentration, he added four to five volumes of 94 per cent. alcohol, evaporated the alcoholic filtrate to a very small bulk and added ammonia; after filtration the crude adrenaline is washed with very dilute ammonia.

Abel [1903, 1] recommends a process illustrated as follows: 11·13 kilos. of minced glands were divided over a number of flasks and to each portion an equal quantity of a solution of 175 grm. trichloracetic acid in 5 litres of absolute alcohol was added, in small quantities at a time, with vigorous shaking. Next day 5-6 litres of filtrate were collected at the pump and evaporated to 380 c.c. After filtering off a flocculent precipitate, ammonia (d = 0·94) was gradually added to the clear filtrate with stirring until the smell of ammonia was permanent. The adrenaline, which separated at once, was filtered off and washed with water, alcohol and ether; yield 23·79 grm. = 0·2 per cent. The product, although nearly white,

[1] Those interested in this question of nomenclature may refer to a letter by T. Maben in the Pharmaceutical Journal (1907, 78, 388-90; "Adrenalin: the Active Principle of the Suprarenal Gland") and to a reply by W. Martin in the same journal (1907, 78, 447 and 514; "Epinephrin or Adrenalin?"), and particularly to a correspondence entitled "Proprietary versus Unprotected Names" between the Council on Pharmacy and Chemistry of the American Medical Association and Messrs. Parke, Davis & Co. (Journ. Amer. Med. Assoc., 1911, 56, 910-5). It is said that 30-40 different trade names for the active principle of the supra-renal gland have been in use. Of these adnephrin, adrenalin, adrin, caprenalin, supra-capsulin and supra-renalin are of American origin; the following are European: atrabilin, chelafrinum, epirenan, hæmostasin, hemisine, ischemin, paraganglin, paranephrin, renoform, supra-nephran, supra-renaden, tonogen, and vaso-constrictin. Suprarenin is used by the Höchst works for their synthetic product.

contained 10-12 per cent. of ash. A second and a third extract, made from the mass of glands with 30-40 grm. of trichloracetic acid in 5-6 litres of 60-70 per cent. alcohol, yielded respectively 8·57 and 3 grm. of base; total = 35·36 grm. or 0·3 per cent. of crude product.

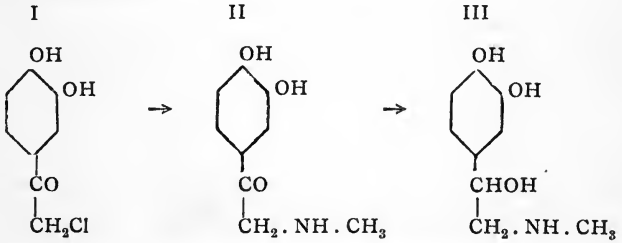
Bertrand [1904, 1] extracted 600 grm. of the minced glands (of horses) with 2 litres of 95 per cent. alcohol, containing 5 grm. of oxalic acid. On evaporation the extract was shaken with petrol to remove lecithin, etc., and the aqueous layer was exactly precipitated with neutral lead acetate and centrifuged. After removal of the excess of lead and evaporation to 100 c.c. a slight excess of ammonia was added. 118 kilos. of fresh minced gland from 3900 horses yielded 125 grm. of adrenaline. This yield is hardly more than one-third of that obtained by Abel (from bullock's glands).

The *purification* of the crude adrenaline may be carried out by dissolving in acid and reprecipitating, but better by Abel's method depending on the solubility of adrenaline oxalate in alcohol. Pauly [1903] used it as follows: 12 grm. of crude adrenaline were ground up with 50 c.c. of 85-90 per cent. alcohol, containing 7 grm. of oxalic acid; the inorganic impurities remain behind. After filtration and dilution with 100 c.c. of water, ammonia precipitated the base in a crystalline condition; the base was freed from ammonium oxalate by thoroughly washing. This process was repeated several times and finally the base was washed with alcohol and ether. A more complicated process which yielded a substance absolutely free from ash, is also described by Pauly [1904].

Syntheses of Adrenaline.

Adrenaline has been synthesised by several methods:—

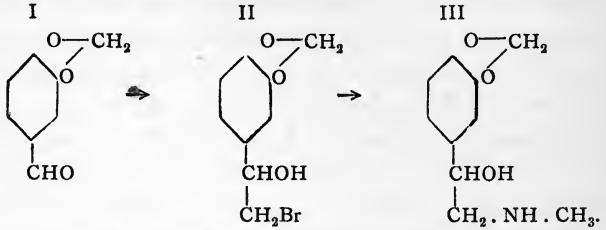
(1) By means of phosphorus oxychloride, catechol is condensed with monochloracetic acid and the resulting chloracetocatechol (I), thus first prepared by Dzierzgowski, is suspended in alcohol (50 c.c. for 100 grm. of the ketone).



A 40 per cent. aqueous methylamine solution (200 c.c.) is then added and on standing methylamino-acetocatechol separates out; the product is washed with water, alcohol and ether. The methylamino-acetocatechol (II) so obtained is reduced to racemic adrenaline (III) by means of aluminium amalgam, or electrolytically. The above process is protected by the German patents Nos. 152814 and 157300 of the Farbwerke vorm. Meister, Lucius und Brüning [1904] and appears to

be the only one which is commercially suitable. The resolution of the racemic adrenaline is effected according to Flächer [1908] by extracting the bitartrate with methyl alcohol; d-adrenaline d-tartrate dissolves and l-adrenaline d-tartrate remains behind. The latter yields commercial synthetic suprarenin.

An attempt to synthesise adrenaline by another method was originated by Barger and Jowett [1905] and continued by Pauly and Neukam [1908], Barger [1908], Böttcher [1909] and Mannich [1910], but has not yielded results of practical value (cf. German patents Nos. 209609, 209610, and 212206). Starting from piperonal (I), Barger and Jowett prepared the bromohydrin (II) which was converted into adrenalin methylene ether (III)



Adrenaline dimethyl ether was prepared from methyl vanillin by a similar method, but neither ether is convertible into adrenaline. Mannich showed that on the addition of methylamine to the bromohydrin, ethers of isoadrenaline $(OH)_2C_6H_3 . CH(NHCH_3) . CH_2OH$ are also formed. The indirect removal of the methylene group by conversion into an unstable cyclic carbonate—e.g. $OCO_2 : C_6H_3 . CH(OH) . CH_2Cl$, has also proved impossible.[1]

Another synthesis of adrenaline which is theoretically possible and has been referred to in the patent literature, consists in methylating the primary base 3:4-dihydroxy-phenylethanolamine $(OH)_2 . C_6H_3 . CH(OH) . CH_2 . NH_2$. This base, which is about as active as adrenaline itself and is known commercially as "arterenol," may be prepared by the reduction of amino-acetocatechol (D.R.P. 155632).

$$(OH)_2C_6H_3 . CO . CH_2 . NH_2 + 2H = (OH)_2C_6H_3 . CH(OH) . CH_2 . NH_2$$

and also by the reduction of the cyanhydrin of protocatechuic aldehyde with sodium amalgam (D.R.P. 193634).

$$(OH)_2C_6H_3 . CH(OH) . CN + 4H = (OH)_2C_6H_3 . CH(OH) . CH_2 . NH_2.$$

Amino-acetocatechol is obtainable in several ways :—

1. From chloro-aceto-catechol and ammonia (the chief method) :—
   $(OH)_2C_6H_3 . CO.CH_2Cl + 2NH_3 = (OH)_2C_6H_3 . CO . CH_2 . NH_2 + NH_4Cl.$
2. By reduction of ω-nitroacetocatechol :—
   $(OH)_2C_6H_3 . CO.CH_2 . NO_2 + 6H = (OH)_2C_6H_3 . CO.CH_2 . NH_2 + 2H_2O.$

The ω-nitroacetocatechol is obtained by hydrolysis of the corresponding methylene- or dimethylether with aluminium chloride in benzene solution. These ethers, ω-nitroacetopiperone and ω-nitroacetoveratrone, may be prepared from piperonal and methylvanillin respectively, by successive treatment with nitromethane, bromine, methylalcoholic potash and acids (D.R.P. 195814).

3. By hydrolysis with hydrochloric acid of the condensation product obtained from veratrole and hippurylchloride by means of aluminium chloride (D.R.P. 185598 and 189483)

$$(CH_3O)_2C_6H_4 + ClCO.CH_2.NH.CO.C_6H_5 = (CH_3O)_2C_6H_3.CO.CH_2.NH.CO.C_6H_5 + HCl.$$

$$(CH_3O)_2C_6H_3 . CO . CH_2 . NH . CO . C_6H_5 + 3HCl + H_2O \rightarrow (OH)_2C_6H_3 . CO . CH_2 . NH_2 .$$

A better yield is obtained by the hydrolysis of the similarly constituted phthalimido-acetoveratrole (D.R.P. 209962 and 216640).

[1] Compare Pauly's repudiation [1909] of Böttcher's claim [1909] to have synthesised adrenaline by this method and D.R.P. 209609, 209610, 212206.

In order to utilise the d-adrenaline, obtained as a by-product in the resolution of the racemic base (according to Flächer [1908] and D.R.P. 222451), the dextro-variety may be racemised by means of acids (according to D.R.P. 220355). For example, 1·5 grm. d-adrenaline is dissolved in 13·5 c.c. normal hydrochloric acid (= 1·65 mol.) and after adding 15 c.c. of water the solution is heated to 80-90° for two to three hours, after which the solution is optically inactive and the crystalline hydrochloride of the racemic base can be isolated by means of alcoholic hydrogen chloride. When the natural base was kept for six weeks at 20-30° with the same concentration of hydrochloric acid, 75 per cent. had been racemised. By repeated resolution and racemisation of the d-base, the whole of the synthetic adrenaline is finally obtained in the l-form.

For an account of the patents relating to the synthesis of adrenaline reference may be made to Friedländer's "Fortschritte der Teerfarbenfabrikation," 1905-7, VIII, 1181-90, and 1907-10, IX, 1024-33; or to the "Chemisches Zentralblatt".

Adrenaline Substitutes.

Numerous bases, more or less closely related to adrenaline, have been synthesised and some of these also resemble adrenaline in physiological action. Only three of them, however, have been recommended as substitutes for the natural active principle, namely

3 : 4-dihydroxy-phenylethanolamine $(OH)_2C_6H_3 . CH(OH) . CH_2 . NH_2$ ("arterenol")
ω-ethylamino-3 : 4-dihydroxy-acetophenone $(OH)_2 . C_6H_3 . CO . CH_2 . NH . C_2H_5$
("homorenon")
3 : 4-dihydroxy-phenylethyl-methylamine $(OH_2)\ C_6H_3 . CH_2 . CH_2 . NH . CH_3$ ("epinine")

Of these, arterenol is according to Schultz [1909, I] about as active on the blood pressure as natural l-adrenaline (and therefore more active than the racemic base). Homorenon and epinine are much less active, the former base having according to Schultz only about one-eightieth of the pressor action of l-adrenaline.

Physical and Chemical Properties of Adrenaline. Salts and Derivatives. Constitution.

Adrenaline, when pure, crystallises in colourless sphærocrystals consisting of superposed lamellæ; crystals suitable for crystallographic measurement have not been obtained. It melts at 211-212° (uncorr.) with decomposition. According to Bertrand the solubility in water at 20° is 0·0268 per cent. The base is somewhat more soluble in boiling water, but less in alcohol; it is practically insoluble in most organic solvents but dissolves in glacial acetic acid, in warm ethyl oxalate (Abel) and in benzaldehyde. In the latter solvent Barger and Ewins [1906] found at 90° the molecular weight 170.

Adrenaline is lævo-rotatory. The more trustworthy determinations in solution in dilute mineral acids are tabulated below:—

Author.	Source.	Temperature.	$[\alpha]_D$
Bertrand [1904, 2]	horse; in N/10 H_2SO_4	—	− 53·3°
Abderhalden and Guggenheim [1908] .	bullock	20°	− 50·72°
Flächer (with Korndörfer) [1908] .	bullock	19·8°	− 51·40°
Schultz (with Taveau) [1909, 1] . .	bullock	26·4°	− 53·40°
Abel and Macht [1912] . . .	parotid gland of *Bufo Agua*	20°	− 51·30°
Weidlein [1912]	whale	25°	− 52·00°
Flächer [1908]	synthetic l-adrenaline	—	− 51·40°
,, ,, ...	,, d- ,,	—	+ 51·88°

d-Adrenaline has the same physical and chemical properties as l-adrenaline and melts also at 211-212°, but is much less active physiologically.

Adrenaline is a fairly strong base and can be dissolved in the theoretical quantity of a mineral acid, or even in somewhat less than one equivalent (Gunn and Harrison [1908]). Being a phenol, it is also soluble in caustic alkalies, but not in ammonia or sodium carbonate. The chief chemical characteristic of adrenaline is the readiness with which it undergoes oxidation, on account of the presence of a catechol nucleus. A large number of mild oxidising agents colour adrenaline solutions pink, rose red, and brown, and the same change takes place on exposure to air, slowly in acid, rapidly in alkaline solution. Adrenaline is most stable in solutions containing a slight excess of acid, for instance one and a half equivalents of acid to one equivalent of the base. The coloration takes place much more rapidly when minute traces of iron are present (Gunn and Harrison [1908]). A number of colour reactions, depending on this oxidative change, are described below (pp. 89-91). According to Abel [1902, 3] extracts of the supra-renal gland are more stable to Fehling's solution than solutions of the pure active principle. Adrenaline solutions do not give precipitates with the common alkaloidal reagents, but on heating with dilute acids, or by the action of concentrated hydrochloric acid in the cold, adrenaline is transformed into a substance yielding alkaloidal reactions (Abel's epinephrine).

The salts of the optically active adrenalines are mostly amorphous and deliquescent; the *borate* prepared by evaporating 1·83 gr. of the base and 0·93 gr. of boric acid in 5 c.c. of water is said to be more stable (D.R.P. 167317). The chief crystalline salt of adrenaline is the *bitartrate*, employed in the resolution of the synthetic product, Pauly [1904] prepared a crystalline *urate*. The racemic base yields, in addition, a crystalline *hydrochloride*, mp. 157° (D.R.P. 202169), and a crystalline *oxalate*, but the corresponding salts of both d-and l-adrenaline are amorphous (Flächer [1908]).

No crystalline derivatives of adrenaline are known. Abel and Pauly prepared benzoyl derivatives of somewhat uncertain composition. Von Fürth obtained a tri-benzenesulphonyl derivative which contains the alcoholic hydroxyl of the side chain intact, for Friedmann [1904, 1906] converted it into m-nitrobenzoyl-tribenzenesulphonyl-adrenaline and oxidised it to tribenzenesulphonyl-adrenalone. Stolz obtained a tri-p-chlorbenzoyl derivative.

The constitution of adrenaline was ascertained from the following reactions ;—

On fusion with potash catechol and protocatechuic acid are formed; on heating with acids or caustic soda methylamine is eliminated. On methylation and subsequent oxidation with permanganate veratric acid, vanillin and trimethylamine were obtained. The constitution is further proved by Friedmann's work (see above, p. 83) and finally of course by synthesis and resolution.

The alleged production of skatole on potash fusion is probably due either to the presence of protein impurities, or to that of a benzoyl

nucleus (in Abel's epinephrine). The constitution of the "alkaloidal" substance formed by the action of acids on adrenaline has not been elucidated, nor of the base $C_3H_4ON_2$ obtained by Abel [1904] on oxidising adrenaline with nitric acid. Adrenaline is readily attacked by various oxidases [Neuberg, 1908; Abderhalden and Guggenheim, 1908].

Colour Reactions of Adrenaline.

The principal colour reactions were already observed by Vulpian and have more recently been used for the estimation of adrenaline. A general review of the various quantitative colorimetric methods has lately been furnished by Borberg [1912]. The reactions are as follows:—

I. Ferric chloride produces in neutral or slightly acid solution a *grass green coloration*, changing to violet, reddish violet, and red on the careful addition of dilute alkali. This is a reaction characteristic of catechol derivatives. The green coloration is the more fugitive and the less strongly marked, the more acidic the solution is. The limit of sensitiveness is about 1 : 30000, but the addition of sulphanilic acid increases the sensitiveness tenfold and changes the green colour to reddish brown or brown yellow (Bayer [1909]). Falta and Ivcovic [1909] describe another sensitive modification of the ferric chloride reaction. For the detection of adrenaline in urine Borberg [1912] gives the limit for the green ferric chloride reaction as 1 : 100,000. On standing a red coloration is produced up to 1 : 300,000.

II. A *pink* or *rose red coloration* ("tout à fait remarquable," Vulpian) is produced in adrenaline solutions on prolonged exposure to air and, almost immediately, by various oxidising agents. The change of colour is less rapid in faintly acid solution than in neutral solution, and more rapid in alkaline solution. It is also brought about by oxidases; from the behaviour of adrenaline to tyrosinase, Gessard [1904] first deduced a relationship to tyrosine. Neuberg [1908] found that an enzyme from the ink-bag of *Sepia officinalis* produces a black pigment from adrenaline, and Abderhalden and Guggenheim [1908] observed that adrenaline solutions are coloured red by a tyrosinase from the fungus *Russula delica;* the lævo-, the dextro-, and the racemic forms are all coloured at the same rate. The formation of pigments from adrenaline has been considered by some to be connected with the pigmentation of the skin in Addison's disease.

The oxidising agents employed for the red colour reaction for adrenaline are:—

A. *Iodine* or *iodic acid.* The excess of iodine may be removed by shaking with ether and the sensitiveness is then according to Schur [1909] 1 : 1,500,000. Abelous, Soulié and Toujan [1905] removed the excess of iodine by means of sodium thiosulphate, but according to Bayer [1909] the reaction, when carried out in this way, is not very delicate and the red colour is not permanent.

Another modification of the iodine reaction was suggested by L. Krauss [1909] who used iodic acid. Subsequently Fränkel and Allers [1909], independently of Krauss, employed an equal volume of 0·001 N-potassium bi-iodate and added a few drops of phosphoric acid; by heating the mixture nearly to the boiling point, the reaction is said to be obtainable at a dilution of 1 : 300,000. Hale and Seidell [1911] recommend this test, but do not add phosphoric acid. Fränkel and Allers consider their test to be quite distinct from that of Vulpian; they state that at no stage of the reaction is iodine set free, but both Krauss and Ewins [1910] deny this. Bayer [1909] claims to have greatly increased the sensitiveness of the Fränkel-Allers reaction by adding sulphanilic acid, which, however, changes the red coloration to an orange or yellow one, which is less specific; Bayer gives 1 : 5,000,000 as the limiting dilution.

B. Another oxidising agent, which colours adrenaline solutions red, is *mercuric chloride*, recommended by Comessatti [1909]. Boas [1909] and Fränkel and Allers [1909] could not obtain the reaction at all readily, but Ewins [1910] has pointed out that Comessatti used solutions of mercuric chloride in tap water, and that the calcium bicarbonate present in the latter acts as a catalyst; it may be replaced by solutions of other salts of weak acids. This observation is of considerable interest in connection with the discovery of Euler and Bolin that the oxidase from *Medicago* consists of calcium salts of organic hydroxy-acids. It was moreover already noticed by Vulpian, that the spontaneous coloration of the adrenal chromogen by exposure to air takes place slowly in distilled water, but much more rapidly in tap water.

Ewins suggests the following conditions for carrying out Comessatti's reaction. To 1 c.c. of adrenaline (1 : 100,000) an equal volume of a 1 per cent. sodium acetate solution is added and then four to five drops of a 0·1 per cent. solution of mercuric chloride in distilled water. A pale rose tint is produced at room temperature in 4 to 5 minutes. Here the sodium acetate solution replaces tap water, in order to secure uniformity.

C. The most sensitive oxidising agent is probably a persul-

phate. Pancrazio [1909, 1910] has used the sodium salt and Ewins [1910] the potassium salt. Ewins adds *potassium persulphate* solution to the adrenaline solution until the concentration of the persulphate is about 0·1 per cent., and then immerses the test tube for a short time in a boiling water bath. Under these conditions a distinct reaction is still obtained at a dilution of 1 : 5,000,000. The persulphate reaction for adrenaline seems therefore to be more delicate than any other, with the possible exception of Bayer's modification of the Fränkel-Allers reaction (see above) for which an equal degree of delicacy is claimed. According to Ewins potassium persulphate has an additional advantage in the estimation of adrenaline in extracts of the gland, since it discharges the colour of these extracts to a considerable extent, the colour interfering with the Bayer-Fränkel-Allers test. With persulphate a clean and distinct red tint results, which is permanent for a considerable time.

D. *Other oxidising agents* which colour adrenaline solutions red, are potassium ferricyanide (Cevidalli [1908]), brown oxides of manganese (Zanfrognini [1909]), sodium nitro-prusside and ammonia, bleaching powder, chlorine, bromine, ammoniacal silver solutions, and osmic acid (Mulon [1905]). According to Borberg [1912] all the "red" colour reactions for adrenaline are similar and depend on the formation of the same oxidation product. Borberg gives the limit as 1 : 300,000, thus perhaps underestimating the sensitiveness of some of the reactions.

Ewins [1910] examined the effect of iodine and persulphate and of the Comessatti, Fränkel and Allers, and Bayer reagents on a number of synthetic bases, closely related to adrenaline. He found that aminoethanol-catechol (arterenol), as well as dihydroxy-phenylethylamine and its N-alkyl derivatives (including epinine) give the various reactions with about the same degree of sensitiveness as adrenaline, but none of these reactions are given by ketone bases, such as amino-aceto-catechol and its derivatives (including homorenon). Among these synthetic bases there is therefore no close parallelism between chemical reactivity and physiological action.

E. Folin, Cannon and Denis [1912] have recently described a new and very sensitive *colour reaction for uric acid*, which is also given by adrenaline with three times as great a sensitiveness (1 : 3,000,000). One hundred grm. of sodium tungstate is dissolved in 750 c.c. of water, and after adding 80 c.c. of 85 per cent. phosphoric acid, the solution is boiled gently for one and a half to two hours and then made up to 1 litre; $\frac{1}{300}$-$\frac{1}{400}$ mg. adrenaline can be detected.

Colorimetric Estimation of Adrenaline.—The green coloration with ferric chloride has been employed by Batelli [1902] who found by this means 0·174 per cent. in fresh bullock's glands. Von Fürth [1901] has used the carmin red coloration produced by ferric chloride in the presence of sodium carbonate and sodium potassium tartrate. The ferric chloride reaction is, however, not very suitable for quantitative work (cf. Cameron [1906]) and the same applies, according to the author's experience, to the iodine-thiosulphate method of Abelous, Soulié and Toujan [1905]. Comessatti [1909] has employed the mercuric chloride reaction a good deal for quantitative purposes, and Cevidalli [1908] and Zanfrognini [1909] have used their reactions in the same way; their methods have been adversely criticised by Borberg [1912]. Ewins [1910] found a distinct parallelism between the depth of colour produced by potassium persulphate and the pressor activity of supra-renal extracts. This physiological control has not been applied sufficiently to most other colorimetric methods.

A notable exception is found in a recent paper by Folin, Cannon, and Denis [1913] and the colorimetric method of these authors based on the reaction described above (under E) appears to be almost or quite as accurate as the blood pressure method with which its results agree within a few per cent. of the total adrenaline present. The method is even sufficiently sensitive to demonstrate the increase of adrenaline in the supra-renal vein by stimulation of the splanchnic nerve (cf. p. 95). It is not necessary to have pure adrenaline as a standard, for uric acid gives an identical coloration with one-third of the intensity.

Amount of Adrenaline in the Supra-renal Gland; Yield; Distribution in other Organs; Origin.

By the physiological blood pressure method, which is probably the most accurate, Elliott finds that the adult *human* gland in health contains about 0·1 per cent. (unpublished observation, referred to below).

By the same method Elliott [1912] has found that the normal *cat's* supra-renal, weighing 0·2 grm., contains on the average 0·22 mg. of adrenaline, or 0·11 per cent. Folin, Cannon and Denis [1913] found in the gland of young cats 0·122-0·152, of the dog and monkey 0·2-0·25, of the calf 0·25-0·35, of sheep, cattle, rabbits, 0·3 per cent.

Houghton [1902] found Takamine's original adrenaline to be 600 to 800 times as active as fresh *bullock's* gland; according to Takamine [1901, 4] the specimen contained mineral impurities and pure adrenaline is probably 1000 times as active as the fresh gland, which would therefore contain 0·1 per cent. of the base.

For the *horse* we have Bertrand's statement [1904, 1] that 118 kilos. of the fresh gland yielded 125 grm. of adrenaline or 0·106 per cent.

As an example of actual yields obtained in the manufacture of adrenaline from *bullock's* glands, the following figures may be quoted which are percentages of the weight of the fresh gland after dissecting away the fat: 0·095, 0·086, 0·103. (The weight of a fresh bullock's gland dissected in this way, is 10-12 grm.)

In manufacture the yield from *sheep's* is the same as that from bullock's glands, or slightly less (0·08 per cent. ?).

From 100 bullocks' glands von Fürth [1903] obtained 0·78-1·74 grm. of adrenaline; on the average 1·13 grm.; 100 glands weighed about 1000 grm., therefore the adrenaline isolated was 0·113 per cent. Weidlein [1912] obtained 0·247 per cent. crude adrenaline from the *whale's* supra-renal.

The results of colorimetric determinations, except those of Folin, Cannon and Denis quoted above, are probably the least reliable. By the persulphate method Pancrazio [1909] found 0·133 per cent. in the calf's gland and Batelli [1902] by the ferric chloride method found 0·174 per cent.

Abel [1903, 1] obtained 0·3 per cent. of crude adrenaline from fresh bullock's supra-renals; the product contained 10 to 12 per cent. of ash and probably also organic impurities, but nevertheless this appears to be by far the highest yield recorded, and Abel [1903, 2] estimates that fresh beeves' supra-renals contain at least 0·3 per cent. of the active principle. Hunt [1906], experimenting with a decoction of dried glands, found by physiological means (blood pressure) that these glands contained 1·5 per cent. of adrenaline; according to the United States Pharmacopeia one part of the dried gland corresponds to six parts of the fresh gland, so that Hunt's results would indicate a content of 0·25 per cent. in the latter.

For the following observations on the occurrence of adrenaline in man I have to thank Dr. T. R. Elliott, F.R.S., of University College Hospital.

At birth adrenaline is almost absent from the supra-renals, but a large load of it is found in the paraganglion aorticum.[1] Thus in a full term child examined three hours after death :—

paraganglion, 0·11 grm. = ·24 mg. adrenalin
left supra-renal, 2·7 grm. = ·01 mg. adrenalin.

The normal weight of each adult supra-renal gland is about 5 grm.; in cases of sudden accidental death it contains about 5 mg. of adrenaline, or about 0·1 per cent.

The adrenaline content rapidly sinks in fevers; in fatal cases of pneumonia it may be reduced to 1 or 2 mg. Similar exhaustion occurs with the prolonged septicæmia of malignant endocarditis, but in no fever does it proceed to the minimal values found in Addison's disease, so that death in fevers cannot be ascribed simply to supra-renal failure.

In chronic kidney disease, accompanied by high blood pressure, there is no hypertrophy of the supra-renals, and the glands yield much

[1] Compare Elliott [1913]. Fenger [1912, 2] finds on the other hand, by a colorimetric method, that the gland of the young fœtal calf contains as much adrenalin as the adult organ. If the discrepancy is not due to the difference in species, it might be that the fœtal gland contains a physiologically inert precursor of adrenaline, giving a similar colour reaction.

the same residual load of adrenalin, 2 or 3 mg., as would be found in any other individual dying similarly without kidney disease.

The supra-renal gland of mammals is made up by the close association of two tissues, the cortex and the medulla, corresponding respectively to the inter-renal and adrenal tissues of the lower vertebrates, in which the two kinds of tissue are less closely associated. In fishes they occur separately. The medullary substance, also called chromophil or chromaffin on account of its being stained brown by chromates, alone contains adrenaline (see for example Gaskell [1912]). This tissue is also present in the paraganglia, associated with the sympathetic system of mammals, including the carotid gland, and the fœtal organs described by Zuckerkandl. An extract of these paraganglia has been shown to possess the physiological action of adrenaline. Further details concerning the distribution of chromophil tissue are contained in Vincent's article in the "Ergebnisse der Physiologie" [1910, under general references to Ch. VI] and Biedl's "Innere Sekretion" [1913, general references to Ch. VI]. Recently the remarkable discovery has been made by Abel and Macht [1911, 1912] that adrenaline occurs in the secretion of the so-called "parotid gland" (on the skin behind the ear) of a tropical toad, *Bufo agua*. The amount of adrenaline in the dried venom is as much as 5 per cent.; the substance is chemically and physiologically identical with the adrenaline from the supra-renal gland of mammals; in particular the rotation was found to be $[\alpha]_D$ at 20° = − 51·30°, in perfect agreement with the value given by Flächer (− 51·40°, see above).

Bufo agua is not immune to its own poison and reacts to adrenaline in the same way as the frog. As might be expected the tissue of the poison gland gives an intense chromophil reaction with chromic acid. According to Gunn [1911] cobra venom injected intravenously has a pressor action like that of adrenaline.

Adrenaline is continuously secreted by the supra-renal gland and is therefore present in appreciable quantity in the blood of the supra-renal vein; Cybulski [1895] first demonstrated the pressor action of the blood from this vein, in which the adrenaline concentration is of the order of 1 : 1,000,000. Adrenaline must therefore also be present in the blood of the general circulation, but the amount is so small that it cannot be demonstrated with certainty (O'Connor [1912, 1], Stewart [1912]). Adrenaline has been said to occur in the urine in nephritis, but the evidence is doubtful, and this also applies to pathological sera.

It has lately been shown that the secretion of adrenaline is controlled by the splanchnic nerves (Asher [1912], O'Connor [1912, 2], Elliott [1912], Dale and Laidlaw [1912, 2]). Cutting these nerves stops the secretion. The supra-renals may be exhausted by fright, by tetrahydro-β-naphthylamine and by morphia, but if one of the splanchnic nerves is cut, the gland on that side is not exhausted (Elliott). Peripheral electrical stimulation of a cut splanchnic nerve produces the same effects as an injection of adrenaline. An injection of nicotine and other alkaloids also stimulates the gland to excrete adrenaline (Cannon, Aub and Binger [1912], Dale and Laidlaw [1912, 2]).

Asphyxia also increases the adrenaline secretion (Cannon and Hoskins [1911-2]). The constriction of peripheral blood vessels on stimulation of the splanchnic nerves (von Anrep [1912]) and the effect of carbon dioxide on the vascular system (Itami [1912]) are both due to increased secretion of adrenaline.

Cannon and de la Paz [1911] were the first to show that the secretion may be stimulated by emotion; they placed a cat near a barking dog and found that the blood from the cat's supra-renal vein contained an increased amount of adrenaline, as shown by its action on strips of muscle from the rabbit's intestine. It is possible that the supra-renals obtained from slaughterhouses for this reason contain less adrenaline than is normally present. Connected with this is emotional glycosuria (Cannon, Shohl and Wright [1911-2]).

Nothing is known of the nature of the parent substance from which adrenaline is derived. The base is obviously more closely related to tyrosine than to any other known constituent of protein, and Halle [1906] has asserted that the adrenaline content of the supra-renals is increased when they are incubated with tyrosine, but this assertion has been disproved by Ewins and Laidlaw [1910, 1]. Abelous and his pupils considered at one time that adrenaline is formed by incubating supra-renals with muscle, but the increased pressor activity of the mixture was later found to result from the meat alone, which underwent putrefaction so that p-hydroxyphenyl-ethylamine was formed (see p. 26). It has been suggested that adrenaline might be derived from a di-hydroxyphenyl-methyl-serine (by decarboxylation), but for this there is not the slightest evidence. It should, however, be noted that Guggenheim [1913] has isolated the amino-acid 3 : 4-dihydroxyphenylalanine, $(OH)_2C_6H_3 . CH_2 . CH(NH_2) . COOH$, from the pods of *Vicia Faba*.

Physiological Action of Adrenaline.

A. Action on the Circulatory System.

Oliver and Schäfer [1894, 1895, 1] and soon afterwards Cybulski [1895] and Szymonovicz [1895] found that intravenous injection of supra-renal extracts causes a very marked rise of arterial blood pressure; this effect is due to the adrenaline contained in such extracts. Oliver and Schäfer showed that the rise of blood pressure is mainly due to the constriction of the arterioles, but that the action of the mammalian heart is also accelerated and augmented in a remarkable manner, the acceleration being most prominent when the vagi have been cut (cf. Gottlieb [1897]). The vaso-constriction is chiefly of peripheral origin, due to the action of the drug on the walls of the arterioles, but some authors have asserted that the vaso-motor centre also plays a part. Oliver and Schäfer [1895, 2] further showed that the activity is confined to extracts of the supra-renal medulla, those of the cortex being inactive or nearly so; the extracts of the gland in two cases of Addison's disease were also found by them to be inactive.

Cybulski [1895] detected the pressor action of the blood from the supra-renal vein.

Very minute doses of adrenaline are sufficient to produce a distinct effect; according to Cameron [1906] 0·0003 mg. per kilo. is enough in rabbits. The latent period is short and the rise of blood pressure begins a few seconds after intravenous injection. The rise is very transitory and the blood pressure soon falls again to the normal level, at first rapidly, then more slowly. In Oliver and Schäfer's experiments, the rise lasted in dogs for at most 4 minutes, and in rabbits for at most 6 minutes. This rapid cessation of the pressor action, which is very characteristic of adrenaline, was first attributed to a disappearance of the base from the blood, but Weiss and Harris [1904] were able to show that after the blood pressure has returned to the normal, the blood still contains adrenaline, capable of raising the blood pressure when injected into another animal (cat), and of producing vaso-constriction, when allowed to flow into a previously ligatured limb of the animal experimented upon (hind limb of frog).

In man a rise of blood pressure may be produced by subcutaneous injection of adrenaline, but the effect is much less marked than with intravenous doses, since the local vaso-constriction, set up at the site of injection, does not allow a sufficiently rapid absorption of the drug. This prevents the maintenance of a sufficiently steep gradient of concentration between the adrenaline in the blood and that in the arterial

walls, and it is this gradient which according to Straub's theory is necessary for the action of certain alkaloids, which only act during and by virtue of their penetration into the sensitive cells. If the gradient is maintained by a continuous slow flow of adrenaline into the blood stream, the pressure may be kept at a high level for hours at a time, as shown by Kretschmer [1907]. Compare also Straub [1909].

When given by the mouth, adrenaline is without pressor action. Applied to a mucous surface, it causes marked local vaso-constriction and blanching; on this property depends the chief use of adrenaline as a hæmostatic in surgery. The repeated intravenous injection may cause serious damage to the arterial walls and bring about arterio-sclerosis.

B. Action on other Organs containing Involuntary Muscle and on Glands.

Besides affecting the heart and blood vessels, adrenaline acts on plain muscle in many organs of the body. Thus the muscles in the wall of the alimentary canal, excepting the sphincters, become relaxed and their automatic movements cease. The bladder in most animals is relaxed, but in some it contracts. The uterus is also very sensitive to adrenaline; that of the rabbit and of the pregnant cat contract, but the non-pregnant cat's uterus is relaxed. The amounts of adrenaline which bring about these effects are as minute as those required for the pressor action, or even more minute. Kehrer [1908] obtained tetanic contraction of the pregnant cat's isolated uterus in a bath containing adrenaline in a concentration of 1 in 350,000,000.

The plain muscle which has perhaps been most commonly employed as a test object for adrenaline is that of the pupil. The mydriatic action of adrenaline after intravenous injection was noted cursorily by Vincent [1897-8] and was first described in detail by Lewandowsky [1898, 1899]. S. J. and C. Meltzer [1904, 1] suggested the reaction of the frog's eye as a means for determining the strength of adrenaline solutions, and Ehrmann [1905] subsequently worked out a method, based on this reaction, which enabled him to detect quantities of adrenaline as small as 0·000000002 grm.

The above apparently divergent actions of adrenaline on plain muscular organs may be viewed from a common standpoint if it is borne in mind that these organs are innervated by branches of the sympathetic system and that the electrical stimulation of sympathetic nerves produces effects similar to those caused by adrenaline (Lewandowsky, Boruttau, Langley, Elliott). The action of adrenaline (and

of a large number of related amines) resembles that of the sympathetic nervous system and has accordingly been termed by Dale [Barger and Dale, 1910, 1] "sympathomimetic". Adrenaline does not, however, affect the sympathetic nerves themselves, for, as has been shown by Lewandowsky [1899, 1900], Langley [1901] and Elliott [1905], the reactivity of plain muscle to adrenaline is not diminished (but rather increased) by cutting the sympathetic nerve supply and allowing the nerves to degenerate. Moreover apocodeine, as Dixon has shown, abolishes the excitability of muscle by sympathetic nervous impulses, *and by adrenaline*, but leaves all other irritability unaffected. The blood vessels of the lungs, which have no sympathetic innervation, are on the other hand not affected by adrenaline, according to Brodie and Dixon [1904].[1] In order to account for the persistence of the adrenaline action after degeneration of the sympathetic nerve supply, Elliott [1905] has invoked a hypothetical structure, the "myo-neural junction," which does not degenerate with the nerve and is the seat of the action of adrenaline. Langley's conception of a "receptive substance" for adrenaline is in most essential respects identical with Elliott's. The nature of the myo-neural junctions determines the response to adrenaline, i.e. whether inhibition or augmentation takes place. Thus these structures would differ in different animals; in some species the augmentor elements would predominate, so that adrenaline causes contraction, in others the reverse condition would prevail. Similarly, during pregnancy, in the cat, the augmentor elements of the uterine myo-neural junctions would achieve preponderance over the inhibitor elements, which predominate in the non-pregnant animal.

The existence, side by side, of two kinds of elements, augmentor and inhibitor, receives considerable support from the discovery by Dale [1906], that the alkaloid ergotoxine paralyses one set of elements without greatly affecting the other. Thus the large rise of blood pressure which adrenaline causes in the normal animal is replaced by a (smaller) depressor effect, if ergotoxine has been previously administered. The ergotoxine paralyses the augmentor elements only (which normally overcome the inhibitor effect) so that, after ergotoxine, the inhibition becomes evident and a "vaso-motor reversal" occurs.

[1] A different conclusion was reached by Wiggers [1909] who attributes Brodie and Dixon's results to their use of a perfusion fluid of smaller viscosity than that of the blood. Older experiments of Plumier and of Langendorff also indicate that adrenaline causes the pulmonary vessels to contract, but Cow [1911] using O. B. Meyer's method (p. 103) finds that the intravisceral portion of the pulmonary, the cerebral and the coronary arteries are not constricted. The action of adrenaline on the pulmonary vessels has also been studied by Baehr and Pick [1913, 2, Ch. I].

In this connection it is of some interest that Ogawa [1912] has recently shown that when the blood vessels of certain isolated organs (e.g. kidney of dog, cat and rabbit) are perfused with very dilute adrenaline solutions (1 : 50 millions) these vessels are dilated. With slightly more concentrated solutions a constriction occurs followed by a secondary dilatation ; larger doses at once produce constriction without subsequent dilatation.

Adrenaline, injected intravenously, causes the *bronchioles* to dilate and abolishes the contraction due to muscarine (Januschke and Pollak [1911]; confirmed by Dixon and Ransom [1912]; see also Jackson [1912]; Golla and Symes [1913]; Baehr and Pick [1913, I, Ch. I]). Hence adrenaline is used in the treatment of asthma.

Action on Glands.—Langley [1901] has shown that an injection of adrenaline excites the secretory activity of salivary and other glands, and this action, as in the case of plain muscle, apparently persists after the degeneration of the sympathetic nerve supply.

C. Action on Carbohydrate Metabolism.

As was first shown by Blum [1901], subcutaneous or intravenous injections of supra-renal extract (in sufficient doses) cause glycosuria ; this action is due to the adrenaline and does not occur after oral administration. The latent period is much longer than in the case of the pressor action and sugar may occur in the urine for several days after the injection. In other respects there is a close analogy to the pressor action. Straub [1909] found adrenaline could be injected continuously at the rate of 0·002 mgm. per minute without causing glycosuria, but that sugar appeared in the urine when the rate of injection was doubled. This is about the same as found by Kretschmer [1907] for the pressor action. Although much work has been done on the subject, the mechanism of adrenaline glycosuria, like that of other forms of glycosuria, has not yet been cleared up. It appears that adrenaline causes a greatly increased production of glucose by the liver and that adrenaline glycosuria is independent of the pancreas. (Compare for instance experiments on birds, after extirpation of the pancreas, by Paton [1903, 1904].)

Pollak [1909] concludes from his experiments on hungering rabbits that adrenaline causes an accumulation of glycogen in the liver. Any injection of the drug will also increase the sugar content of the blood, but glycosuria does not necessarily occur ; it will do so more probably if diuresis is also set up. In a later paper Pollak [1910] denies the alleged special protective action of d-adrenaline against the

diabetic effect of the natural l-variety. The minimal dose of the latter which produces glycosuria in rabbits of 2 kilos. is 0·4-0·5 mg.

D. Toxic Action of Adrenaline.

The effects which have so far been described are all brought about by minute doses of adrenaline. Larger, although still quite small doses cause death, and adrenaline is therefore a powerful poison. For guinea-pigs, rabbits, and dogs the fatal intravenous dose is about one-tenth to one-quarter of a milligram per kilo. of body weight. For cats the corresponding dose is 0·5-0·8 mg. per kilo. The subcutaneous lethal dose is very much higher; for white rats Cushny [1909] found 10-20 mg. per kilo. and Schultz [1909, 1] for mice 8 mg. per kilo. of body weight. For guinea-pigs the corresponding dose is 10 mg. according to Crawford [1907]. For the toxicity to dogs and cats, reference may also be made to Lesage [1904, 1, 2].

The Physiological Action of Dextro- and of Racemic Adrenaline.

Cushny, who discovered the difference in the physiological activity of optical enantiomorphs in the case of hyoscyamine and hyoscine, also first drew attention to the quantitative differences in the action of natural l-adrenaline and the synthetic racemic substance.[1] He [1908] found racemic adrenaline to be about half as active as the natural variety and concluded therefore that d-adrenaline is inactive. Later [1909], having at his disposal a specimen of the dextro-variety, he was able to estimate its activity directly, instead of by difference, and he slightly revised his preliminary conclusion. The specimen of d-adrenaline examined had $\frac{1}{12}$-$\frac{1}{15}$ of the activity of l-adrenaline in raising the blood pressure of dogs and cats. The ratio of the pressor activities of racemic and natural adrenaline is therefore not 1 : 2 but between 13 : 24 and 16 : 30. The ratio of the activities of the two isomerides in producing glycosuria was very similar, namely 1 : 12-18, and the minimal lethal doses for white rats were in about the same ratio.

The different physiological activity of the two enantiomorphous adrenalines has also been dealt with in a series of papers by Abderhalden, in collaboration with Müller [1908], Thies [1909], Kautzsch [1909], Slavu [1909], and Kautzsch and Müller [1909]. Some of the conclusions arrived at are that l-adrenaline is fifteen times as

[1] Cf. Dixon, Pharm. Journ., 1908, XXVI, 723; Biberfeld, *ibid.*, p. 626; Cushny, *ibid.*, p. 668.

active on the blood pressure as d-adrenaline, that the effects of the two isomerides on the frog's eye and in producing glycosuria are different, and that d-adrenaline establishes a tolerance to the toxicity of l-adrenaline. Of these experiments those on the frog's eye, by Abderhalden and Thies, and the toxicity experiments, by Abderhalden and Slavu, have been criticised by Schultz [1909, **2**].

Schultz had previously [1909, **1**] carried out an extensive series of very careful experiments on the relative activity of racemic and l-adrenaline; it is a matter for regret that he was not also in possession of a pure specimen of the dextro-variety. He found the pressor effect of the natural base to be one and a half times that of the racemic synthetic product. Dale [Barger and Dale, 1910, **1**] obtained the same ratio (6·5 : 10) but does not regard the discrepancy from Cushny's ratio (16 : 30) as having any significance. Biberfeld's original statement [1908] that the racemic base is as active as the lævo-variety is certainly erroneous. Schultz [1909, **2**] states that the ratio of the activities of dl- and l-adrenaline on the frog's eye and the toxicity ratio for white mice is the same as that of the pressor activities, namely 1 : 15.

Various authors have suggested that d-adrenaline renders the organism less sensitive to the action of the natural l-variety and to some extent confers an "immunity," so that subsequent doses of l-adrenaline have a much smaller effect than is normally the case. This has been claimed for the pressor action by Fröhlich [1909], for the toxicity (to mice) by Abderhalden in collaboration with Slavu [1909] and with Kautzsch [1909], and for the diabetic action by Waterman [1909, 1911]. With regard to the last-named effect Pollak [1909, 1910] has, however, come to a different conclusion and considers that d-adrenaline is as little able to prevent glycosuria by l-adrenaline as a previous dose of l-adrenaline itself. A phenomenon, similar to that observed by Fröhlich, has recently been described by Ogawa [1912] who finds that the secondary vaso-dilatation referred to above (p. 99), when due to d-adrenaline, is not so readily abolished by l-adrenaline as the dilatation caused by (smaller doses of the more active) l-adrenaline.

Physiological Methods of Estimating Adrenaline.

At a time when little was known of the chemistry of adrenaline, the methods employed in its estimation were perforce physiological, and even now the best physiological methods are preferable to the

colorimetric processes which have been suggested more recently (see p. 92). The quantitative estimation of adrenaline is of importance in many physiological and pathological investigations.

The most obvious, accurate and reliable method is based on a comparison of the pressor effects of intravenous injections; the peculiarly evanescent nature of this adrenaline action greatly favours accurate comparison, and in a suitably prepared animal equal submaximal doses will produce time after time practically identical effects; this method is, however, inapplicable to very dilute adrenaline solutions. The blood pressure of a cat, with brain and spinal cord destroyed and without anæsthetic, reacts according to Elliott [1912] "with mechanical accuracy," and by comparison with a standard solution, Elliott assays the adrenaline content of the cat's supra-renal gland with an error of 0·01 mg., which is 3-4 per cent. of the total amount present.

The accurate pharmacological assay of preparations of the supra-renal gland by means of the blood pressure was first carried out by Houghton [1901]; the blood pressure has further been used especially by Elliott [1912], Hunt [1906], Sollmann and Brown [1906], Cushny [1908, 1909], Schultz [1909, 1], and Dale [Barger and Dale, 1910, 1]. Schultz employed dogs (with morphine, ether and curari) and cats (with ether), Elliott and Dale almost exclusively decerebrate cats. The doses are $\frac{1}{100}$-$\frac{1}{30}$ mg. for dogs and $\frac{1}{50}$-$\frac{1}{20}$ mg. for cats (which are more resistant than dogs). Other blood-pressure methods, such as the determination of the dose required to compensate for the vaso-dilator action of a given quantity of nitroglycerine (Cameron [1906]) and the determination of the minimal dose necessary to give a perceptible pressor effect, are much less accurate.

A second method employing the circulatory system but depending on vaso-constriction instead of on blood pressure is due to Läwen [1903-4] and has been improved by Trendelenburg [1910]. The rate is measured at which, under a constant hydrostatic pressure, blood flows through the vessels of a frog, of which the brain and spinal cord have been destroyed; the adrenaline to be estimated is added to the blood. This method appears to yield moderately accurate results, but is laborious when many estimations have to be performed. The significance of determinations by this method of adrenaline in serum has recently been questioned by O'Connor [1911, 1912, 1] who finds that serum itself causes vaso-constriction, quite apart from the addition of adrenaline (see also Handovsky and Pick [1913, Ch. I]). Stewart [1912], and Dale and Laidlaw [1912, 2] agree with O'Connor's objections to the

use of serum. According to Stewart it is possible to prove the presence of adrenaline only in the blood from the supra-renal vein.

Besides those on the circulatory system, the other effects of adrenaline on plain muscle, described in a previous section, are to some extent available for the quantitative estimation of the drug; the methods which have been suggested, based on these effects, are much less accurate than the blood-pressure method, but, on the other hand, some of them are more suitable for the very rough estimation of extremely minute quantities of adrenaline, such as may occur in the blood or in tissue extracts. In such cases it is, however, necessary to avoid confusion with other ill-defined substances (such as vaso-dilatin, p. 30) which may produce similar effects in plain muscle (cf. Hoskins [1911] and O'Connor [1912, 1]).

O. B. Meyer [1906] has employed isolated rings of the subclavian or carotid artery of the ox, which contract in solutions of adrenaline up to 1 : 1,000,000,000 (0·000015 mg. in 15 c.c. Ringer's solution). Cow [1911] has investigated other arteries by this method and finds that the only arteries not constricted by adrenaline are the intravisceral portion of the pulmonary, the coronary and the cerebral arteries. Argyll Campbell [1911] also finds by this method that adrenaline causes marked constriction of the vessels of all organs, except those of the heart and lungs. A slight constriction occurs occasionally in the heart and more frequently in the lung vessels.

A. Fränkel [1909] used the isolated uterus of the rabbit, which still reacts to adrenaline at a dilution of 1 : 20,000,000, but Hoskins [1911] states that this reaction is not specific and that contractions are caused by a large number of glandular and tissue extracts; the use of the rabbit's uterus for testing serum has also been criticised by Stewart [1912].

Cannon and de la Paz [1911] employed longitudinal strips of muscle from the rabbit's intestine and Hoskins [1911] a short length of small intestine from the same animal. These two methods depend on the *inhibition*, by adrenaline, of the spontaneous contractions. In Hoskins's experiments this inhibition occurred regularly at 1 : 100,000,000 and sometimes even at 1 : 500,000,000. Hoskins considers his method and that of O. B. Meyer (above) to be the most sensitive methods known. According to O'Connor [1912, 1] substances are formed during the coagulation of blood with actions simulating this and other effects of adrenaline, but by using the plasma, instead of the serum, and rabbit's intestine as test object, he finds that the blood from the

supra-renal vein contains one part of adrenaline in 1 to 5 millions; he could not demonstrate adrenaline with certainty in the peripheral blood. Stewart, who employed this method and that depending on the contraction of the rabbit's uterus, also concludes that adrenaline is not detectable in the general circulation, or indeed in blood from the supra-renal vein, except during massage of the gland or stimulation of the splanchnics, when there was respectively 1 : 500,000 and 1 : 1,000,000.

Dale and Laidlaw [1912, 2] have used as a test object another organ which is inhibited by adrenaline, viz. the non-pregnant uterus of the cat. In a cat under chloroform and ether they find that the blood from the supra-renal vein contains one part of adrenaline in from 1 to 2 millions. After injection of pilocarpine this amount was increased tenfold.

The method which has been most widely used for the detection of small quantities of adrenaline is based on mydriatic action, particularly as applied to the excised eye of the frog. This test object was first employed by S. J. and C. Meltzer [1904, 1, 2]; later Ehrmann [1905] brought it into prominence by his experiments on body fluids and by his claim that the excised eye, being much more sensitive than the intact eye, can reveal adrenaline in a concentration of 1 : 10,000,000. According to Borberg [1912] the sensitiveness is only one-tenth of this. Schultz [1909, 1] has elaborated the technique of this method by measuring the pupil under the microscope. Hoskins [1911] dissected the eye, removed the lens and applied the fluid under examination directly to the iris; in this way results were obtainable at a dilution of 1 : 5,000,000 and sometimes a positive result was noted at 1 : 100,000,000, but a mydriatic effect is also shown by pituitary extract, iodothyrin, etc., which renders the method very uncertain when applied to the detection of adrenaline in the blood. Schultz [1909, 2] considers that Ehrmann overstated the sensitiveness of the method. He writes: "At its very best the excised frog's eye as a pharmacological assay for adrenaline is inferior to the blood-pressure method. As a qualitative test it is perhaps one of the most sensitive test-objects known, but it is not a characteristic test (Comessatti, Meltzer) and observations convince me that too much weight ought not to be attached to results with it in clinical diagnosis". This adverse opinion is shared by Cameron [1906] and by Borberg [1912], but the method at least has the advantage that it is applicable to very dilute solutions and that it can be used by the chemist who cannot undertake more elaborate animal experiments. According to Schultz the dilatation time is a better index than the degree of mydriasis and one should

aim at making this time equal for both of a pair of eyes. In a recent article on the estimation of adrenaline in the blood, Gottlieb and O'Connor [1912] place the blood-pressure method first in point of accuracy, provided the adrenaline solution is sufficiently concentrated. Next comes the perfusion of the frog's blood vessels, which may be used quantitatively and is more sensitive (up to 1 : 30,000,000). For the qualitative recognition of the minutest quantities the inhibition of the cat's small intestine is very specific and, in particular, it is not produced by serum (limit 1 : 400,000,000).

CHAPTER VII.

BASES OF UNKNOWN CONSTITUTION.

THE constitution of nearly all the bases dealt with in the preceding chapters is known with certainty. In addition a large number of bases of unknown constitution have been described at various times. In many cases even their composition has not been fully established. Nevertheless some of the latter class will be included here on account of their great physiological interest. It is of course impossible to say whether they have a "simple constitution," but in any case the methods by which their isolation may be attempted are similar to those used for the other bases of this monograph.

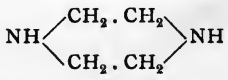
Spermine.

The phosphate of this base crystallises out when semen dries, and constitutes over 5 per cent. of the solids. It has been most fully investigated by Schreiner [1878] who prepared it in a pure condition by boiling fresh human semen with alcohol, filtering off and drying the precipitate so formed, extracting the latter with very dilute warm aqueous ammonia and then concentrating. The phosphate is hardly soluble in cold, and only a little in hot water, but soluble in dilute acids and alkalies. The salt contains two atoms of nitrogen to one of phosphorus, and at 100° $3H_2O$ are given off; it melts at 170°.

Schreiner found that the crystals on the surface of old anatomical preparations (Böttcher's crystals) are identical with spermine phosphate; he obtained them by scraping them off the surface of calves' livers and hearts and bulls' testes, kept in alcohol for three months.

It has further been suggested that the crystals discovered by Charcot in the spleen, liver, and blood in cases of leucocythæmia, and also found in the sputum in cases of bronchial asthma, are identical with spermine phosphate, but this does not appear to be the case.

Schreiner assigned to spermine the formula C_2H_5N; Ladenburg and Abel [1888] considered it to be most probably identical with piperazine $C_4H_{10}N_2$, which has the constitution:—

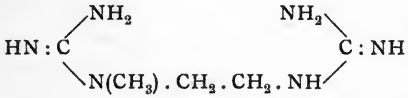


By direct comparison with a specimen of Schreiner's preparation they found a great similarity to piperazine but also some differences. Schreiner's specimen was found to be slightly impure and to contain calcium. Ladenburg and Abel considered that Schreiner's phosphate might conceivably be $(C_4H_{10}N_2)_2CaP_2O_8$ which agrees better with his analyses. Poehl [1891] arrived at the formula $C_{10}H_{26}N_4$ for spermine after analysing the platinichloride and the aurichloride, but the formula $C_5H_{12}N_2$ would also fit his results.

Bases from Muscle.

In addition to creatine, methylguanidine, carnosine, carnitine, neosine, betaine, myokynine, and trimethylamine-oxide, all described previously, the following may be mentioned:—

Vitiatine, $C_5H_{14}N_6$, has been obtained by Kutscher [1907] from meat extract and is regarded by him as a guanidine derivative of the possible constitution:—



Crangitine, $C_{13}H_{20}O_4N_2$, and *crangonine*, $C_{13}H_{26}O_3N_2$, have been obtained by Ackermann and Kutscher [1907, 4, Ch. III, betaine] from shrimps.

Creatosine has been obtained from commercial meat extract by Krimberg and Izraïlsky [1913] and yields an aurichloride

$$C_{11}H_{28}O_4N_3Au_2Cl_8.$$

Bases from Urine.

The following bases, already described, have been isolated as normal or occasional constituents of human or animal urine: trimethylamine, isoamylamine, putrescine, cadaverine, iminazolylacetic acid, urocanic acid, kynurenic acid, methylpyridinium hydroxide, γ-picoline, butyrobetaine, carnitine (= novaine), reductonovaine, creatine, creatinine, methylguanidine, dimethylguanidine, vitiatine. In addition the following may be mentioned:—

Mingine, $C_{13}H_{18}O_2N_2$. Kutscher [1907, Ch. III, butyrobetaine] obtained 0·45 grm. of the di-aurichloride from 100 litres of women's urine.

Gynesine, $C_{19}H_{23}O_3N_3$. Kutscher and Lohmann [1906, 4, Ch. III, butyrobetaine] obtained 1·5 grm. of the aurichloride $C_{19}H_{23}O_3N_3$, $2HAuCl_4$, from 100 litres of women's urine.

Kynosine, $C_{13}H_{26}O_4N_4$, was isolated from normal dog's urine as the aurichloride $C_{13}H_{26}O_4N_4$, $2HAuCl_4$ by Kutscher [1906].

Putrefaction Bases.

In addition to the amines of Chapter I and some other bases mentioned in the previous chapters a large number of less well characterised putrefaction bases have been described. A few of these may be mentioned here:—

Viridine, $C_8H_{12}O_3N_2$, was obtained by Ackermann [1908, **2**] from putrid pancreas. The hydrochloride has an intense green colour; on heating the odour of quinone is perceptible. The aurichloride is blackish green to yellow and melts at 176°; the platinichloride is intense yellow and melts at 212-216°.

Marcitine, $C_8H_{19}N_3$, also obtained by Ackermann [1907, **2**] from putrid pancreas, gives an aurichloride $C_8H_{19}N_3$, $2HAuCl_4$ melting at 175-178°. It is perhaps a guanidine derivative.

Putrine, $C_{11}H_{26}O_3N_2$, likewise isolated by Ackermann [1907, **2**] from putrid pancreas, gives a dark orange aurichloride melting at 109-110°. The formula of this base contains one carbon atom and two oxygen atoms less than the so-called diamino-trihydroxy-dodecanic acid $C_{12}H_{26}O_5N_2$ of Fischer and Abderhalden from which it is perhaps derived by decarboxylation.

Skatosine, $C_{10}H_{16}O_2N_2$, has been described by Baum [1903] and Swain [1903] as a product of pancreatic autolysis. It is stated to give a benzoyl derivative melting at 169° and a hydrochloride forming leaflets melting at 345°. To the latter the improbable formula $C_{10}H_{16}O_2N_2$, $3HCl$ was given. Mr. A. J. Ewins (private communication) has lately failed to obtain this base by Baum's process.

The Active Principle of the Pituitary Body.

Soon after their discovery of the pressor action of supra-renal extracts Oliver and Schäfer [1895, **3**] found that an extract of the pituitary body or hypophysis cerebri (a small appendage at the base of the brain) has the power of raising the blood pressure, when injected intravenously. The active principle is only contained in the infundibular or posterior lobe of this organ. At first stress was laid in the literature on the similarity of the action to that of adrenaline, and some authors even imagined that the two active principles must have a similar chemical constitution. During the last few years pituitary extracts have come more and more into therapeutic use on account of their great power of producing contractions of the uterus, and the isolation of the active principle has been attempted. Although these attempts have perhaps not been wholly successful as yet, they seem to prove that the active substance is a base; little else is definitely known about its chemical

constitution. Its physiological action has, however, been studied in some detail and such correspondence as exists between the action of the pituitary body and of adrenaline has been found to be "superficial and illusory".

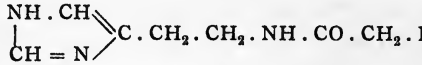
The chemical investigation of the pituitary active principle is greatly hampered by its instability and by the difficulty of procuring enough material. The infundibular portions, dissected clean from fresh glands, are ground up with sand and boiled with water acidulated with acetic acid. After filtration a clear colourless extract is obtained, which contains a little protein and some phosphates. By the addition of uranyl acetate the phosphates may be precipitated and most of the protein is carried down with the precipitate, but the solution remains physiologically active. Almost the only precipitant for the active principle itself is phosphotungstic acid, as has for instance been found by Engeland and Kutscher [1911] and by Meister, Lucius and Brüning (see Führer [1913]). The chemists of the Hoechst firm, on decomposing the phosphotungstate with baryta, and removing the excess of baryta with sulphuric acid, obtained on concentration *in vacuo* a pale yellow crystalline sulphate, which was physiologically active and apparently homogeneous, but was afterwards separated by fractional crystallisation into four different substances, all crystalline, and all having some physiological activity. Two of these were more active than the others; the more abundant of the two is a colourless sulphate, readily soluble in water, but only slightly so in alcohol, acetone, or ethyl acetate. It gives Pauly's histidine reaction with p-diazobenzene-sulphonic acid and also the biuret reaction. Its picrate is readily soluble in water. In contact with alkali a volatile amine is at once given off.

According to Führer, who has examined physiologically the various substances from the phosphotungstate, they all contribute to the activity of the gland; thus there would be four active principles.

The facts at present available do not, however, absolutely exclude the possibility that these four substances all owe their activity to contamination, in various degrees, with one and the same highly active substance which has so far escaped isolation. The further chemical examination of the most active of the four substances should prove of great interest. That this substance gives the biuret reaction may be considered in conjunction with an observation by Dale [1909] that the activity of pituitary extracts is rapidly destroyed by trypsin and much less rapidly by pepsin. This would point to a polypeptide structure. The activity is also fairly rapidly lost when an aqueous solution is evaporated to dryness; perhaps this is owing to hydrolysis.

The fact that the bases from a pituitary extract give the Pauly reaction suggests a connection with histidine, and moreover β-iminazolylethylamine, which is obtained from histidine by decarboxylation, also causes powerful contractions of the uterus. Possibly, therefore, the pituitary active principle is a polypeptide-like derivative of histidine.

Guggenheim [1913] has lately synthesised a number of bases by combining amines with chloracetylchloride and treating the product with ammonia. In this way, for example, glycyl-β-iminazolylethylamine



was prepared. The bases of this type, for which the name *peptamine* is suggested, are therefore decarboxylated polypeptides; their physiological action is of the same kind as the amine from which they are derived, but much weaker.

The physiological action of pituitary extracts has been investigated chiefly by Schäfer, in conjunction with Oliver [1895, 3], Magnus [1901], Herring [1906] and Mackenzie [1911], and further by Dale [1909], von Frankl-Hochwart and Fröhlich [1910], Pankow [1912] and others. Pituitary extract produces a direct stimulation of involuntary muscle, without any relation to innervation. Here there is, therefore, an important difference from adrenaline which stimulates sympathetic nerve endings (see p. 98). The action of pituitary is most nearly allied to that of the digitalis series, but the effect on the heart is slight, that on plain muscle intense. The rise of blood pressure caused by pituitary is thus due to the stimulation of the plain muscle of the arterioles. The rise is much smaller than in the case of adrenaline and lasts much longer. A further difference is, that when the blood pressure has returned to the normal, the rise caused by adrenaline can at once be reproduced by a second dose, but in the case of pituitary the effect of a second dose is much smaller, unless it is administered after a considerable interval of time. In the birds pituitary extract causes a fall of blood pressure, which is antagonised by adrenaline and by barium (Paton and Watson [1912]). The powerful stimulation of uterine plain muscle was first pointed out by Dale [1909] and also studied by von Frankl-Hochwart and Fröhlich [1910] and was first applied clinically by Bell [1909] in England and soon afterwards by Foges and Hofstätter in Germany. The supposed pure substances have been used clinically by Herzberg [1913].

Pituitary extracts bring about contraction of the uterus in the cat, dog, guinea-pig, rat, and rabbit, in all functional conditions. Adrenaline, on the other hand, in some of these species has a motor effect on the pregnant uterus only and inhibits the non-pregnant organ. The effect of pituitary extracts on the uterus can be shown both by intravenous injection into the anæsthetised animal and by means of the surviving uterus in a bath of oxygenated Ringer's solution. The latter method, applied to the uterus of the young virgin guinea-pig, has been worked out by Dale and Laidlaw [1912, 1] to a process for standardising pituitary extracts and has also been used more recently by Fühner [1913]. It has the great advantage over blood pressure experiments that tolerance is practically absent. Dale and Laidlaw find that $\frac{1}{200}$ c.c. of an extract obtained by boiling infundibula with five parts of water will produce almost maximal tonus of the uterus in a bath of 250 c.c. Ringer solution. Since such an extract only contains about 0·6 per cent. of solids, this represents a concentration of little more than 0·1 mg. of solid matter per litre, most of it being inert material. The pituitary active principle is therefore a very powerful uterine stimulant, the activity being probably at least of the same order as that of β-iminazolyl-ethylamine.

In addition to the above effects on plain muscle, pituitary extracts bring about a profuse flow of urine and also greatly increased secretion of milk. The diuretic action was discovered by Schäfer in conjunction with Magnus and with Herring and was at first attributed to a different substance from that causing the rise of blood pressure; later observers, however, consider that the active principle is the same in both these cases. According to Houghton and Merrill [1908] diuresis is merely a secondary effect of the rise in blood pressure and is also brought about by injecting adrenaline. The galactagogue action was first observed by Ott and Scott [1911] and has subsequently been described by Schäfer and Mackenzie [1911], and Hammond [1913]. For the effect on the mammary gland in the human subject see Schäfer [1913].

Vitamine, Oryzanine, Toruline.

A polyneuritis, resembling the tropical disease beri-beri, can, as Eykman discovered, be induced artifically in fowls by feeding them on an exclusive diet of polished rice. The condition is due to the lack of a substance present in the outer coating of the rice and removed in the process of polishing. During the last year or two several attempts have been made to isolate this curative substance from various sources.

Funk [1911] in England, and Suzuki with Shimamura and Odake [1912] in Japan, showed independently and about the same time that the substance is a base, is present in very small amount, and has great curative action. To Funk belongs the further credit of having been the first to analyse the substance and to isolate the same or a similar body from yeast. Chemical work in this direction has also been done by Schaumann [1912, 1], Moore and his collaborators [1912], Cooper [1913] and others.

In spite of the discrepancies which exist between the statements of various authors, it seems fairly well established that the curative substance in rice polishings, for which Funk has suggested the name *vitamine* and which Suzuki and his collaborators call *oryzanine*, is a base which can be extracted by water and by alcohol, but not by acetone or ether. It is precipitated by phosphotungstic acid, by tannin, by mercuric chloride in alcoholic solution and by silver nitrate and baryta. The latter property indicates the presence of an imino-group. The mercurichloride is soluble in boiling water.

Suzuki, Shimamura and Odake describe a crystalline picrate of their substance, which they did not however analyse. Funk, by utilising the properties indicated above, obtained from rice polishings a minute yield of a crystalline substance, to which he assigned the formula $C_{17}H_{20}O_7N_2$, but more recently [1913], by fractional crystallisation, he separated it into two substances; one of these was found to give the following average analytical results: C = 58·85 per cent., H = 3·9 per cent., N = 10·6 per cent.; it melted at 233°. The other gave on the average C = 58·4 per cent., H = 4·0 per cent., N = 11·05 per cent., and melted at 234°. The latter was identified as nicotinic acid, $C_6H_5O_2N$, which, in the pure state, is inactive and had already been obtained from rice by Suzuki. To the former substance Funk gave the formula $C_{26}H_{20}O_9N_4$ and he stated that it is a tetrabasic acid. It is considered by Funk to be the chief curative substance in rice polishings. Funk separated the "vitamine" fraction of yeast, which he at first considered to be identical with that of rice, into nicotinic acid and an active principle melting at 229° (corr.) which when dried *in vacuo* at room temperature has the formula $C_{26}H_{21}O_9N_5$, but dried at 100° changes to $C_{24}H_{19}O_9N_5$, implying the somewhat unusual loss of two carbon and two hydrogen atoms.

It will be seen that the substance $C_{26}H_{20}O_9N_4$ obtained from rice has a very close resemblance to nicotinic acid, both as regards melting point and chemical composition, and at present the possibility does not seem completely excluded, that this body is merely nicotinic acid con-

$C_{12}H_{17}ON_4SCl, HCl$: B_1

taminated with a small quantity of a highly active substance richer in carbon. Further work will therefore be of the greatest interest.

Funk and also Schaumann consider that there are a number of substances capable of preventing and curing polyneuritis. The former [1912, **2**] has found that certain purine and pyrimidine derivatives have a weak activity in this direction. The crystalline and apparently homogeneous vitamine fraction from rice and from yeast is active in doses of a few centigrams, and when injected subcutaneously such doses will restore a severely paralysed pigeon within a few hours. A substance curing polyneuritis is also present in ox brain, in milk (Funk [1912, **1**]), and in muscle (Eykman [1897], Cooper [1913]). Edie, Evans, Moore, Simpson, and Webster [1912] have given the name *toruline* to an antineuritic base from yeast having the formula $C_7H_{17}O_5N_2$. A concomitant effect of a diet of polished rice is a loss of body weight which has been taken into account more particularly in the experiments of Suzuki and his colleagues. In this connection attention may be drawn to the work of Hopkins [1912] which shows that growth is greatly influenced by some as yet undetermined constituents of food.

Sepsine.

The name sepsine was given more than forty years ago by Schmiedeberg to a poisonous putrefaction product which was more recently isolated by Faust [1903-4] as a crystalline sulphate. Faust used putrid yeast and obtained under the most favourable conditions only 0·03 grm. of sepsine sulphate from 5 kilos. of yeast. The process of isolation is a complicated one, one of its chief features being that the sepsine is precipitated by mercuric chloride from an aqueous solution rendered strongly alkaline by means of sodium carbonate. Later the sulphate separates out in a crystalline condition by fractional precipitation of the alcoholic solution of the base by means of sulphuric acid dissolved in alcohol. The sulphate can be recrystallised and then forms well-developed crystals having according to Faust the composition $C_5H_{14}O_2N_2$, H_2SO_4; his analyses, however, fit equally well or slightly better the formula $C_5H_{12}O_2N_2$, H_2SO_4. The free base is a syrup readily soluble in water.

Sepsine is very unstable; on repeated evaporation of the aqueous solution of the sulphate on the water bath this salt is transformed according to Faust into cadaverine sulphate, and the substance loses its physiological activity. This transformation, which involves the loss of two oxygen atoms, is without any analogy and very difficult to

understand. Perhaps the identification of the inactive substance as cadaverine is erroneous, as it is apparently only based on the platinum content of a platinichloride. Perhaps the analyses of sepsine sulphate have been wrongly interpreted. However this may be, it seems clear that a crystalline substance of remarkable physiological properties was obtained, corresponding to those originally possessed by the putrid yeast and described by Schmiedeberg.

Twenty mg. of sepsine sulphate injected into a dog of 7-8 kilos. weight very soon cause vomiting and defecation; finally almost pure blood is passed and the poisoning ends fatally; sepsine is a capillary poison.

Fornet and Heubner [1908] have isolated organisms which they imagined produce sepsine and the chief of these they named *Bacterium sepsinogenes*, but in a later paper [1911] they greatly modified their original conclusions. The organism referred to was found not to produce sepsine but a colloidal poison having a similar action and being in some respects comparable to the toxin formed in anaphylaxis.

A further chemical investigation of Faust's sepsine appears to be very desirable, particularly if it could reveal the constitution of this interesting substance.

Secretine.

This substance, which causes secretion of pancreatic juice when injected intravenously, appears to be a base, judging from a method of purification described by Dale and Laidlaw [1912, 3]. This is founded on the solubility of the mercury compound in moderately dilute acid and its insolubility in neutral or weakly acid solution. Dale and Laidlaw's method may be given as an additional example of the technique of using mercuric chloride for the separation of bases (cf. p. 119).

The mucous membrane of the intestine of dogs is scraped off weighed and ground up with one-fifth of its weight of solid mercuric chloride to a smooth paste; then two parts of water are added for every part of the mucous membrane taken. This mixture can be accumulated and kept indefinitely; the mercuric chloride coagulates the protein and acts as an antiseptic. To work up the mixture it is boiled, filtered through paper or muslin, and pressed dry. The press cake is suspended in an aqueous 1 per cent. mercuric chloride solution containing acetic acid; 4 c.c. of this are used for every gram of moist mucous membrane taken. The mixture is boiled and filtered, and the filtrate should be nearly clear. Ten per cent. sodium hydroxide is added

until the filtrate is nearly neutral, i.e. until the yellow mercuric oxide just fails to be permanent. The white flocculent precipitate formed is collected at the pump, suspended in hot water, and decomposed by hydrogen sulphide; after neutralising and boiling off the hydrogen sulphide the solution is filtered and then furnishes a strongly active secretine solution. The active substance can further be precipitated from this solution by excess of picric acid, but attempts to obtain it chemically pure have so far been unsuccessful.

CHAPTER VIII. (APPENDIX.)

PRACTICAL CHEMICAL METHODS AND DETAILS.

A. General Methods for the Separation and Isolation of Bases.

With few exceptions the simple natural bases are readily soluble in water, but not in ether or chloroform. As a rule they cannot therefore be extracted from alkaline solution by shaking with organic solvents, and the methods of Stas and Dragendorff, employed for the isolation of vegetable alkaloids and based on the use of solvents immiscible with water, are therefore not applicable. The earliest work on putrefaction bases, therefore, suffered from too close adherence to the methods used for alkaloids; amylamine and phenyl-ethylamine which are readily soluble in ether and in chloroform, and p-hydroxy-phenyl-ethylamine which dissolves in amylalcohol, are among the few simpler bases which can be isolated in this manner.

In general, therefore, the isolation of these bases is effected by means of an insoluble salt or other derivative, a method which in the case of putrefaction bases was first extensively used by Brieger, with conspicuous success.

The simplest (aliphatic) monamines are volatile with steam and can therefore easily be separated by *steam distillation*, first from acid solution in order to remove non-basic volatile products and subsequently from alkaline solution. Some non-volatile bases, particularly betaines, are decomposed by strong alkalies with evolution of trimethylamine; if such bases are present the solution should only be made alkaline with magnesium oxide and the distillation should be carried out at a low temperature under reduced pressure. This precaution is for instance important in the estimation of trimethylamine in urine.

When bases have to be isolated from a complex mixture such as a tissue extract, it is necessary to remove first proteins and peptones as far as possible. The oldest method employed for this purpose is to evaporate the aqueous extract to a small bulk and add *alcohol* which precipitates the proteins, but leaves the salts of organic bases in solution. The separation is, however, not very complete; in some cases it may be improved by using *acetone* instead of alcohol. The aqueous solution

containing proteins and bases is evaporated to a thin syrup, and this is mixed with sand and then ground up under acetone. Dry acetone does not dissolve the salts of most organic bases, but enough water remains behind in the aqueous extract to prevent precipitation of the salts by acetone.

The *preliminary purification* of a tissue extract after removal of coagulable protein is, however, best effected by means of lead acetate or by tannin. In the former case the solution is first treated with normal *lead acetate* and then with the basic salt ; the joint precipitate of these reagents is then filtered off and the excess of lead is removed from the filtrate as sulphide, sulphate, or phosphate. The *tannin method* has been largely employed by Kutscher and his pupils ; it completely removes peptones and proteoses, but bases are also carried down by the bulky precipitate ; according to Krimberg the yield of bases from meat extracts is much smaller after purification with tannin than with lead acetate. Many bases form tannates insoluble in neutral solution, so that the reaction before precipitation should be made distinctly acid by adding phosphoric acid, if necessary. A 20 per cent. aqueous tannic acid solution is then added until no further precipitation occurs ; at this stage the precipitate ceases to be milky and flocculates ; a considerable excess of tannic acid must be avoided since it redissolves the precipitate (it is a case of the mutual precipitation of two colloids). On standing overnight the bulky precipitate shrinks to the consistency of pitch and the clear supernatant solution can easily be poured off. In order to remove the excess of tannin, a warm saturated baryta solution is added until, after stirring, the surface of the liquid shows a reddish or purple colour. The barium tannate is filtered off at the pump, the filtrate is acidified with sulphuric acid, and without removing the barium sulphate formed, freshly prepared lead hydroxide, suspended in distilled water, is stirred in. This removes the last traces of tannin and the excess of sulphuric acid, and now, after filtration, the solution should contain at most only traces of lead and should be alkaline to litmus.

The last operations illustrate the general principle that as far as possible no ions should be introduced into the solution which cannot afterwards be removed, for the separation of bases from inorganic salts is often difficult.

Kossel and Weiss [1910] use a solution containing 70 grm. of tannic acid, 100 grm. of sodium chloride and 50 c.c. of glacial acetic acid per litre for the precipitation of peptones.

The solution of bases which has been purified by one or other of

the above methods is now evaporated to a small volume, when on standing some bases, such as creatine, may crystallise out. Generally, however, they are too soluble in water and must be separated by some general precipitant. The most important reagent for this purpose is *phosphotungstic acid*, introduced into physiological chemistry by Drechsel. The acid is readily soluble in ether, in acetone and in water. It precipitates all nitrogen bases from their aqueous solution if the latter contains 5 per cent. by weight of sulphuric acid. Ammonia is also precipitated and should therefore be expelled, if present in quantity. It is important to employ a good preparation of phosphotungstic acid, such as that of Kahlbaum, which dissolves in water with hardly any opalescence. A method for preparing the acid has been given by Winterstein ("Chemiker Zeitung," 1898, p. 539). In order to obtain the bases from an aqueous solution, sulphuric acid is added to the latter to make 5 per cent. and a concentrated aqueous solution of phosphotungstic acid, which should also contain 5 per cent. of sulphuric acid, is added until no further immediate precipitation occurs. After standing for a day the precipitate is filtered off at the pump and thoroughly washed with 5 per cent. sulphuric acid. Often the precipitate is partially or wholly soluble in acetone, and more readily in a mixture of acetone and water. (Compare Wechsler, below.) By pouring the solution of the precipitate into a large bulk of 5 per cent. sulphuric acid, the phosphotungstates of the bases are reprecipitated and in this way they can be purified more readily than by washing at the pump. In synthetic work, and when only one or two bases are present, a phosphotungstate may occasionally be crystallised from a large volume of boiling water (for instance in the case of iminazolyl-propionic acid).

The bases are again liberated from their phosphotungstates by means of baryta, finely powdered or dissolved in water. For this purpose the phosphotungstate precipitate must be carefully suspended in water in as fine a state of division as possible; where possible it is much quicker to dissolve the precipitate in dilute acetone and then add an aqueous baryta solution. Wechsler [1911] recommends a mixture of three volumes of acetone with four volumes of water; this dissolves arginine phosphotungstate to the extent of 120-130 per cent. and of the histidine salt even 160 per cent. of its own weight, but albumose phosphotungstates only to the extent of 2-7 per cent. The precipitate of barium phosphotungstate and sulphate settles down rapidly. Several drops of the clear supernatant fluid are sucked up into a capillary pipette and tested on a glass plate. When they no

longer give a precipitate with baryta, but precipitate both with sulphuric acid and with sodium carbonate solutions, enough baryta has been added to liberate the bases. The barium phosphotungstate is then filtered off on the pump and washed out thoroughly with hot water until the washings no longer give a precipitate with a phosphotungstic-sulphuric acid solution. The excess of barium is at once removed from the filtrate and washings by passing carbon dioxide through them; on filtration and evaporation the organic bases are obtained either in the free state or as carbonates.

Should it be necessary to remove the excess of phosphotungstic acid from the filtrate, after precipitation of bases as phosphotungstates, this can be done either by precipitation with excess of baryta, or, according to Jacobs [1912], by extracting the acid solution with amyl-alcohol, which may be conveniently mixed with up to four parts of ether. This method may also be used for decomposing the phosphotungstates of bases if they are soluble in hot water.

Mercuric chloride is next in importance to phosphotungstic acid as a precipitant of bases. It is not so universal a precipitant and is most frequently used after phosphotungstic acid to separate the recovered bases into several fractions. With suitable precautions mercuric chloride may, however, often replace phosphotungstic acid altogether. It was first used extensively by Brieger for isolating putrefaction bases, before phosphotungstic acid had come into general use.

Mercuric chloride is generally used in saturated alcoholic solution which is added to an alcoholic or sometimes to an aqueous solution of the bases to be precipitated. Some bases are precipitated from neutral solution, but others only after the solution has been made slightly alkaline. In aqueous solution sodium carbonate is used, in alcoholic solution fused sodium acetate, dissolved in alcohol, is added, or the solution is saturated with powdered sodium acetate. If such a solution is afterwards also saturated with powdered mercuric chloride, very few bases escape precipitation. Generally the mercuric chlorides are much more soluble in hot water than in alcohol; Brieger extracted the precipitate formed in alcoholic solution with boiling water, when the mercuric chloride compounds of peptones remained undissolved. On filtration and cooling choline mercurichloride crystallised out. Another example of the use of mercuric chloride is the preparation of histidine from blood, by Fränkel's method. After the blood (or hæmoglobin) has been hydrolysed by boiling with concentrated hydrochloric acid, most of the acid is distilled off and the residue, after being nearly

neutralised with sodium hydroxide, is filtered. The filtrate is then made alkaline with sodium carbonate and the histidine is precipitated by adding alcoholic mercuric chloride solution. Engeland has worked out a method for separating the bases of meat extract in which all the bases are first precipitated by the alternate addition of cold saturated solutions of mercuric chloride and of sodium acetate. The precipitate dissolves for the most part in hot water acidulated with hydrochloric acid and is freed from mercury by means of hydrogen sulphide. After evaporation of the aqueous filtrate the residue is dissolved in alcohol and alcoholic mercuric chloride is added ; finally the solution is saturated with the powdered salt. This precipitates neosine, carnitine and vitiatine as mercurichlorides which are removed by filtration. Alcoholic sodium acetate solution is now added and precipitates the mercury salts of histidine, methyl guanidine and β-alanine. Cf. also p. 114.

Silver nitrate is principally used to precipitate bases containing an imino-group and is of great value for their separation. As in the case of mercuric chloride, the degree of acidity or alkalinity of the solution is the determining factor. In the presence of (nitric) acid only purine bases are precipitated as insoluble silver compounds ; in a slightly alkaline solution, i.e. after the addition of a limited quantity of baryta, the silver compounds of histidine and allied bases are thrown down ; excess of baryta then precipitates the silver compound of arginine. The separation of arginine and histidine in this manner may be rendered quantitative and if silver sulphate is used instead of the nitrate, the process affords a means of estimation by determination of the nitrogen in the various fractions (see Plimmer's " Chemical Constitution of the Proteins," Part I, pp. 35-8). The practical details in the application of silver nitrate may be illustrated by a description of Kutscher's method for the isolation of bases from meat-extract. After purification by means of tannin, as described above, and concentration to a small volume, creatine and some creatinine crystallise out. Then, after filtration, the solution is acidified with sulphuric acid and the resulting precipitate of lead sulphate is filtered off. Now a 20 per cent. silver nitrate solution is added to the filtrate and this causes the precipitation of the purine bases (as compounds with silver nitrate), together with a little silver chloride. After standing for some time this precipitate is filtered off and enough silver nitrate is added to the solution to enable the whole of the bases capable of forming silver compounds to be precipitated as such by subsequent addition of baryta. Enough silver nitrate has been added for this purpose when a drop of the solution, mixed on a watch glass with cold saturated baryta water,

shows no longer a white precipitate (silver compound of bases) but at once a brown precipitate (of silver oxide). The addition of barium hydroxide in excess would now precipitate both the histidine and the arginine fraction, but a separation of these may be effected by utilising the fact that histidine silver is precipitated by an ammoniacal silver solution but arginine silver is not. Hence, after adding enough silver nitrate, baryta is added in small quantities until a drop of the clear supernatant or filtered solution no longer gives a white precipitate with a reagent which is prepared by adding ammonia to 10 per cent. silver nitrate until the silver oxide has just dissolved.

The histidine fraction, which is thus precipitated by baryta, is filtered off, and the precipitate, after washing, is suspended in water in as fine a state of division as possible. If a suitable centrifuge is available this means of separation is greatly to be preferred. The silver is then removed with hydrogen sulphide, or with hydrochloric acid, a little sulphuric acid being first added to precipitate adherent baryta. The barium sulphate formed can be readily filtered off with the silver sulphide or chloride.

Baryta in excess is now added to the filtrate of the "histidine" fraction, and precipitates the silver compounds of the "arginine" fraction, which are treated in the same way.

The former fraction may contain histidine, β-iminazolyl-ethylamine, carnosine and creatinine, the latter arginine, agmatine and methyl-guanidine. The separation is not always quite sharp, however. Thus Reuter found adenine (a purine base) in the histidine fraction of the bases from *Boletus edulis* and trimethyl-histidine in the arginine fraction from this same fungus. In Kutscher's examination of mushroom extract trimethyl-histidine altogether escaped precipitation by silver and appeared in the lysine fraction.

After the silver precipitate of the arginine fraction has been filtered off, the solution may still contain various bases constituting the so-called "lysine" fraction. The excess of baryta is removed by sulphuric acid and that of silver by hydrochloric acid; then the bases remaining in solution are precipitated by phosphotungstic acid, and after recovery from the phosphotungstic precipitate, they are separated by mercuric chloride or by other means.

Potassium bismuth iodide and *potassium tri-iodide* are more or less general precipitants for bases and have been chiefly used in investigations on plant alkaloids, but only to a slight extent for the separation of animal bases. Potassium bismuth iodide (Dragendorff's reagent, modified by Kraut) gives brick red and generally amorphous precipitates

with organic bases. The reagent is prepared by dissolving 80 grm. of bismuth subnitrate in 200 c.c. of pure nitric acid of density 1·18, and pouring this solution slowly, with stirring, into a concentrated aqueous solution of 227 grm. of potassium iodide. A precipitate forms and dissolves on stirring to a deep orange solution. This is cooled strongly to allow potassium nitrate to crystallise out as far as possible. The clear solution is poured off and made up to 1 litre; the more concentrated solution may also be employed. The reagent should be kept in the dark. Kossel and Weiss [1910] recommend a solution of 50 grm. sodium iodide and 100 grm. bismuth iodide in 100 c.c. of 0·5 per cent. aqueous hydriodic acid.

To regenerate the bases, the precipitate caused by addition of Dragendorff's reagent is ground up with freshly precipitated lead hydroxide, which is transformed to lead oxyiodide. After filtration the last traces of lead are removed by hydrogen sulphide; the solution is then concentrated to a syrup, which is extracted with alcohol.

To precipitate bases as periodides a concentrated solution of iodine in potassium iodide is employed (compare the estimation of choline and betaine by Staněk's method). The periodides may be decomposed by sodium bisulphite or thiosulphate, but this introduces into the solution a good deal of inorganic matter. It is better to grind up the periodide in warm water with finely divided copper, so-called "molecular copper," prepared by Gattermann's method, as follows: Zinc dust is added through a sieve to a cold saturated solution of copper sulphate in a porcelain dish, until the solution is only faintly blue. The precipitated copper settles down and is repeatedly washed by decantation. To remove traces of metallic zinc, the copper is placed under several times its volume of distilled water and quite dilute hydrochloric acid is added until no more hydrogen is evolved and the copper is no longer carried up to the surface of the solution but remains quietly at the bottom. The copper is then collected on a filter at the pump, washed until neutral and kept in a well-stoppered bottle in the moist state. It is very easily oxidised.

For the isolation of individual bases from the fractions obtained by any of the above methods, it is necessary to prepare a crystalline derivative. *Benzoylation* is occasionally resorted to (in the case of diamines from urine, p-hydroxyphenyl-ethylamine, etc.) but generally a salt of the base is crystallised. The *hydrochlorides* of putrescine and of betaine are almost insoluble in alcohol, in contradistinction to the corresponding cadaverine and choline salts. The *nitrates* of some bases (guanidine, methylguanidine, arginine, hypaphorine, certain

purine bases) can be readily crystallised from water and are particularly little soluble in dilute nitric acid.

Much more frequently *picrates* are prepared. The picric acid is added in aqueous and also in alcoholic solution; the precipitated picrate is recrystallised from water, from dilute or from strong alcohol. Often, on cooling a hot solution, it separates first in oily drops which only become definitely crystalline on standing. Ammonium salts, when present, may sometimes lead to confusion owing to the formation of ammonium picrate, which is not very soluble in water and forms long thin *pale* yellow needles; these have no proper melting point, but decompose suddenly on heating. When a base is insoluble in ether (as is the case with most of the simpler natural bases) it can be readily recovered from its picrate by dissolving the latter in hot dilute hydrochloric acid and, after cooling, extracting the picric acid with ether or with benzene. On the large scale most of the picric acid generally separates and can be filtered off. The estimation of picric acid in picrates can be carried out very conveniently and with enough accuracy by means of the "nitron" reagent of Busch [1905]. This process has the further advantage over a combustion that the base is recovered unchanged.

Picrolonates are much less soluble than picrates and generally crystallise well, but to some extent this advantage is neutralised by the slight solubility in water of picrolonic acid itself. An alcoholic solution of the acid is generally added to an aqueous solution of the base. The precipitate is at first often amorphous, but readily crystallises from hot water in some cases. The high molecular weight of picrolonic acid renders the melting points and analyses of picrolonates of less significance than those of picrates.

Platinic chloride is used in concentrated aqueous or (more frequently) alcoholic solution. The platinichlorides of the simplest bases are often readily soluble in water, but not in alcohol, and may be crystallised from dilute alcohol.

Gold chloride is generally used in a 30 per cent. aqueous solution. Aurichlorides sometimes partially decompose on recrystallisation, gold being set free. In order to avoid this and obtain a gold salt of normal composition, the salt should be recrystallised from $\frac{1}{2}$ – 1 per cent. hydrochloric acid to which a little gold chloride has been added.

In special cases *zinc chloride* or *cadmium chloride* are used for forming double salts in alcoholic solution, or the base is isolated as *chromate*, *perchlorate* or *metaphosphate*.

B. Special Methods. Properties of Individual Bases and of their Salts.

Bases Volatile with Steam.

Methyl-, dimethyl-, and trimethylamine, isobutyl- and the amylamines can all be readily distilled by passing steam into their alkaline solutions. The last two can be separated from the others by extracting an alkaline solution with chloroform or ether and distilling; isobutylamine boils at 68°, isoamylamine at 95°.

The separation of the first three bases from one another can be accomplished in various ways. Delépine [1896, Ch. I] dissolves the mixture of their salts in cold concentrated formaldehyde solution. An equal volume of potassium hydroxide is added and the solution is distilled. Trimethylamine passes over as such, dimethylamine forms $CH_2[N(CH_3)_2]_2$ and $CH_2(OH)N(CH_3)_2$, b.p. 80-85°, and monomethylamine yields $(CH_2 : NCH_3)_2$, b.p. 166°.

For the quantitative determination of trimethylamine and ammonia, Budai (Bauer) [1913] has worked out a titration method with formaldehyde. The neutral aqueous solution of the mixed hydrochlorides is treated with an excess of formalin (10 c.c.), previously neutralised to phenolphthalein. The solution is then titrated with standard potassium hydroxide until pink with phenolphthalein; this gives the amount of ammonia present. The solution, together with the hexamethylene tetramine formed from the ammonia, is strongly acidified with concentrated hydrochloric acid and boiled down to one-third of its original volume. It is then distilled with excess of potassium hydroxide. This gives ammonia + trimethylamine; the latter is estimated by difference.

The quantitative separation of ammonia, mono-, di-, and trimethylamine is carried out by processes due to Bresler [1900], Bertheaume [1910, **1, 2**], and François [1907, **1, 2**] and is chiefly based on the fact that trimethyl- and dimethylamine hydrochloride alone are soluble in boiling chloroform. 1-2 grm. of the mixed hydrochlorides are dried at 110°, weighed out, dissolved in a little very dilute hydrochloric acid, mixed with at least 20 grm. of pure silver sand, dried *in vacuo* over sulphuric acid, and extracted with hot chloroform in a small funnel tube over glass wool.

The chloroform is evaporated, the residue is weighed and dissolved in 2000 parts of water; 200-300 c.c. of the solution are measured, cooled to 0° and for every 100 c.c. of solution taken, at least 30 c.c. of an ice cold solution of 12·7 grm. of iodine and 15 grm. of potassium iodide in 100 c.c. of water are added. After one hour the

crystals of the periodide of trimethylamine are sucked off on to glass wool, washed with 3-4 c.c. of a mixture of one part of the above potassium tri-iodide solution with three parts of water. The crystals are then dissolved in sodium thiosulphate solution, and after adding excess of sodium hydroxide, the trimethylamine is distilled; the distillate is titrated with acid. The mother liquor of the crystals of trimethylamine periodide yields by a similar treatment the dimethylamine on distillation.

The separation of ammonia and monomethylamine, which are left behind as hydrochlorides mixed with the sand, is effected by François's process, of which the following is an example: 70 grm. of methylamine + 7 grm. of ammonia (both in the free state) in 2000 c.c. of water are shaken for one hour with 200 grm. of yellow mercuric oxide. The solution is decanted and the precipitate is washed. The filtrate and washings contain all the methylamine, but almost the whole of the ammonia is in the mercury precipitate. To remove the remainder, 40 c.c. of caustic soda and 40 c.c. of saturated potassium carbonate solution are added, together with 100 grm. of mercuric oxide. The solution now only contains monomethylamine.

Methylamine can be distinguished from ammonia by means of Nessler's reagent; the amine gives a cream-coloured precipitate, ammonia a brown one.

The estimation of small quantities of amines in the presence of much ammonia has been described by Bertheaume [1910, **2**].

Fleck [1896] recommends the separation of trimethylamine from ammonia by means of the sulphates, rather than the chlorides. Ammonium sulphate is insoluble in absolute alcohol, in which ammonium chloride is distinctly soluble; trimethylamine salts dissolve readily in alcohol.

de Filippi [1906] has estimated *trimethylamine* in urine by destroying ammonia, primary and secondary amines by means of sodium hypobromite; this reagent leaves tertiary amines intact. Dorée and Golla [1910] by a slightly modified method found 0·014 per cent. trimethylamine in urine. They state that this amine cannot be distinguished from choline by the alloxan test, nor by the bismuth iodide or periodide test.

Melting points and solubility of trimethylamine salts:—

Hydrochloride	271-275°	soluble in boiling chloroform.
Picrate . .	216°	soluble in 77 parts of cold water.
Picrolonate .	250-252°	in 1121 parts of cold and 166 parts of boiling water, 794 of cold and 233 of boiling alcohol.
Aurichloride .	228°	yellow monoclinic crystals, readily soluble in hot alcohol, slightly in water.
Platinichloride	240-245°	regular orange crystals, little soluble in boiling alcohol.

Isobutylamine hydrochloride does not melt at 160°, as stated in Beilstein, but at 177-178° (Thoms and Thümen [1911]).

The *platinichloride* forms golden yellow crystals, very soluble in alcohol and in water, decomposing at 224-225° and melting at 230-232°.

Isolation of isoamylamine from putrid horse meat.—The material had undergone putrefaction anærobically for eight to ten days at 37°. The proteins were coagulated, the filtrate was evaporated to a syrup, mixed with sand and extracted with acetone. After distilling off the acetone, hydrochloric acid was added to the residue, which was washed with chloroform to remove fatty acids, etc., and then rendered alkaline and again extracted with chloroform. After evaporation of the solvent the base was distilled and converted into the crystalline oxalate.

Isoamylamine hydrochloride forms deliquescent crystals; the *hydrobromide* is non-deliquescent. The *acid oxalate* $C_5H_{13}N$, $H_2C_2O_4$ is obtained by mixing ethereal solutions of oxalic acid and of the base; m.p. 169°; it slowly loses amylamine at 100° and should be dried *in vacuo*.

The *platinichloride* forms golden yellow leaflets, readily soluble in hot water.

Isolation and Separation of Putrescine and Cadaverine.

Both bases are very common in putrefaction. They are not readily volatile with steam, nor can they readily be extracted from aqueous solution by ether or by chloroform. They can be precipitated by phosphotungstic acid, and after treatment with silver nitrate and baryta they are found in the lysine fraction (see above). From this they can be precipitated by mercuric chloride in alcoholic solution, or they may be precipitated directly by this reagent, as was done by Brieger, without previous use of phosphotungstic acid. He precipitated both bases from an alcoholic extract of a putrefaction mixture by means of alcoholic mercuric chloride and afterwards fractionally crystallised the platini- and aurichlorides (putrescine aurichloride is the less soluble in water). It is, however, more convenient to separate the hydrochlorides, that of putrescine being but little soluble in 96 per cent. alcohol, whereas the corresponding cadaverine salt dissolves readily.

From urine Udránszky and Baumann [1888, I, 1889] separated both bases as dibenzoyl compounds by shaking with benzoyl chloride in sodium hydroxide solution; this process is quantitative even in a 1 : 10,000 solution of the base. The benzoyl derivatives are washed with

water and dissolved in a little boiling alcohol. After concentration the alcoholic solution is poured into thirty volumes of water when the benzoyl compounds crystallise. The concentrated alcoholic solution of the crystals is then poured into twenty volumes of ether when dibenzoyl putrescine separates and the cadaverine compound remains dissolved. Another method is due to Loewy and Neuberg [1904]. After filtering off the cystine the bases in the urine are precipitated with phosphotungstic acid and after regeneration are treated in alkaline solution with phenylisocyanate. The precipitated compounds of the diamines are very little soluble in most organic solvents, and are boiled out with alcohol, dried and dissolved in warm pyridine. On adding dry acetone the putrescine compound crystallises at once, the cadaverine compound only on standing.

Properties and Compounds of Putrescine.

The base is obtained synthetically by reduction of ethylene dicyanide (succino-nitrile), but more conveniently by reduction of succindialdoxime (Willstätter and Heubner [1907]).

Putrescine is a liquid of semen-like odour; m.p. 27-28°; b.p. 158-160°; slightly volatile with steam; very soluble in water, miscible with alcohol, very little soluble in ether.

The *dihydrochloride*, $C_4H_{12}N_2 . 2HCl$, crystallises in leaflets and needles and is insoluble in absolute alcohol. On destructive distillation it yields pyrrolidine (rigid proof of the constitution) (Ackermann [1907, 1]).

The *platinichloride*, $C_4H_{12}N_2 . H_2PtCl_6$, needles or six-sided plates, is sparingly soluble in water (Brieger [1885, **2**, p. 26]).

The *aurichloride*, $C_4H_{12}N_2 . 2HAnCl_4 . 2H_2O$, is less soluble than the cadaverine salt (Brieger [1886, **1**, p. 51]).

The *mercurichloride* is readily soluble in water, but not in alcohol.

The *dipicrate*, $C_4H_{12}N_2 . 2C_6H_3O_7N_3$, silky needles, hardly soluble in cold water, decomposes at 250°.

The *dipicrolonate*, $C_4H_{12}N_2 . 2C_{10}H_8O_5N_4$, dissolves in 13,157 parts of cold and 653 parts of boiling water, and in 17,857 parts of cold and 954 parts of boiling alcohol; decomposes at 263° (Otori [1904, 3]).

The *dibenzoyl derivative*, $C_4H_8(NHCOC_6H_5)_2$, crystallises in long needles; m.p. 178°; almost insoluble in ether; sparingly in cold, readily in hot alcohol.

The *phenylisocyanate*, $C_4H_8(NH . CO . NH . C_6H_5)_2$, forms sheaves of needles from pyridine acetone; m.p. 240° (corr.). Insoluble in water and most organic solvents; hardly soluble in boiling alcohol.

Properties and Compounds of Cadaverine.

Cadaverine or pentamethylene diamine was obtained by Ladenburg [1886] by the reduction of trimethylene dicyanide, but is now most easily obtained from potassium phthalimide and pentamethylene dichloride; the latter compound is readily formed from benzoyl piperidine and phosphorus pentachloride, by von Braun's method [1904]. Cadaverine is also formed in small quantity by the destructive distillation of lysine (Neuberg [1905]). Cadaverine is a liquid with the odour of semen and of piperidine; b.p. 178-179°; somewhat volatile with steam, readily soluble in water and in alcohol, hardly in ether; is precipitated by alkaloidal reagents.

The *dihydrochloride*, $C_5H_{14}N_2 . 2HCl$, needles, non-deliquescent according to Gulewitsch [1894], is readily soluble in 96 per cent. alcohol, sparingly in absolute alcohol. On destructive distillation it yields piperidine.

The *platinichloride*, $C_5H_{14}N_2 . H_2PtCl_6$, forms orange coloured rhombic prisms, somewhat resembling ammonium platinichloride (for details see Brieger [1885, **2**, p. 37]); they blacken at 195° and decompose at 215°; soluble in 70·8 parts of water at 21° (Gulewitsch [1894]), in 113 to 114 parts of water at 12° (Udránszky and Baumann).

The *aurichloride*, $C_5H_{14}N_2 . 2HAuCl_4$, forms long needles and also flat prisms; m.p. 186-188°; fairly readily soluble in water and containing water of crystallisation.

The *mercurichloride*, $C_5H_{12}N . 2HCl . 4HgCl_2$, prepared with excess of mercuric chloride, crystallises from hot water and melts at 214·5° (Gulewitsch [1894]). It already loses mercuric chloride at 95°. Soluble in 32·5 parts of water at 21°; not appreciably soluble in alcohol.

The *dipicrate*, $C_5H_{14}N_2 . 2C_6H_3O_7N_3$, forms long needles; m.p. 221°; sparingly soluble in hot water, hardly at all in boiling alcohol.

The *dipicrolonate*, $C_5H_{14}N_2 . 2C_{10}H_8O_5N_4$, darkens at 220° and melts at 250°; soluble in 7575 parts of cold water and 357 parts of boiling water, 5952 parts of cold and 475 parts of boiling alcohol (about twice as soluble as the putrescine salt) (Otori [1904, **3**]).

The *dibenzoyl derivative*, $C_5H_{10}(NHCOC_6H_5)_2$, long needles, hardly soluble in ether, melts at 135°.

The *phenylisocyanate*, $C_5H_{10}(NHCONHC_6H_5)_2$, is somewhat more soluble in pyridine acetone than the putrescine compound and melts at 207-209° (corr.).

Tetramethyl-putrescine, $C_8H_{20}N_2$.

This base occurs along with hyoscyamine, in *Hyoscyamus muticus*. It is a strongly alkaline liquid, boiling at 169° and miscible with water, alcohol and ether in all proportions. Pharmacologically it is inert (0·05 grm. given as salt hypodermically to frogs and 0·5 grm. intravenously to rabbits was without effect).

The *dihydrochloride*, m.p. 273°, is neutral and deliquesces in moist air; the *dipicrate* is fairly readily soluble in water; m.p. 198°.

The *platinichloride*, $C_8H_{20}N_2 . H_2PtCl_6 . 2H_2O$, is readily soluble in hot, but much less in cold water; m.p. 234°. The *aurichloride*, of similar solubility in water, dissolves very readily in acetone and forms golden yellow anhydrous prisms decomposing at 206-207°. The constitution $(CH_3)_2 : N . CH_2 . CH_2 . CH_2 . CH_2 . N : (CH_3)_2$ was established by synthesis (Willstätter and Heubner [1907]).

Agmatine.

On treatment with silver nitrate and baryta, in the way described in section A of this chapter, this base is precipitated in the arginine fraction.

Agmatine salts.—The *sulphate*, $C_5H_{14}N_4 . H_2SO_4$, forms long needles, m.p. 229°; the *dipicrate*, $C_5H_{14}N_4 . 2C_6H_3O_7N_3$, forms crystals melting at 238° and decomposing at 244°; the *aurichloride*, $C_5H\ N_4 . 2HAuCl_4$, crystallises in yellow needles. The *carbonate* separates from aqueous solution on concentration as a chalky mass.

Phenyl-ethylamine.

From a putrefaction mixture this base is best isolated in the manner described above for isoamylamine, from which it is separated by its much higher boiling point.

Phenyl-ethylamine and its salts.—The base is easily obtained synthetically, by the reduction of benzylcyanide; the highest recorded yield by this reaction is 53 per cent. of the theory (Wohl and Berthold [1910]). It is also obtainable from phenylacetic acid, *via* the amide, by Hofmann's reaction and *via* the hydrazide and urethane, by Curtius's method; it is further one of the products of the destructive distillation of phenylalanine.

The synthetic base is a liquid of slight amine-like odour and readily absorbs carbon dioxide from the air, forming the crystalline carbonate. The boiling point of the base is 196° at 747 mm., 197-198° at 754 mm.; it is somewhat lighter than water, and dissolves in 24 parts of water at 20°; it is miscible with alcohol and with ether.

The *hydrochloride*, $C_8H_{11}N$. HCl, is soluble in alcohol and melts at 217°; with mercuric chloride a sparingly soluble crystalline compound is formed. Other salts are the *acid oxalate*, $C_8H_{11}N$. $C_2H_2O_4$, m.p. 181°; the *normal oxalate*, $(C_8H_{11}N)_2C_2H_2O_4$, m.p. 218°; and the *picrate*, $C_8H_{11}N$. $C_6H_3O_7N_3$, tetragonal prisms, m.p. 171-174°, readily soluble in warm water.

The *benzoyl derivative*, C_6H_5 . CH_2 . CH_2 . NH . CO . C_6H_5, melts at 114°.

p-Hydroxy-phenyl-ethylamine.

Small quantities of this amine are most readily prepared by heating tyrosine under reduced pressure in test tubes dipping into a bath of fusible metal at 260-270°; the amine sublimes; the yield is 50 per cent. (cf. F. Ehrlich and Pistschimuka [1912]). For the isolation from complex mixtures such as are obtained in putrefaction, the base can be precipitated with phosphotungstic acid, but the phosphotungstate is rather soluble. On fractionation with silver and baryta, the base is obtained as platinichloride from the lysine fraction. A better way is to utilise its phenolic properties by washing its solution in ·5N sodium hydroxide with amyl alcohol, neutralising, adding sodium carbonate and extracting the amine with amyl alcohol. After distilling off the solvent with steam, the dibenzoyl derivative is obtained by the Schotten-Baumann method.

In sufficient quantity p-hydroxy-phenyl-ethylamine is best purified by distillation; it boils at 161-163° at 2 mm. and 175-181° at 8 mm. It is also readily purified by crystallisation from boiling xylene in which it is very sparingly soluble. It forms colourless hexagonal leaflets melting at 161°, soluble in 95 parts of water at 15° and in about 10 parts of boiling ethyl alcohol. The base is fairly soluble in amyl alcohol, but hardly at all in ether or chloroform. It gives Millon's and Mörner's reaction for tyrosine, but no coloration with triketohydrindene hydrate.

The *hydrochloride*, $C_8H_{11}ON$. HCl, is very soluble in water and may be crystallised from concentrated hydrochloric acid; m.p. 268°.

The *phosphate*, $C_8H_{11}ON$. H_3PO_4 . $1\frac{1}{2}H_2O$, forms white prisms, readily soluble in water; m.p. 209-210°.

The *picrate*, $C_8H_{11}ON$. $C_6H_3O_7N_3$, forms short prisms; m.p. 200°.

The *platinichloride*, $(C_8H_{11}ON)_2H_2PtCl_6$, forms six-sided leaflets.

The *N-monobenzoyl derivative* crystallises from alcohol in hexagonal plates; m.p. 162°.

The *dibenzoyl derivative*, $C_6H_5CO.O.C_6H_4.CH_2CH_2.NH.CO.C_6H_5$,

is the most useful and characteristic derivative of the base. Formed by the Schotten-Baumann reaction, it crystallises readily from alcohol and melts at 170°; this derivative gives Mörner's reaction, but not Millon's.

Yeast transforms p-hydroxy-phenyl-ethylamine to the corresponding alcohol, tyrosol, $OH . C_6H_4 . CH_2 . CH_2OH$ (Ehrlich and Pistschimuka [1912]). p-Hydroxy-phenyl-ethylamine is attacked by various oxidases and converted to pigments, but does not always behave in the same way as its parent substance tyrosine. Thus Neuberg [1908, Ch. VI] found that a ferment from a melanoma attacked the amine, but not the amino-acid, whereas an extract of the ink-bag of *Sepia* acts on tyrosine more readily than on the amine. Compare also J. Chem. Soc., Abstr., 1908, **94**, i., 236.

Hordenine.

Gaebel's *process of isolation* was as follows: The extract of 3 kilos. of malt germs with 95 per cent. alcohol was evaporated to a syrup and extracted with 1 litre of water. After filtration the aqueous extract was made alkaline with sodium carbonate, shaken once with a little ether to remove a colouring matter, and then ten times with large quantities of ether. The concentrated ethereal extract was dried with potassium carbonate and evaporated, when the residual syrup soon crystallised. On recrystallisation from dry ether, with charcoal, the pure base is obtained; the yield is 0·2 per cent. of the air dry germs.

Properties.—Hordenine forms colourless crystals melting at 117·8° (corr.) and boiling at 173-174° and 11 mm. Distillation under reduced pressure is the most convenient method of purification. The base dissolves readily in alcohol and in chloroform, and fairly readily in ether and in water; it is hardly soluble in benzene. Hordenine gives Millon's and Piria's reactions for tyrosine, and reddens phenolphthalein; it is not coloured by concentrated sulphuric acid, but reduces potassium permanganate in the cold and ammoniacal silver nitrate on warming.

The *sulphate*, $(C_{10}H_{15}NO)_2 . H_2SO_4 . H_2O$, the *hydrochloride* and the *hydrobromide* are sparingly soluble in alcohol. The *quaternary iodide*, hordenine methiodide, obtained by the action of methyl iodide in methyl alcoholic solution on hordenine (or on p-hydroxy-phenyl-ethylamine), forms large glassy prisms, sparingly soluble in cold water; m.p. 230-231°.

Indolethylamine.

The free base, on recrystallisation from a mixture of alcohol and benzene, forms long colourless needles, melting at 145-146°. It is readily soluble in alcohol and in acetone, but is almost insoluble in water, ether, benzene and chloroform. It gives very intensely Hopkins and Cole's reaction with glyoxylic and sulphuric acids, characteristic of tryptophane; the bluish-violet coloration is still obtainable with the base in a dilution of 1 : 300,000. Unlike tryptophane, aminoethylindole is not coloured by bromine water, nor does it react with triketohydrindenehydrate.

The *hydrochloride*, $C_{10}H_{12}N_2 . HCl$, forms thin prisms melting at 246° and is soluble in about 12 parts of water at 18°.

The *picrate* is the most characteristic salt of the base. It has the composition $C_{10}H_{12}N_2 . C_6H_3O_7N_3$ and is obtained by adding a cold saturated solution of picric acid to a solution of the hydrochloride in water; the mixture at once becomes turbid and orange-red in colour, and dark red crystals, consisting of fern-like aggregates of needles or prisms (resembling in shape those of ammonium chloride) rapidly separate. This picrate is almost insoluble in water and very sparingly so in alcohol and most organic solvents, but dissolves readily in acetone; it melts and decomposes at 242-243°.

The *picrolonate* crystallises readily from hot water in deep chrome-yellow prisms melting at 231°.

The *monobenzoyl derivative* of 3-β-amino-ethylindole is difficult to crystallise, and therefore not suitable for characterising the base; it forms stout prisms melting at 137-138°.

β-Iminazolyl-ethylamine.

Bacterial Preparation.

Ackermann [1910, **1**] dissolved 49 grm. of histidine hydrochloride in 4 litres of water, added 10 grm. of Witte peptone, 20 grm. of glucose, a few drops of magnesium sulphate and sodium phosphate solutions, and excess of calcium carbonate to keep the reaction alkaline. After inoculation with putrid pancreas the solution was kept fifty-two days at 35°. It yielded 61·6 grm. of iminazolyl-ethylamine dipicrate which is 42 per cent. of the theoretical; a very small quantity of iminazolyl-propionic acid was also formed.

When working with small quantities of histidine and pure cultures of certain bacteria one can occasionally obtain solutions of which the physiological activity indicates an almost complete conversion. How-

ever, it seems generally impossible to isolate more of the amine than Ackermann obtained and the yield is often very much less. The mode of action of one and the same organism seems to depend on conditions which are as yet imperfectly understood, so that this method is rather uncertain.

Mellanby and Twort [1912] have isolated a bacillus of the typhoid-coli group from the intestine of various mammals (from the duodenum downwards) which is capable of decarboxylating histidine. The best yields of the amine were obtained by inoculating histidine solutions with adequate quantities of a vigorous twenty-four hours' culture of the organism on glycerine-agar and incubating for one week at 37°. The solutions contained histidine 1 per cent., ammonium tartrate 1 per cent., dipotassium phosphate 0·1 per cent., magnesium sulphate 0·02 per cent., calcium chloride 0·01 per cent., but no peptone. Solutions containing 0·1 per cent. histidine give a better yield. See also patents by Hoffmann, La Roche & Co. [1912] and papers by Berthelot and Bertrand [1912, **1, 2**; 1913, **1, 2**] and by Bertrand and Berthelot [1913] describing the isolation of *Bacillus aminophilus intestinalis*, a Gram-negative capsulated organism, resembling *B. lactis aerogenes* and the bacillus of Friedländer, but differing from these in its great power of decarboxylating amino-acids. For isolation of the organism they used 0·2 grm. K_2SO_4, 0·2 grm. $MgSO_4$, 0·5 grm. K_2HPO_4, 0·25 grm. KNO_3, 0·02 grm. $CaCl_2$ and 1·5 grm. histidine hydrochloride per litre.

To isolate β-iminazolyl-ethylamine when pure histidine has been submitted to putrefaction, it is hardly necessary to precipitate with phosphotungstic acid. Instead one can precipitate at once with picric acid, having removed ammonia, and recrystallise the picrate. For the isolation of the base from complex mixtures such as ergot, it is necessary to fractionate with silver nitrate and baryta. The base is then found in the histidine fraction. Its hydrochloride is conveniently separated from inorganic salts by extraction with methyl alcohol.

Salts of β-iminazolyl-ethylamine.—The *dihydrochloride*, $C_5H_9N_3 . 2HCl$, is extremely soluble in water and sparingly soluble in ethyl alcohol; it crystallises in prisms; m.p. 240°. The *dihydrobromide* has similar solubilities and forms stout prisms sintering at 265° and melting at 284° (corr.). The *acid phosphate*, $C_5H_9N_3 . 2H_3PO_4$, is somewhat less soluble in water and crystallises very well; it decomposes indefinitely at 120-140°.

The *platinichloride*, $C_5H_9N_3 . H_2PtCl_6$, orange coloured prisms readily soluble in hot water and hardly at all in alcohol, blackens and de-

composes between 200° and 240° without melting. The *aurichloride*, $C_5H_9N_3 . (HAuCl_4)_2$, melts with decomposition at 200-210°.

The *dipicrate*, $C_5H_9N_3 . (C_6H_3O_7N_3)_2$, is the most convenient salt for purposes of isolation. It forms deep yellow rhombic leaflets, melting at 238-242° (corr.) according to the rate of heating. It is very sparingly soluble in cold water and can be recrystallised from hot water. The *monopicrate*, $C_5H_9N_3 . C_6H_3O_7N_3$, m.p. 233-234°, forms bunched, slightly curved, pointed needles.

The *dipicrolonate*, $C_5H_9N_3(C_{10}H_8O_5N_4)_2$, dissolves in about 450 parts of boiling water, from which it crystallises in sheaves of needles, melting at about 264°.

Reactions of β-iminazolyl-ethylamine.—In common with histidine, this amine gives Pauly's reaction with p-diazobenzene sulphonate; a very distinct rose pink coloration is still obtainable at a dilution of 1 : 10,000. It also gives Knoop's histidine reaction, a claret coloration, on boiling with bromine water. It is precipitated by ammoniacal silver oxide, by mercuric chloride in the presence of potassium hydroxide, and by phosphotungstic acid. On the other hand, it is distinguished from histidine in not giving the biuret reaction, nor Ruhemann's reaction with triketohydrindenehydrate, and it further behaves differently on benzoylation. When shaken with benzoyl-chloride in potassium hydroxide solution, the glyoxaline ring is ruptured and tribenzoyl-butentriamine is formed, of the following constitution :—

$$\begin{array}{l} CH . NH . CO . C_6H_5 \\ \ddot{C} . NH . CO . C_6H_5 \\ \dot{C}H_2 . CH_2 . NH . CO . C_6H_5. \end{array}$$

Histidine, on the other hand, yields a monobenzoyl derivative.

β-Alanine.

The substance may be obtained synthetically in several ways, the best being from succinimide (Holm [1904]); 1 mol. of succinimide in 10 per cent. potash solution containing 6 mol. of KOH and 1 mol. of KOBr is warmed for two hours to 50-60°. The resulting β-alanine is purified by esterification.

Abderhalden and Fodor [1913] isolated β-alanine according to Fischer's ester method. The free ester boils at 54° and 10 mm. The hydrochloride melts at 64°. On distilling the ester at ordinary pressure it gives the pungent smell of ethyl acrylate which is a good mode of recognition of β-alanine.

Synthetic β-alanine forms prisms, melting at 206-207° and decomposing into acrylic acid and ammonia.

The *hydrochloride* melts at 122·5°.

The *sulphate*, $(C_3H_7O_2N)_2H_2SO_4$, decomposes at 150°.

The *platinichloride*, $(C_3H_7O_2N)_2H_2PtCl_6$, crystallises from water or hydrochloric acid in deep yellow needles, m.p. 180°; it is soluble in alcohol (Engeland [1908, 1]).

The *copper salt*, $(C_3H_6O_2N)_2Cu + 6H_2O$, forms azure crystals (Holm [1904]).

γ-Aminobutyric Acid.

According to Engeland and Kutscher [1910, 3, Ch. III, butyrobetaine] γ-aminobutyric acid is precipitated in dilute solution by phosphotungstic acid and also by mercuric chloride in the presence of sodium acetate, but not by mercuric chloride alone. These properties it shares with histidine and methyl-guanidine, from which it may be separated by silver nitrate and baryta, when it appears in the lysine fraction. It can also be separated by distillation of its ester, prepared by Fischer's method.

γ-Amino-butyric acid was first obtained by Schotten [1884] by oxidising piperidylurethane with fuming nitric acid and subsequently

hydrolysing the oxidation product (for details see Abderhalden and Kautzsch [1912]). The *free acid* forms leaflets melting at 183-184° with conversion into the anhydride pyrrolidone, NH$\begin{matrix}\diagup CH_2 . CH_2 \\ \diagdown CO \ . \ CH_2.\end{matrix}$

The *hydrochloride* crystallises in stout prisms; m.p. 135°.

The *platinichloride* forms orange prisms; m.p. 220°.

The *aurichloride* crystallises in glistening plates; m.p. 138°. The *ethyl ester* boils at 75-77°/12 mm.

δ-Amino-valeric Acid.

E. and H. Salkowski obtained this substance from putrid blood fibrin by evaporating the mixture repeatedly with water, adding barium chloride to remove some fatty acids as soaps, acidifying the filtrate, washing with ether, evaporating to dryness, and extracting the residue with alcohol. On standing for a long time in a desiccator the residue from the alcoholic solution gave the crystalline hydrochloride of δ-amino-valeric acid, from which the platinichloride and finally the aurichloride was isolated.

Formation from proline.—Ackermann [1911, **2**] obtained 3·6 grm. of δ-amino-valeric acid aurichloride from 34 grm. of proline after putrefaction for nine days with glucose, peptone and salts. Neuberg isolated the acid by means of *α*-naphthylisocyanate and obtained at the same time n-valeric acid, which would result from the deamination of δ-amino-valeric acid. Neuberg [1911, **1**] used a 1 per cent. proline solution, made and kept alkaline by repeated addition of sodium bicarbonate, and containing a few drops of saturated magnesium sulphate, potassium chloride and sodium phosphate solutions, but no glucose or peptone; from 23 grm. of proline 12·1 grm. was recovered unchanged, together with 2·7 grm. δ-amino-valeric acid hydrochloride, and 2·3 grm. of silver n-valerate.

δ-Amino-valeric acid crystallises in pearly leaflets, extremely soluble in water, and melting at 157-158° when they undergo transformation to piperidone. The aqueous solution is faintly acid and has an astringent taste. The substance is precipitated in dilute solution by phosphotungstic acid, but not by cupric acetate or ammoniacal silver solution. The *hydrochloride*, $C_5H_{11}O_2N$. HCl, forms rhombic leaflets which on heating distil for the most part without change.

The *platinichloride*, $(C_5H_{11}O_2N)_2H_2PtCl_6$, forms long rhombic leaflets, readily soluble in hot water but only slightly in cold water and in alcohol.

The *normal aurichloride*, $C_5H_{11}O_2N \cdot HAuCl_4 \cdot H_2O$, crystallises in monoclinic orange coloured crystals; m.p. 86-87°; an *abnormal aurichloride*, $C_5H_{11}O_2N \cdot AuCl_3$, is also known; it forms pale yellow crystals decomposing at 130° and is transformed to the more deeply coloured normal salt by recrystallisation from dilute hydrochloric acid.

Benzoyl-δ-amino-valeric acid is formed by the oxidation of benzoyl piperidine with potassium permanganate and by the benzoylation of δ-amino-valeric acid. It melts at 94° and at 105°.

δ-Amino-valeric acid does not yield a blue copper salt on boiling with cupric oxide or on adding cupric acetate.

β-Iminazolyl-propionic Acid.

This substance was isolated by Ackermann from the filtrate of the β-iminazolyl-ethylamine picrate [1910, 1] obtained in the putrefaction of histidine. The picric acid was removed from this filtrate, the solution was evaporated, the residue was extracted with alcohol and to the alcoholic solution platinic chloride was added. A slight precipitate was filtered off and the alcoholic solution was evaporated to dryness. The residue, dissolved in a minimum quantity of boiling water, deposited the crystals of the platinichloride of β-iminazolyl-propionic acid.

β-Iminazolyl-propionic acid is readily soluble in water, less so in alcohol and crystallises from dilute acetone; m.p. 208-209°.

The *nitrate*, $C_6H_8O_2N_2 \cdot HNO_3$, readily soluble in methyl alcohol, forms elongated six-sided leaflets; m.p. 143-148°.

The *platinichloride*, $(C_6H_8O_2N_2)_2 \cdot H_2PtCl_6$, melts at 209°.

The *phosphotungstate* crystallises from hot water in characteristic rectangular leaflets, decomposing above 300°.

The *copper salt* forms blue needles.

Carnosine (Ignotine).

Carnosine is obtained from the regenerated phosphotungstic acid precipitate (after neutralisation with nitric acid) by means of silver nitrate and excess of baryta. After decomposing the silver precipitate with hydrogen sulphide and removing the baryta by carbon dioxide, the solution is neutralised with nitric acid and concentrated; carnosine nitrate crystallises out after the addition of alcohol.

Krimberg, by Gulewitsch's method, obtained 15·3 grm. of the free base from 1 lb. of Liebig's extract, or 3·4 per cent.; by Kutscher's process he only obtained 3 grm. of carnosine from the same quantity

of meat extract, and Kutscher himself obtained 3 grm. of ignotine from 1 lb. of Liebig's extract.

The *free base*, $C_9H_{14}O_3N_4$, crystallises in needles, soluble in 3·2 parts of water at 25° and appreciably so in alcohol; m.p. 248·5 – 250°; $[\alpha]_D^{20°} = 21°$, independent of the dilution. A 2·5 per cent. aqueous solution gives no precipitate with platinic chloride, but it causes turbidity with picric acid and a precipitate with gold chloride and potassium bismuth iodide.

The *nitrate*, $C_9H_{14}O_3N_4 . HNO_3$, melts at 219° and dissolves in 1·04 parts of water at 25°; $[\alpha_D^{20°}]$ in 1·48 per cent. solution = +24·2°, in 8 per cent. solution = +22·8°; excess of nitric acid lowers the rotation [Gulewitsch, 1913].

The *copper salt*, $C_9H_{14}O_3N_4 . CuO$, forms deep blue six-sided plates, resembling cystine crystals in shape. It is sparingly soluble in hot water and results when carnosine is boiled with copper carbonate.

Carnosine yields a sparingly soluble dipicrolonate, of which Mauthner [1913] has attempted to use the mono-sodium salt as a means of estimating carnosine in the histidine fraction of muscle extracts.

Carnosine resembles arginine and differs from histidine in requiring a fixed alkali for its precipitation as *silver compound* from a solution of carnosine nitrate containing an equimolecular amount of silver nitrate. With silver nitrate in excess the silver compound is also precipitated by careful addition of ammonia, but is soluble in excess. Demjanowski [1912, Ch. V, methyl-guanidine] gives the following limits of precipitation in aqueous solution: mercuric chloride, 1 : 2000; mercuric sulphate, 1 : 100,000; mercuric nitrate, 1 : 100,000; 25 per cent. phosphotungstic acid, 1 : 20,000.

Urocanic Acid.

Preparation.—Jaffé obtained the substance by a very simple method. The urine was evaporated to a syrup and the latter was extracted with hot alcohol; after evaporation of the alcohol the residue was acidified with sulphuric acid; after washing with ether to remove impurities the urocanic acid crystallised from the aqueous layer. Hunter used phosphotungstic acid for the isolation of urocanic acid and this is probably also the most certain method of obtaining it from urine. The amount when present in urine is not inconsiderable; Jaffé obtained 2-3 grm. per day and Siegfried found the urine to contain 0·18 per cent. of the substance.

Jaffé gave the formula $C_{12}H_{12}O_4N_4, 4H_2O$ to the free acid, but this

must be halved. The free acid is slightly soluble in cold water (0·15 per cent. at 18° according to Siegfried) and readily soluble in hot water. The melting point depends greatly on the rate of heating; after crystallisation from dilute acetone Barger and Ewins found 235-236° (uncorr.). Hunter gives 231-232° (corr.), Jaffé 212-213°, Siegfried 229°. Hunter obtained the acid in slender, beautifully iridescent needles or tetragonal prisms. With sodium p-diazobenzene sulphate it gives the red coloration of histidine. The acid is precipitated from solution by silver nitrate; the precipitate dissolves in excess of ammonia and in nitric acid.

The *barium salt*, $(C_6H_5O_2N_2)_2Ba \cdot 8H_2O$, crystallises in needles and loses $6H_2O$ at 100° and the rest at 150°.

The *nitrate*, $C_6H_6O_2N_2 \cdot HNO_3$, is the most characteristic salt. It is sparingly soluble in dilute nitric acid and crystallises in small sickle-shaped plates frequently united to cross- or rosette-shaped aggregates (figured by Hunter, p. 541); m.p. 198° with explosive decomposition (Barger and Ewins).

The *picrate*, $C_6H_6O_2N_2 \cdot C_6H_3O_7N_3$, forms golden yellow prisms; m.p. 213-214°, 224-225° (corr.).

The *picrolonate*, $C_6H_6O_2N_2 \cdot C_{10}H_8O_5N_4$, crystallises from dilute alcohol; m.p. 268° (corr.).

The *phosphotungstate* forms small rectangular plates from dilute acetone or from hot water.

Kynurenic Acid.

To obtain kynurenic acid, Kretschy [1881] fed a dog of 34 kilos. weight daily with 1 kilo. of horse meat, 70 grm. of bread and 1 litre of water. At first the daily production of the acid was 0·1 grm. but after 1 month 0·8 grm. The best method, however, is to give tryptophane by the mouth. The urine is acidified and the precipitate formed in twenty-four hours is filtered off and purified by dissolving in ammonia, acidifying slightly with acetic acid and leaving for twenty-four hours to allow a brown impurity to precipitate. After filtration the solution is acidified with 4 per cent. hydrochloric acid. Adherent uric acid may be removed by Hopkins's method and the kynurenic acid may be finally recrystallised from 800 parts of boiling alcohol (Homer [1913]). The pure acid forms long glistening needles, of the formula $C_{10}H_7O_3N, H_2O$. The water of crystallisation is given off at 140-145°. The highest melting point obtained by Miss Homer was 288-289° (uncorr.). The acid is practically insoluble in cold water and 100 parts of boiling water only dissolve 0·09 parts;

100 c.c. of boiling alcohol dissolve 0·1 grm. The following salts are crystalline: $C_{10}H_6O_3NK + 2H_2O$, $(C_{10}H_6O_3N)_2Ba + 4\frac{1}{2}H_2O$, $(C_{10}H_6O_3N)_2Ca + 2H_2O$ and $(C_{10}H_6O_3N)_2Cu + 2H_2O$. The barium salt is fairly soluble in hot water, but the copper salt is almost insoluble in it. The crystalline hydrochloride $C_{10}H_7O_3N$, HCl easily loses hydrochloric acid (Brieger [1879]); the basic properties of the substance are further evident from its precipitation by phosphotungstic acid (Hofmeister [1880, Ch. V, creatine]).

Kynurine, formed in a 90 per cent. yield by heating kynurenic acid to 253-258°, is little soluble in cold water, more so in alcohol. The hydrated substance C_9H_7ON, $3H_2O$ melts at about 52°, the anhydrous substance at 202°. It is a feeble base yielding a platinichloride $(C_9H_8ON)_2PtCl_6 + 2H_2O$ and a crystalline hydrochloride; with bromine the substance $C_9H_4Br_3ON$ is formed (Brieger [1879]).

Jaffé's reaction for kynurenic acid [1883].—A solution of the acid is evaporated on the water bath with hydrochloric acid and potassium chlorate; the red residue becomes brownish green with ammonia, soon changing to an intense emerald green; the chief product is tetrachlorooxykynurine, $C_9H_3O_2NCl_4$.

A convenient *method of estimation* has been described by Capaldi [1897, **2**].

Betaine (Acetobetaine).

The *isolation* by Schulze's method is described along with that of choline (p. 150) as is also Staněk's method of estimation (p. 151).

For the *estimation in crude sugar and in molasses* Staněk [1904] dissolves 20-30 grm. of the former or 3-5 grm. of the latter in 50 c.c. of 10 per cent. sulphuric acid previously saturated with sodium chloride. This yields in either case a 1-3 per cent. solution of betaine which is completely precipitated by the potassium tri-iodide reagent (if the precipitate is oily, it may be rendered filterable by adding finely powdered iodine); the nitrogen is determined in the precipitate as described in the section on choline (p. 151).

For the estimation of betaine in plants Staněk and Domin [1910] may also be consulted.

In order *to prepare betaine from molasses* Staněk [1901-2] utilises the great stability of the base by mixing the molasses with an equal volume of concentrated sulphuric acid and heating for three hours to 130°. After neutralisation with lime, evaporation to dryness and extraction of the residue with alcohol, the alcoholic extract is treated with charcoal, concentrated to a syrup and saturated with gaseous hydrogen chloride, when betaine chloride crystallises out.

A method of isolating betaine from the desaccharified strontium liquors as the phosphate is given by Andrlik [1903-4] and as the chloride by Stoltzenberg, German patent No. 243332 and [1912].

The last-named method is similar to that given by Urban [1913], but the best method of all is apparently that due to Ehrlich [1912 and D.R.P. 157173 of 1904]. From the desaccharified residue ("Melasse Schlempe") the betaine is extracted as base by means of 96 per cent. alcohol, and after evaporation of the alcohol, the free base is converted into the chloride which is crystallised. The commercial product *acidol* is prepared according to this method.

Chemical Properties and Derivates of Betaine.

Betaine crystallises from alcohol in deliquescent crystals containing one molecule of water which is lost at 100°. The hydrated substance

probably has the constitution $(CH_3)_3N(OH).CH_2.COOH$, of which the other substance is a cyclic anhydride.

Betaine and its isomeride, the methyl ester of dimethyl-amino-acetic acid, are interconvertible at temperatures between 135° (the boiling point of the ester) and 293°; over this range betaine is the more stable and it is formed in good yield by heating the ester in a sealed tube to 200°. On the other hand a 50 per cent. yield of the ester is obtainable by heating betaine to 300°, when the ester distils out. At or above 293° betaine begins to be decomposed into trimethylamine and other substances (Willstätter [1902, 1]).

Betaine is a very feeble base, forming a series of stable salts. The salts with mineral acids have a strongly acidic reaction, and for this reason the chloride is sold as a solid substitute for hydrochloric acid under the name "acidol".

The *chloride*, $C_5H_{12}O_2NCl$, forms leaflets, melting and decomposing at 227-228° (243°); it is very soluble in water and differs from the hydrochlorides of most organic bases in being almost insoluble in absolute alcohol (1 grm. dissolves in 365 c.c. of absolute alcohol at room temperature; Schulze [1909, Ch. IV, choline]).

The *iodide*, $C_5H_{12}O_2NI$, non-deliquescent crystals; m.p. 188-190°; very soluble in hot alcohol, but little in cold (Willstätter [1902, 1]). The *periodide*, $C_5H_{12}O_2NI.I_5$, loses iodine on exposure to the air [Staněk, 1912]. Compounds with potassium iodide of the formulæ $C_5H_{11}O_2N.KI.2H_2O$ and $(C_5H_{11}O_2N)_2.KI.2H_2O$ have also been described (see Willstätter [1902, 1]).

The *phosphate*, $C_5H_{11}O_2N.H_3PO_4$, melts at 199-200° and decomposes at 234° (Andrlik [1903-4]).

The *perchlorate*, $C_5H_{11}O_2N.HClO_4$, is much less soluble than the corresponding choline salt; at 19° 17·73 parts dissolve in 100 parts of water (Hofmann, Roth, Höbold and Metzler [1910, Ch. IV, choline]; Hofmann and Höbold [1911, Ch. IV, choline]).

The *picrate*, $C_5H_{11}O_2N.C_6H_3O_7N_3$, forms yellow needles; m.p. 180-181°; it is suitable for the separation of the base from mixtures (Schulze and Trier [1910, 1]).

The *picrolonate*, $C_5H_{11}O_2N.C_{10}H_8O_5N_4$, forms yellow needles readily soluble in alcohol and in water, and decomposes at 200° (Otori [1904, 3, Ch. I]).

The *platinichloride*, $(C_5H_{11}O_2N)_2H_2PtCl_6.4H_2O$., crystallises from concentrated aqueous solution in the cold in large rhomb-shaped tables with truncated angles, and effloresces in air; m.p. 242°; insoluble in alcohol, very soluble in hot water from which it crystallises

in pale orange-yellow prisms with varying water content. In contact with the aqueous mother liquor, the anhydrous needles which separate from a hot solution are transformed into the four-sided tables with $4H_2O$. This constitutes a test for betaine [Trier, 1913, 5]. It is possibly dimorphous (Willstätter [1902, 2]).

The *aurichloride*, $C_5H_{11}O_2N \cdot HAuCl_4$, is the most characteristic salt and is dimorphous (Willstätter [1902, 2]).

(*a*) Regular system; from a 5 per cent. solution in hot water on slow cooling, best in the presence of a slight excess of gold chloride; it generally separates in dull yellow, star-shaped aggregates; m.p. 200-209° (uncorr.) according to the rate of heating.

(*b*) Rhombic system; bright yellow leaflets, prisms and plates with one truncated angle; m.p. 248-250° (uncorr.). This form always separates in the presence of hydrochloric acid.

By recrystallisation from pure water a pale yellow salt of an inferior gold content is obtained, possibly due to admixture with a hydrated salt. For purposes of identification it is therefore best to recrystallise from 0·5-1 per cent. hydrochloric acid, in order to obtain the rhombic variety of high melting point (Willstätter [1902, 2], Fischer [1902]).

The *mercurichloride*, $(C_5H_{11}ON \cdot HCl)_2 \cdot HgCl_2$, is fairly readily soluble in water, sparingly in alcohol.

Stachydrine.

The *preparation* of stachydrine from *Stachys* tubers and from orange leaves was carried out by Schulze and Trier [1909, 1] by purifying an aqueous extract with basic lead acetate, precipitating the bases in the filtrate with phosphotungstic acid, removing the "histidine" and "arginine" fractions of the recovered bases by means of silver, again precipitating the bases from the filtrate of these fractions as phosphotungstates, extracting the recovered hydrochlorides with absolute alcohol and then precipitating with mercuric chloride; the stachydrine is separated from choline by Staněk's method (see p. 151).

The yield from fresh tubers of *Stachys* was 0·036 per cent. of stachydrine; from dried orange leaves 0·19 per cent. The tubers also contain a minute quantity of trigonelline. Jahns [1896] isolated stachydrine by means of potassium bismuth iodide (Kraut's reagent).

For the properties of the base and its salts consult Schulze and Trier's paper [1910, 2]. Like other betaines, the base loses a molecule of water of crystallisation at 100°; the anhydrous base has the composition $C_7H_{13}O_2N$ and melts at 235°. Stachydrine is readily

soluble in water and in alcohol, but not in cold chloroform or in ether; its aqueous solution is neutral.

The *hydrochloride*, $C_7H_{13}O_2N$. HCl, crystallises in large prisms and dissolves in 12·7 parts of cold absolute alcohol at 17-18°; it is therefore much more soluble than betaine hydrochloride.

The *acid oxalate*, $C_7H_{13}O_2N$. $C_2H_2O_4$, forms needles, insoluble in cold absolute alcohol; m.p. 105-107°.

The *picrate*, $C_7H_{13}O_2N$. $C_6H_3O_7N_3$, m.p. 195-196°, is only precipitated from a concentrated solution.

The *aurichloride*, $C_7H_{13}O_2N$. $HAuCl_4$, precipitated in aqueous solution, soon crystallises and forms characteristic four-sided leaflets of rhombic habit; m.p. 225° on rapid heating.

The *platinichloride*, $(C_7H_{13}O_2N)_2H_2PtCl_6$, with 0, 2 and $4H_2O$, readily soluble in water, insoluble in alcohol; m.p. indefinite at 210-220°.

Mercuric chloride causes a precipitate in solutions of the hydrochloride (best in alcoholic solution), but not in those of the free base.

Stachydrine methyl and *ethyl esters* are only soluble in acid solution.

Betonicine and Turicine.

The *hydrochloride* of betonicine is less soluble in absolute alcohol than the hydrochloride of turicine, but the free bases have a reverse order of solubility.

Betonicine, $C_7H_{13}O_3N + H_2O$ has $[\alpha]_D = -36{\cdot}60°$ and decomposes at 243-244°. *Turicine* $[\alpha]$ has $_D = +36{\cdot}26°$ and decomposes at 249°. Betonicine hydrochloride gave $[\alpha]_D = -24{\cdot}79°$ and turicine hydrochloride $[\alpha]_D = +24{\cdot}65°$.

Betonicine *aurichloride* decomposes at 242°, that of turicine at 232°. Betonicine *platinichloride* crystallises with $2H_2O$ and decomposes at 226°, turicine platinichloride contains only $1H_2O$ and decomposes at 223°. Both bases heated with zinc dust give a pyrrole reaction with pine wood.

Trimethyl-histidine.

Reuter found this base in the arginine fraction, Kutscher curiously enough in the lysine fraction. It is best isolated as aurichloride.

The base from *Boletus* has $[\alpha]_D = +41{\cdot}1°$ (in the presence of 8 mol. HCl).

The *nitrate* forms large transparent plates and octahedra. The *monopicrate*, $C_9H_{15}O_2N_3$. $C_6H_3O_7N_3$. H_2O, thin felted needles, m.p. 201°, is readily soluble in water; the *dipicrate*, $C_9H_{15}O_2N_3$. $2C_6H_3O_7N_3$. $2H_2O$,

is much less soluble in water (in 25 parts at 100°); it melts at 123°; when anhydrous the melting point is 213-214°.

The normal *aurichloride*, $C_9H_{15}O_2N_3 . 2HAuCl_4$, forms large orange yellow crystals, m.p. 184°, by crystallisation in the presence of dilute hydrochloric acid and excess of gold chloride. Reuter mentions two other gold salts of abnormal composition.

Ergothioneine.

For the preparation of the base according to Tanret [1909] ergot is extracted with 90 per cent. alcohol; after evaporation of the alcohol, the aqueous residue is freed from fat and resin by filtration; 20 per cent. sulphuric acid is then added to precipitate colouring matters, and after removal of the acid by baryta, the filtrate is precipitated with basic lead acetate. After filtering again, the excess of lead is removed with sulphuric acid and the solution is made alkaline and extracted with chloroform to remove the complex ergot alkaloids. It is then acidified with acetic acid and precipitated completely with a warm 8 per cent. solution of mercuric chloride. The mercury precipitate is filtered off, washed, suspended in a large bulk of water and decomposed by hydrogen sulphide. After removal of the mercuric sulphide, the filtrate is evaporated under reduced pressure to a syrup from which ergothioneine hydrochloride soon crystallises. After washing with alcohol the substance is recrystallised from water. The yield is 0·1 per cent. of the ergot employed. From the hydrochloride the base can be obtained in various ways, for instance by boiling with excess of calcium carbonate, filtering, concentrating and adding alcohol. The free base is recrystallised from boiling 60 per cent. alcohol.

Ergothioneine crystallises in leaflets and needles containing two molecules of water of crystallisation. It is soluble in 8.6 parts of water at 20°, but requires more than a thousand parts of boiling 95 per cent. alcohol, and is insoluble in ether, chloroform and benzene. The base is dextro-rotatory, $[\alpha]_D = +110°$. The melting point on the Maquenne block is 290°.

Ergothioneine does not act on litmus; the salts are precipitated even in dilute solution by potassium mercuric iodide, by iodine in potassium iodide and by mercuric chloride. With sodium p-diazobenzene sulphonate a cherry-red coloration is produced (Pauly's histidine reaction). The most characteristic reaction is with excess of alcoholic iodine solution which forms crystals of a less soluble iodide (p. 46). On evaporation of the alcohol these crystals take up iodine and become steel grey or blue.

Hypaphorine.

The isolation from the seeds of *Erythrina Hypaphorus* is carried out, according to Greshoff, by adding dilute nitric acid to an aqueous or alcoholic extract of the powdered cotyledons; this causes the very sparingly soluble nitrate to crystallise out. The yield is 3 per cent. of the dried seeds.

The free base is obtained from the nitrate by adding concentrated sodium carbonate solution; the base then separates as an oily upper layer which soon crystallises. Hypaphorine is also obtainable from an aqueous extract, after purification with lead acetate and concentration. The mother liquor of the crystals of the free base is treated with nitric acid and yields a further quantity as nitrate.

Hypaphorine crystallises from water in large monoclinic transparent crystals of the composition $C_{14}H_{18}O_2N_2 . 2H_2O$ which effloresce in a desiccator.

The anhydrous substance melts at about 255° with decomposition. It is dextro-rotatory; in 1-3 per cent. solution in water $[\alpha]_D = +93°$. Hypaporine dissolves very readily in water and also readily in alcohol, but not in other organic solvents. The aqueous solution is neutral to litmus and, if not very dilute, yields precipitates with most alkaloidal reagents. Gold chloride is reduced and coloured red even by dilute solutions; potassium permanganate is decolourised and a solution containing ferric chloride and potassium ferricyanide yields Prussian blue. The solution of the base in concentrated sulphuric acid yields with various oxidising agents (potassium dichromate, ferricyanide, etc.) an intense violet coloration which soon disappears. The close relationship between hypaphorine and tryptophane is shown by the fact that the former substance also gives Hopkins and Cole's reaction with glyoxylic and sulphuric acids, but hypaphorine does not react with triketohydrindenehydrate. In spite of the similarity of its structure to that of tryptophane, hypaphorine yields on oxidation with ferric chloride only traces of β-indole aldehyde.

The most characteristic salt is the *nitrate*, $C_{14}H_{18}O_2N_2 . HNO_3$, which melts with decomposition at 215-220° and dissolves at room temperature in about 170 parts of water; other crystalline salts and the free base are much more soluble.

The *quaternary iodide*, $C_{15}H_{21}O_2N_2I$, obtained by methylation from both tryptophane and hypaphorine, forms glistening plates from boiling water and dissolves in 200 parts of water at 18°.

Trigonelline.

Jahns extracted *Trigonella* seeds with 70 per cent. alcohol, purified with basic lead acetate, concentrated to a syrup and precipitated with potassium bismuth iodide. The precipitate was decomposed with soda, and after filtration the solution was exactly neutralised; mercuric chloride was then added until mercuric iodide appeared. This precipitates only choline, but on acidification the crystalline double salt of trigonelline separates.

Schulze [1909; Ch. IV, choline] used phosphotungstic acid and alcoholic mercuric chloride for approximately quantitative estimation of trigonelline.

Trigonelline, $C_7H_7O_2N \cdot H_2O$, becomes anhydrous at 100°, when the crystals become opaque without losing their shape. The hydrated base melts at about 130°, the anhydrous at 218°.

The *platinichloride*, hardly soluble in alcohol, crystallises from water. There are two characteristic *aurichlorides;* one, of normal composition, $C_7H_7O_2N \cdot HAuCl_4$, leaflets, m.p. 198°, changes on recrystallisation from water to the basic salt $(C_7H_7O_2N)_4 \cdot 3HAuCl_4$, needles, m.p. 186°, which recrystallised in the presence of gold chloride and hydrochloric acid, may be reconverted to the normal salt.

Butyrobetaine.

Brieger precipitated the alcoholic mother liquors of putrescine hydrochloride with alcoholic mercuric chloride and extracted the precipitate with boiling water. On cooling cadaverine mercurichloride crystallised out, while the butyrobetaine salt remained in solution. After removal of the mercury with hydrogen sulphide and concentration to a syrup, the butyrobetaine was precipitated as sparingly soluble aurichloride.

According to Willstätter the *free base* crystallises from dilute alcohol in leaflets, probably with three molecules of water. Dried over sulphuric acid, the composition is $C_7H_{15}O_2N$; the crystals begin to soften at 130° and froth up at 222°, decomposing into trimethylamine and γ-butyrolactone.

The *hydrochloride*, $C_7H_{15}O_2N \cdot HCl$, forms needles, almost or quite insoluble in absolute alcohol; m.p. 200° (Takeda), 203° (Engeland and Kutscher).

The *aurichloride*, $C_7H_{15}O_2N \cdot HAuCl_4$, is precipitated on adding gold chloride to an aqueous solution of the hydrochloride; it crystallises in needles and leaflets and melts at 176° (Brieger; his formula contains two more hydrogen atoms).

The *platinichloride*, $(C_7H_{15}O_2N)_2 . H_2PtCl_6$, is readily soluble in warm water, but hardly in hot alcohol, and forms light red plates, melting at 224-225°.

The *ethyl ester* yields a characteristic *platinichloride* $(C_9H_{19}O_2N)_2 . H_2PtCl_6$, melting at 220° (Takeda, Engeland and Kutscher).

Apart from the synthesis, the constitution is established by the formation of an ester, by the optical inactivity (Takeda) and by the liberation of trimethylamine on distillation with baryta.

Solutions of the hydrochloride are not precipitated by picric acid, but by phosphomolybdic and phosphotungstic acids, by potassium mercuric iodide, potassium cadmium iodide, and potassium tri-iodide; in all cases the precipitate, which is at first amorphous, soon crystallises in needles (Brieger). Takeda also observed the gradual crystallisation of the precipitate with potassium bismuth iodide.

Carnitine.

This substance is best prepared from meat extract by Gulewitsch and Krimberg's method. After removal of carnosine and other bases by means of silver nitrate and baryta, the solution is freed from silver and barium, and the carnitine is precipitated with potassium bismuth iodide (see p. 121).

The free base, the *hydrochloride* $C_7H_{15}O_3N . HCl$ and the *nitrate* $C_7H_{15}O_3N . HNO_3$ are all readily soluble in water; a 10 per cent. solution of the hydrochloride in excess of free acid has $[\alpha]_D = -20{\cdot}9°$.

The *platinichloride*, $(C_7H_{15}O_3N)_2 . H_2PtCl_6$, crystallises from 80 per cent. alcohol in short prisms; m.p. 214-218°.

The *aurichloride*, $C_7H_{15}O_3N . HAuCl_4$, forms citron yellow needles; m.p. 153-154°.

There are two *double salts with mercuric chloride:* $C_7H_{15}O_3N . 2HgCl_2$, from the free base and mercuric chloride, both in alcoholic solution; sparingly soluble in water and crystallising fairly readily; m.p. 204-205°. $C_7H_{15}O_3N . HCl . 6HgCl_2$ is formed in the presence of a slight excess of hydrochloric acid; it is an oil, crystallising with difficulty; m.p. 211-215°.

Carnitine ethyl ester, $C_9H_{19}O_3N$, was according to Krimberg [1908, **2**] mistaken by Kutscher for a new base from meat extract under the name *oblitine;* Kutscher gave it the formula $C_{18}H_{38}O_5N_2$. Krimberg [1907, **2**] showed that oblitine is formed by evaporating an alcoholic solution of carnitine with hydrochloric acid, which is one of the steps in Kutscher's process of separation. At first Krimberg considered oblitine to be the ethyl ester of an anhydride, formed from two

molecules of carnitine by loss of one molecule of water, but the composition of the base is not $C_{18}H_{38}O_5N_2$ but $C_9H_{19}O_3N$ and the substance is merely carnitine ethyl ester. It is therefore not surprising that "novaine" (= carnitine) is formed from oblitine by bacterial action, and is the only product which can be isolated (Kutscher [1906, **2**]), nor that oblitine is partially transformed in the intestine to "novaine" (Kutscher and Lohmann [1906, **1**]).

Preparation of Choline from Natural Sources.

The best source is egg-yolk. Crude lecithin, obtained by extracting the yolk with alcohol and ether, is hydrolysed by boiling with saturated baryta solution for one hour; after removal of the barium, the solution is evaporated and the residue extracted with alcohol. After acidification of the alcoholic solution with hydrochloric acid, the choline is precipitated by alcoholic platinic chloride solution.

According to the German patent No. 193449 of J. D. Riedel [1908] lecithin is heated with twice its weight of 40 per cent. sulphuric acid, and after removal of the acid with baryta, choline is precipitated with mercuric chloride (cf. also Moruzzi [1908] and MacLean [1908]). To convert the platinichloride into the hydrochloride, the aqueous solution of the former salt is evaporated after adding the calculated quantity of potassium chloride, and then the choline chloride can be extracted by absolute alcohol.

Schulze's Method of Separating Choline and other Plant Bases.

This method [1909] for the more or less quantitative isolation of choline, betaine and trigonelline, is more trustworthy than that of Staněk (described below) when other bases are present, and is correspondingly more complicated.

An aqueous extract of the material (which is preferable to an alcoholic one since it excludes phosphatides more completely) is purified with lead acetate, strongly acidified with sulphuric acid and precipitated with phosphotungstic acid. After regeneration of the precipitate with baryta, the purine bases and the histidine and arginine fractions are removed by means of silver nitrate in the usual manner, and the last filtrate is again precipitated with phosphotungstic acid; after regeneration the mixture of chlorides is dissolved in 95 per cent. alcohol and precipitated with alcoholic mercuric chloride. Choline mercurichloride is very little soluble in boiling water, the betaine compound more so. The separation is completed by converting the more and the less soluble mercurichlorides into the dry hydrochlorides

and extracting with anhydrous alcohol, which leaves betaine hydrochloride undissolved. The method may be shortened by omitting the second precipitation with phosphotungstic acid, and in place of it precipitating the filtrate from arginine at once with mercuric chloride (after removal of the silver). It is also possible to combine Staněk's process with mercuric chloride precipitation.

The properties of trigonelline are similar to those of betaine and the separation from choline is effected in the same way. According to Schulze 3-4 per cent. of these bases escape precipitation with phosphotungstic acid. In alcoholic solution 5 per cent. of the trigonelline and choline escaped precipitation by mercuric chloride, but in the case of betaine the loss was more than double this amount, so that it is advisable to concentrate the filtrate.

Staněk's Method for the Estimation of Choline and Betaine.

The method [1905, 1906, **1, 2**] is based on the fact that betaine, being a very weak base, is set free from its salts by sodium bicarbonate, while choline is not. It is carried out as follows: To 25-40 c.c. of the aqueous solution, containing at most 5 per cent. of the mixed hydrochlorides of choline and betaine, sodium or potassium bicarbonate is added to make 5 per cent. and then a solution of 153 grm. of iodine and 100 grm. of potassium iodide in 200 grm. of water is added until precipitation is complete; the precipitate consists of brown choline ennea-iodide and soon becomes crystalline. It is collected on a paper disk in a Gooch crucible, washed with water and transferred to a Kjeldahl flask for nitrogen determination. If desired, the choline may instead be recovered from the periodide by adding finely divided ("molecular") copper (see p. 122), boiling with cupric chloride and copper and, after filtration, treating the filtrate with hydrogen sulphide. The solution then contains the choline as hydrochloride.

The betaine is estimated by concentrating the filtrate which passed through the Gooch crucible to 25 c.c. and adding enough sulphuric acid to make 10 per cent.; the solution is then saturated with sodium chloride, and the betaine is now precipitated with the potassium tri-iodide solution (already used for choline). After standing for three hours the precipitated betaine per-iodide is collected, washed five times with 5 c.c. of saturated sodium chloride and transferred to a Kjeldahl flask in which its nitrogen content is determined.

For the estimation of choline (and betaine) in plants Staněk extracts the air dry material with 96 per cent. alcohol which is distilled off; the aqueous residue is boiled with baryta and the barium is re-

moved by carbon dioxide; the filtrate is then treated with tannin, of which the excess is removed by baryta. The choline and betaine are then precipitated *together* as periodides from acid solution, and after successive treatment of the precipitate with copper powder and with cupric chloride, the mixture of chlorides is separated as described above. If much betaine is present it is preferable to effect a preliminary separation of the dry chlorides by means of absolute alcohol, in which betaine chloride is insoluble.

Tests, Chemical Properties and Salts of Choline.

An admirable account of choline is given by Gulewitsch [1908, 1]. The free base is very soluble in water, from which it cannot be extracted by organic solvents. (Only amyl alcohol extracts more than traces from an alkaline solution.) Choline is a strong base, liberating ammonia from its salts and preventing the coagulation of proteins.

The most delicate precipitant is potassium tri-iodide (limit according to Gulewitsch 1 : 20,000; according to Kinoshita [1910, 2] the limit (with Staněk's concentrated potassium tri-iodide, see above) is at 1 : 2,000,000. The choline per-iodide on standing forms rhomboidal, almost quadratic, leaflets.

Phosphotungstic acid precipitates at 1 : 20,000 (Gulewitsch). Less sensitive precipitants in aqueous solution are potassium bismuth iodide, mercuric chloride, saturated cadmium chloride and gold chloride. Tannin precipitates only in strictly neutral solution. In absolute alcoholic solution mercuric chloride and platinic chloride are the most delicate reagents (1 : 2,000,000).

The *periodide test* was used by Florence [1897] as a reaction for semen; Bocarius [1901] showed that it is due to choline. The test may be applied in a characteristic way to crystals of choline platinic chloride. After evaporating the solution of this platinum salt in 15 per cent. alcohol on a microscope slide at 40°, potassium tri-iodide solution (20 grm. iodine and 60 grm. potassium iodide per litre) is added; the yellow crystals of the platinichloride disappear and are replaced by dark brown doubly refractive and dichroitic prisms and plates of choline periodide. When the excess of reagent evaporates, the periodide dissociates and the brown crystals liquefy and disappear, but they can be reformed by again adding the reagent (cf. Rosenheim [1905-6]). Joesten [1913] considers that Florence's crystals are perhaps merely iodine, without any choline. His paper should be consulted for an account of the literature of the reaction.

Alloxan reaction.—When a drop of choline hydrochloride is eva-

porated with a drop of a saturated alloxan solution, a reddish violet colour results, which becomes more blue on the addition of caustic soda. The reaction is not characteristic and similar colorations are produced by ammonium salts, proteins and amino-acids (cf. Hurtley and Wootton, Journ. Chem. Soc., 1911, **99**, 288).

Choline, as free base, deliquesces in the air and absorbs carbon dioxide. According to Gulewitsch aqueous solutions may be concentrated by boiling to 4 per cent. concentration, when trimethylamine is given off. The base is not changed rapidly by boiling with alkalies in dilute solution; on keeping for a long time in aqueous solution neurine is formed. Concentrated nitric acid converts it to its nitrous acid ester, pseudo-muscarine (cf. addendum, p. 68). It may be oxidised to betaine ("Oxyneurin," Liebreich [1869, **1**]).

The organisms from a hay infusion probably to some extent convert choline into neurine (Schmidt [1891]). Brieger had already surmised that this change takes place in putrefaction and found that all the choline disappeared within the first week, but Gulewitsch [1864, Ch. I] isolated choline from putrid horse meat after four months' putrefaction at 15°. According to Ackermann and Schütze [1910, 1911, Ch. I] *Bacterium prodigiosum* forms trimethylamine and a little monomethylamine from choline, but *Bacillus vulgatus* does not decompose it. Prolonged anærobic putrefaction yields CO_2, CH_4, N_2, NH_3 and CH_3NH_2 (Hasebroek [1887, Ch. I]).

All known choline salts are readily soluble in water, except the periodide, the phosphotungstate and the double salts with gold and with mercury. Some, as for instance the *chloride* $C_5H_{14}ONCl$, are deliquescent. The chloride also dissolves readily in absolute alcohol (distinction from betaine).

The *sulphate* $(C_5H_{14}ON)_2SO_4$, the *acetate* $C_5H_{14}ON \cdot C_2H_3O_2$, and the *monophosphate* $C_5H_{14}ON \cdot H_2PO_4$ are all readily soluble in water, and crystallise in needles. The first two are readily soluble in alcohol, but the phosphate is not. The acetate is deliquescent (Renshaw [1910]).

The *perchlorate* $C_5H_{14}ON \cdot ClO_4$, m.p. 273°, dissolves in 2·9 parts of water at 15° and is not birefringent. The *perchlorate of the nitric acid ester* of choline is, however, only very slightly soluble (0·62 parts in 100 parts of water at 15°). It is obtained by evaporating 0·1 grm. of choline perchlorate in 50 c.c. of water with 2 c.c. of 65 per cent. nitric acid, dissolving the residue in a little water and adding a few drops of a concentrated aqueous solution of perchloric acid. This latter salt is characteristic; it is strongly birefringent, melts at 185-186° and is suitable for the isolation of choline (Hofmann and Höbold [1911]).

The acid *chromate*, $C_5H_{14}ON \cdot HCrO_4$, is on the other hand much more soluble than the neurine salt (Cramer [1904]).

The *picrate*, $C_5H_{14}ON \cdot C_6H_2O_7N_3$, is fairly soluble in water and more so in alcohol (Brieger [1885, **2**, p. 56; Ch. I]).

The *picrolonate*, $C_5H_{14}ON \cdot C_{10}H_7O_5N_4 \cdot H_2O$, loses water of crystallisation at 130°, melts at 158° and decomposes at 241-245° (Otori [1904, **3**]).

The *platinichloride*, $(C_5H_{14}ON)_2PtCl_6$, is dimorphous. It crystallises from a mixture of equal volumes of absolute alcohol and water in the regular system (octahedra, cubes) and from water in rhomb-shaped six-sided or pyramidal crystals of the monoclinic system; on slow evaporation the latter kind may attain considerable size (Kauffmann and Vorländer [1910]; Gulewitsch [1891, **1**] gives crystallographic details). Both forms of the salt are anhydrous and orange red in colour; they are stable in the dry state, but readily interconvertible by recrystallisation from the proper solvent. Since one form is isotropic and the other anisotropic, the dimorphism of choline platinichloride is readily detected in polarised light and affords according to Kauffmann the surest qualitative means of identification. The platinichlorides of potassium, ammonium, trimethylamine and neurine all crystallise from dilute alcohol in the regular system only; if, after adding water and evaporating, crystals become anisotropic, choline is probably present.

At 21° one part of choline platinichloride dissolves in 5·82 parts of water (Gulewitsch). The melting point is not characteristic; both forms melt at 209-211° on slow heating and at 240-241° when heated rapidly.

The *aurichloride*, $C_5H_{14}ONAuCl_4$, crystallises in deep yellow needles and also (from very dilute alcohol) in octahedra and cubes; it dissolves in 75·2 parts of water at 21° and in hot alcohol (Gulewitsch). The melting point has been variously given as 238-239°, 249°, 244-264°, etc.

The *mercurichloride*, $C_5H_{14}ONCl \cdot 6HgCl_2 \cdot H_2O$, forms crossed hexagonal prisms, loses water above 100° and melts at 249-251°; it is soluble in 56·6 parts of water at 24·5° (Gulewitsch). According to Mörner [1896; Ch. I] the melting point is 242-243° and it dissolves in 67 parts of water at 19·5°. Schulze [1909] found that one part of the mercury salt dissolves in about fifty parts of water at room temperature; the solubility determinations were not concordant, probably owing to hydrolytic dissociation.

The slight solubility of choline mercurichloride in cold water was used by Brieger for its isolation; after complete precipitation by

mercuric chloride in alcoholic solution, the precipitate was extracted with boiling water, in which the mercury compounds of peptones and proteins were completely insoluble. The choline mercurichloride crystallised out almost completely from the filtrate on cooling, and the mercury salts of other putrefaction bases remained in solution.

Double salts of choline chloride with *cadmium* and with *zinc* chloride are precipitated in alcoholic solution.

Staněk [1905] has described two *periodides.* With excess of iodine in potassium iodide an *ennea-iodide* $C_5H_{14}ONI \cdot I_8$ is formed as a brown precipitate, changing to shiny green crystals; in a 0·1-1 per cent. choline solution only 2-3 per cent. of the total escapes precipitation. When choline is in excess a hexa-iodide $C_5H_{14}ONI \cdot I_5$ results.

Amino-ethyl Alcohol.

The free base distils at 160-165° and 718 mm. The *hydrochloride* $C_2H_5ON \cdot HCl$ is hygroscopic. The *aurichloride* $C_2H_5ON \cdot HAuCl_4$ crystallises slowly from concentrated hydrochloric acid containing excess of gold chloride in large crystals, melting at 186-187°. The *platinichloride* is anhydrous.

Amino-ethyl alcohol differs from choline in not being precipitated by potassium bismuth iodide, and not by phosphotungstic acid except in concentrated solutions. Heated with hydriodic acid, as in Herzig and Meyer's method for the determination of N-methyl groups, it gives off a little ethyl iodide [Trier, 1913, 5].

Neurine.

The *separation of neurine from choline* may be carried out by fractional crystallisation of the platinum salts; the large crystals of choline platinichloride are readily obtained pure, but the small, less soluble crystals of the neurine salt are only purified with difficulty (Gulewitsch [1899; under choline]).

The chemical properties of neurine and some of its compounds have been described in detail by Gulewitsch [1898, 2]. It is a strong base and, like choline, it liberates ammonia from its salts and prevents the coagulation of protein. It may be boiled in dilute solution without decomposition, and is not changed by boiling with concentrated baryta. Its behaviour with alkaloidal reagents is very similar to that of choline; generally the reactions are more delicate; thus with phosphotungstic acid and with potassium tri-iodide a micro-crystalline precipitate is produced which is even indicated at a dilution of 1 : 200,000.

The *chloride*, $C_5H_{12}NCl$, forms deliquescent needles, the *iodide* is non-deliquescent; m.p. 196°.

The *perchlorate*, $C_5H_{12}NClO_4$, forms characteristic aggregates of short prisms, which are scarcely birefringent; 100 grm. of water at 20° dissolve 5·764 grm., at 145° 4·89 grm. Hence this salt is much less soluble than the corresponding choline salt, but six times as soluble as the perchlorate of choline nitric acid ester. *q.v.* [Hofmann and Höbold, 1911; under choline].

The acid *chromate*, $C_5H_{12}N \cdot HCrO_4 \cdot H_2O$, forms orange needles from water; m.p. 278° on rapid heating; heated slowly it decomposes explosively at 140-150°. In contradistinction to choline chromate it is little soluble in cold water (Cramer [1904, under choline]).

The *picrate*, $C_5H_{12}N \cdot C_6H_2O_7N_3$, forms long feathery golden yellow needles; m.p. 263-264°; soluble in 91·6 parts of water at 23°, more so in hot water, readily in hot alcohol (Gulewitsch [1898, **2**]).

The *platinichloride*, $(C_5H_{12}N)_2PtCl_6$, forms cubes and octahedra of the regular system; m.p. 196-198° (but according to Nothnagel the melting point is 15-20° higher); the salt is anhydrous and at 20·5° dissolves in 37·6 parts of water (Gulewitsch [1898, **2**]). The solubility is considerably less than that of the corresponding choline salt.

The *aurichloride*, $C_5H_{12}N \cdot AuCl_4$, forms large golden yellow acicular crystals; m.p. 232-238°; soluble in 336·5 parts of water at 21·5°; not very soluble in hot water.

There are two *mercurichlorides* formed by precipitation with alcoholic $HgCl_2$ and not readily separated. (*a*) $C_5H_{12}NCl \cdot 6HgCl_2$, plates and prisms; m.p. 230-234°; is but little soluble in hot water. (*b*) $C_5H_{12}NCl \cdot HgCl_2$, triclinic plates, more readily soluble in water (Gulewitsch [1898, **2**]).

Creatine and Creatinine.

Preparation of creatine for muscle.—Liebig mixed minced meat repeatedly with an equal volume of cold water and pressed out. In the extract the protein was coagulated and, after straining, the solution was treated with baryta until no more precipitate occurred. After filtration and concentration creatine crystallised out in the course of a few days.

It is, however, better to start with commercial meat extract and after dissolving in twenty parts of water, to precipitate peptones, etc., either with basic lead acetate (Mulder and Mouthaan [1869]) or with tannin (Kutscher [1905]). After removal of the excess of lead or of tannin (see p. 117) the filtrate is concentrated to a thin syrup; on standing creatine crystallises and is then washed with absolute alcohol to remove creatinine and is recrystallised with charcoal; the creatinine crystallises from the alcoholic washings on the addition of ether. Creatinine, abundantly present in most commercial meat extracts, is also obtained by Kutscher's method as a silver compound in the histidine fraction. Here it is accompanied by carnosine, from which it is separated by solution in alcohol, which leaves the carnosine behind.

Preparation of creatinine.—Creatinine is most conveniently obtained from urine by precipitation with picric acid (Folin and Blanck [1910]). To each litre of urine 18 grm. of picric acid, dissolved in 45 c.c. of boiling alcohol, is added.

The resulting precipitate, mostly of creatinine potassium picrate, is decomposed by grinding with potassium bicarbonate and, after filtration, the solution is slightly acidified, mixed with two volumes of alcohol, decolourised with a little charcoal and treated with concentrated alcoholic zinc chloride. The crude creatinine zinc chloride, which separates on standing, may be boiled with lead hydroxide, when about equal quantities of creatine and creatinine are obtained; or it may be dissolved in warm 10 per cent. sulphuric acid, when the addition of acetone causes the separation of pure creatinine zinc sulphate, $(C_4H_7ON_3)_2H_2SO_4 . ZnSO_4 . 8H_2O$.

The use of zinc chloride alone was introduced by Pettenkofer [1844], the discoverer of creatinine; Neubauer [1863] and Salkowski [1886, 1890] attempted to make this method a quantitative one, but as such it has been entirely superseded by Folin's colorimetric estimation. The use of picric acid for the precipitation of creatinine from urine was introduced by Jaffé [1886]; other precipitants are mercuric chloride (Maly [1871]) and phosphotungstic acid (Hofmeister [1880]).

Quantitative conversion of creatine to creatinine.—Benedict and Myers [1907, 2] heated a dilute creatine solution containing 6-7 per cent. hydrochloric acid (i.e. $\frac{1}{6}$ volume of the concentrated acid) in an autoclave to 117° for forty-five minutes. Dorner [1907] warmed a 0·1 per cent. creatine solution for 3-4 hours on the water bath with twice its volume of normal hydrochloric acid (hence concentration of acid = 2·44 per cent.). Thompson, Wallace and Clotworthy [1913] recommend adding an equal volume of normal hydrochloric acid and heating on the water bath for 3 hours or in the autoclave to 117-120 for 25 mins.

According to the last named authors pure dextrose, up to 10 per cent., does not affect the estimation of creatine, although 3 per cent. phosphoric acid has been recommended instead of hydrochloric acid, in order to avoid the formation of coloured products. Creatine figures for diabetic urine may come 5 per cent. too low, probably owing to the presence of aceto-acetic acid. The darkening of the urinary pigment by treatment with acid may increase the creatine readings in human urine by 1-2½ per cent., in dog's urine by 10 per cent.

According to Folin and Blanck [1910] creatine crystals may be converted quantitatively into creatinine by heating without a solvent in an autoclave for three hours at 4·5 atmospheres; the water of crystallisation appears to be the active agent.

Physical and chemical properties of creatine.—This substance forms lustrous transparent monoclinic prisms of the composition $C_4H_7O_2N_3$, H_2O. The 12·08 per cent. of water of crystallisation is given off quantitatively at 100-110°, and the crystals become opaque (a determination of the loss of weight may be used for identification).

Creatine dissolves in 74 parts of water at 18°; it is much more soluble in hot water, but hardly at all in absolute alcohol (1 : 9400). The aqueous solution is neutral. The basic properties of creatine are very feeble (dissociation constant $1{\cdot}81 \times 10^{-11}$ at 40·2°, Wood [1903]) and its salts with mineral acids are hydrolysed by water. Creatine is precipitated from aqueous solution by mercuric nitrate, but not by phosphotungstic acid, nor by basic lead acetate · crystal-

lisable compounds with zinc chloride and cadmium chloride are known and are dissociated by water.

Creatine reduces Fehling's solution without separation of cuprous oxide and is oxidised by boiling with mercuric oxide to methyl guanidine oxalate (Dessaignes [1854, 1855]) and also by Fenton's reagent (hydrogen peroxide and ferrous sulphate, Dakin [1906]); in the latter case glyoxylic acid is the chief other product. When it is heated with dilute mineral acids, with water, or by itself, creatinine is formed. On boiling with barium hydroxide it forms urea and sarcosine (Liebig [1847]) and also methyl hydantoin (Neubauer [1866, 1]). Heating with soda lime causes it to give off methylamine.

Physical and chemical properties of creatinine.—Creatinine generally forms anhydrous monoclinic prisms; on slow evaporation of a cold saturated solution it also crystallises with $2H_2O$ in large tables and prisms, which easily effloresce (Wörner [1899]). It is considerably more soluble in water than creatine, the solubility being 1 : 10·6 at 14° and 1 : 10·78 at 17° (Toppelius and Pommerehne [1896]; according to Liebig one part dissolves in 11·5 parts of water at 15°). Creatinine is also more soluble than creatine in cold absolute alcohol, namely 1 : 625 (Toppelius and Pommerehne [1896]). In hot alcohol much more dissolves, but hardly any in ether.

Creatinine solutions have an acrid taste and are hardly alkaline to litmus. The substance is, however, a stronger base than creatine (dissociation constant $3{\cdot}57 \times 10^{-11}$ at 40·2°; Wood [1903]) and is precipitated by phosphomolybdic acid, phosphotungstic acid (limits 1 : 12,000 on prolonged standing, according to Hofmeister [1880], and 1 : 25,000 according to Demjanowski [1912, under methylguanidine]), mercuric nitrate, mercuric chloride (1 : 3000) and by silver nitrate after careful addition of ammonia (hence it occurs in the histidine fraction of bases; Kutscher [1905]). It is not precipitated by potassium tri-iodide.

The reducing properties of creatinine are similar to those of creatine. On boiling with Fehling's solution the cuprous oxide formed at first remains dissolved as a compound with unattacked creatinine (Maschke [1878], Korndörfer [1904, 2]), but after prolonged boiling with excess of the reagent cuprous oxide separates—creatinine is the chief cause of the slight action of normal urine on Fehling's solution. Unlike glucose, creatinine does not reduce alkaline bismuth solutions. Mercuric oxide, potassium permanganate, lead peroxide and sulphuric acid oxidise creatinine to methylguanidine and oxalic acid; Fenton's reagent produces methylguanidine, formaldehyde, formic, carbonic,

and glyoxylic acids. On boiling with baryta methylhydantoin results. Dry distillation of creatinine chloride yields hydrocyanic acid, pyrrole, and dimethylamine (Engeland [1908, **4**]). On standing or boiling with very dilute alkalies, creatine is formed.

Compounds of creatine.—The *nitrate*, $C_4H_9O_2N_3 . HNO_3$, is less soluble than the hydrochloride or the sulphate. The compounds $C_4H_9O_2N_3 . ZnCl_2$ and $C_4H_9O_2N_3 . CdCl_2 . 2H_2O$ are crystalline (Neubauer [1862, **2**]). All these salts are hydrolysed by water.

Compounds of creatinine.—The *hydrochloride*, $C_4H_7ON_3 . HCl$, separates in anhydrous prisms and tables when a solution of creatinine in hydrochloric acid is evaporated on the water bath; from cold solution it crystallises with $1H_2O$. It is not precipitated by zinc chloride except in the presence of excess of sodium acetate.

Creatinine zinc chloride, $(C_4H_7ON_3)_2ZnCl_2$, is the most characteristic derivative and separates immediately as a micro-crystalline precipitate on adding a concentrated neutral zinc chloride solution to an alcoholic or not too dilute aqueous solution of creatinine; on standing, a dilute solution deposits needles and prisms. It is soluble in 53·8 parts of water at 15° and in 27·74 at 100°; it is insoluble in absolute alcohol, readily soluble in hydrochloric acid, from which sodium acetate causes a double salt of creatinine hydrochloride and zinc chloride $C_4H_7ON_3 . HCl . ZnCl_2$ (Neubauer [1861, **2**]) to crystallise in long needles, readily soluble in water. Creatinine zinc chloride dissolves in warm 10 per cent. sulphuric acid and then the addition of acetone causes the separation of a double sulphate of zinc and creatinine $(C_4H_7ON_3)_2H_2SO_4 . ZnSO_4 . 8H_2O$ (Folin and Blanck [1910]).

Creatinine may be regenerated from its double compounds with zinc by boiling with freshly precipitated lead hydroxide.

The *mercury salt* $(C_4H_7ON_3 . HCl . HgO)_4 3HgCl_2$ is formed on the addition of mercuric chloride and sodium acetate to a creatinine solution.

The *picrate*, $C_4H_7ON_3 . C_6H_3O_7N_3$, forms long yellow needles sparingly soluble in cold water; m.p. 213-214° (Toppelius and Pommerehne [1896]), 215-217° (Korndörfer [1904, **2**]).

Creatinine potassium picrate, $C_4H_7ON_3 . C_6H_3O_7N_3 . C_6H_2O_7N_2K$ (formed by saturating urine with picric acid) crystallises in citron yellow needles or thin prisms, and explodes on rapid heating: 100 c.c. of water dissolve 0·1806 grm. at 19-20°; it is also very slightly soluble in hot alcohol (Jaffé [1886]).

An *acid picrate* $C_4H_7ON_3 . (C_6H_3O_7N_3)_2$, m.p. 161-166°, has been described by Mayerhofer [1909].

Creatinine aurichloride, $C_4H_7ON_3 . HAuCl_4$, separates in yellow

leaflets on adding a slight excess of gold chloride to a concentrated solution of creatinine hydrochloride at 40-50°; the gold salt is readily soluble in water and in alcohol and, after drying at 100°, melts at 170-174° (Wörner [1899]), 182-185° (Korndörfer [1904, 2]).

Creatinine platinichloride, $(C_4H_7ON_3)_2H_2PtCl_6$, crystallises in orange red prisms and needles; from water with $2H_2O$, from alcohol anhydrous (Wörner [1899]). It is soluble in about 36 parts of water (Toppelius and Pommerehne [1896]); hardly soluble in cold alcohol; m.p. 220-225° on rapid heating.

Creatinine oxime, $C_4H_6O_2N_4$, m.p. 250°, is according to Schmidt [1912] identical with "nitroso-creatinine" of Kramm.

Colour reactions and estimation of creatine and creatinine.—The only colour reaction for *creatine* is the pink coloration produced by diacetyl, $CH_3 . CO . CO . CH_3$ (Harden and Norris [1911]). This reaction is also given by arginine and some other guanidine derivatives, but *not* by creatinine. Walpole [1911] has used it for the direct estimation of creatine in pathological urines. The usual method, however, is an indirect one; the creatine is converted into creatinine by heating with acids (see above) and then estimated by Folin's method, described below.

The following are colour reactions for *creatinine*:—

(*a*) Weyl's reaction [1878]; a freshly prepared very dilute solution of sodium nitroprusside is added and then a few drops of dilute caustic soda. In the presence of creatinine a ruby red colour is produced; acetone gives a similar coloration, and if present should first be boiled off. The red colour due to creatinine is fugitive and soon changes to yellow; if then glacial acetic acid is added and the solution is boiled, it becomes green and on standing a deposit of Prussian blue is formed (Salkowski [1879]). This reaction is given by hydantoins but not by creatine, and is still obtainable with pure creatinine solutions containing 0·03 per cent. and urine containing 0·066 per cent. of creatinine.

(*b*) Jaffé's reaction [1886]. The addition of aqueous picric acid and a few drops of caustic soda produces in creatinine solutions an immediate red coloration (orange to blood red). The colour increases during the first few minutes and afterwards fades very slowly. Limit 1 : 5000. Acetone gives a somewhat similar but much feebler reddish yellow coloration, and if present should first be boiled off. Aceto-acetic ester, hydrogen sulphide and particularly aceto-acetic acid are the only other pathological substances which may interfere.

According to Chapman [1909] the coloration in Jaffé's reaction

is due to the reduction of the picric acid and is also caused by acetone, acetaldehyde, hydroxylamine and titanium chloride in the cold, and by dextrose, maltose, lævulose, and urea on warming.

(*c*) Maschke's reaction [1878]. The creatinine solution is saturated with sodium carbonate; on warming with Fehling's solution the blue colour is discharged and a white precipitate of creatinine cuprous oxide appears, which is readily soluble in water, but only slightly so in sodium carbonate solution.

Folin's method [1904]. Since the coloration produced by picric acid and sodium hydroxide gradually fades, a half normal solution of potassium bichromate (24·54 grm. per litre) is employed as a permanent standard of colour; this accurately matches the creatinine coloration. Since the intensity of coloration is further influenced by dilution, it is necessary to work within certain limits and the solution to be examined should contain 7-15 mg. of creatinine in 500 c.c.

Folin adds to 10 c.c. of urine in a 500 c.c. measuring flask 15 c.c. of saturated (1·2 per cent.) aqueous picric acid solution and 5 c.c. of 10 per cent. sodium hydroxide; after shaking, the solution is allowed to stand for five minutes to let the colour develop fully, and is then made up to 500 c.c. The solution thus diluted is now matched with a column of the 0·5N bichromate solution 8 mm. high. If the column of creatinine solution required to do this has a height of x mm. there are present in the 10 c.c. of urine employed $\frac{8 \cdot 1}{x} \times 10$ mg. of creatinine. If more than 15 mg. of creatinine is present, only 5 c.c. of urine are taken, if less than 7 mg. 20 c.c. are employed. For substances which interfere with the test, see above, under Jaffé's reaction.

According to Thompson, Wallace and Clotworthy [1913] the maximum colour develops in 5 minutes at 17-20°; at 15-17° seven minutes are required, at 10-15° eight minutes.

The necessity of a constant temperature has been emphasised by Mellanby [1908], Chapman [1909] and others. Mellanby has plotted a curve showing the variation of colour with dilution and Cook [1909] has suggested a correction for dilution, namely the addition of 0·19 mg. to the value found for every 10 c.c. of dilution above the original 10 c.c.; thus for a 100 c.c. solution $9 \times 0 \cdot 19$ mg. should be added. For factors influencing the estimation in urine consult Taylor [1910] who considers that the variation in the light and in the pigmentation of the urine constitute the chief sources of error, and also Thompson, Wallace and Clotworthy [1913]. Under ideal conditions 10 mg. of creatinine may be estimated to within 0·1

mg.; under bad conditions to within 1 mg. Weber [1908] puts the error at 4 per cent. Rona [1910] purifies solutions by means of colloidal ferric hydroxide, which does not adsorb any creatinine.

The estimation of creatine and creatinine in meat and meat extracts by Folin's method has been carried out by Baur and Barschall [1906], Grindley and Woods [1906], Emmett and Grindley [1907], Chapman [1909] and Cook [1909]. It affords a means of distinction from the very similar commercial yeast extracts which contain no creatine or creatinine (at most 0·08 per cent.).

Chapman [1909], for the estimation of creatine + creatinine, mixes 10 c.c. of a 10 per cent. meat extract solution with 10 c.c. of normal hydrochloric acid, and heats to 120° in an autoclave for half an hour. After cooling to 20°, 30 c.c. of saturated picric acid and 15 c.c. of 10 per cent. sodium hydroxide are added; after five minutes the solution is made up to 500 c.c. and estimated colorimetrically.

For the actual isolation of creatinine from small quantities of extracts, see Micko [1910].

Glycocyamine and Glycocyamidine.

Glycocyamine, $C_3H_7O_2N_3$, forms anhydrous crystals which gradually decompose above 220° without melting. At 14·5° 1 part dissolves in 218 parts of water (Ramsay). The substance is a stronger base than creatine (dissociation constant $2{\cdot}32 \times 10^{-11}$ at 40·2°; Wood [1903, under creatine]) and yields a *hydrochloride*, $C_3H_7O_2N_3 . HCl$, m.p. 191°; a *picrate*, $C_3H_7O_2N_3 . C_6H_3O_7N_3$, m.p. 199-200°, very little soluble in water; a readily soluble *platinichloride*, $(C_3H_7O_2N_3)_2 . H_2PtCl_6 . 2H_2O$, m.p. 198-200°, and an *aurichloride*, m.p. 173°. Glycocyamine solutions give with copper acetate a pale blue precipitate $(C_3H_6O_2N_3)_2Cu . H_2O$, and with $HgCl_2$ in the presence of sodium acetate a white precipitate; no compound with zinc chloride is known.

Glycocyamidine, $C_3H_5ON_3$, is formed by heating glycocyamine hydrochloride to 160-170°; small quantities are more readily prepared by heating 1 grm. of this hydrochloride with 5 c.c. of concentrated hydrochloric acid to 140° in a sealed tube. The free base is obtained by boiling the resulting hydrochloride with freshly precipitated lead hydroxide. An alcoholic solution (but not an aqueous solution) of glycocyamidine hydrochloride gives with alcoholic zinc chloride a crystalline salt $(C_3H_5ON_3)_2ZnCl_2$. The *picrate*, $C_3H_5ON_3 . C_6H_3O_7N_3$, forms yellow needles; m.p. 206-210°. The *normal aurichloride* is very soluble and easily changes to the less soluble *gold salt* $C_3H_5ON_3 . AuCl_3$; m.p. 153-154° (Korndörfer [1905]).

Glycocyamidine, like creatinine, gives Weyl's and Jaffé's reactions; there is, however, this point of difference, that whereas the red or yellow coloration produced by creatinine, sodium nitroprusside, and caustic soda is discharged by acetic acid or changed to green on boiling (formation of Prussian blue), glycocyamidine yields with acetic acid a stable burgundy red coloration.

Guanidine.

Guanidine is a strong base, absorbing atmospheric carbon dioxide to form the well crystallised *carbonate*, $(CH_5N_3)_2 . H_2CO_3$, soluble in water but not in alcohol. Of the salts with mineral acids the *nitrate* $CH_5N_3 . HNO_3$ is among the least soluble; it forms large plates, melting at 214°.

The *picrate*, $CH_5N_3 . C_6H_3O_7N_3$, when pure forms characteristic irregular aggregations of leaflets; m.p. 315°, on rapid heating up to 320°. The solubility in cold water is 1 : 2630 at 9° and the salt may be used for the estimation of guanidine (Emich [1891]). From complex mixtures, particularly when arginine is present, guanidine is not so readily precipitated by picric acid; the arginine should first be precipitated by alcoholic picrolonic acid solution, and then, after removal of the excess of picrolonic acid from the filtrate, the guanidine may be precipitated by aqueous picric acid (Kutscher and Otori [1904]).

The *picrolonate*, $CH_5N_3 . C_{10}H_7O_5N_4$, dissolves in excess of alcoholic picrolonic acid solution (separation from arginine, above). With aqueous picrolonic acid an amorphous precipitate is formed, which crystallises from hot water in clusters of thin needles; m.p. 272-274° (Schenck [1905, 2]).

The *aurichloride*, $CH_5N_3 . HAuCl_4$, forms deep yellow needles, little soluble in water.

With alcoholic cadmiumchloride a double salt $CH_5N_3 . HCl . 2CdCl_2$ results; m.p. 390-395° (Schenck [1904]).

Guanidine is precipitated in the "arginine" fraction by silver nitrate and baryta as a *silver compound* $CH_5N_3 . Ag_2O$ which may be crystallised (Kutscher and Otori [1904]). Guanidine salts in concentrations down to 0·01 per cent. give a white or pale yellow precipitate with Nessler's reagent; arginine gives a similar precipitate.

Methylguanidine.

Methylguanidine may be synthesised by heating cyanamide and methylamine hydrochloride in alcoholic solution to 60-70°. It forms deliquescent crystals. The *nitrate*, $C_2H_7N_3 . HNO_3$, forms rhombic

leaflets, melting at 150° (155°), not very soluble in cold alcohol, and less in water and particularly in dilute nitric acid. The *picrate* $C_2H_7N_3 . C_6H_3O_7N_3$, m.p. 201·5°, crystallises in two modifications according to Gulewitsch [1906] and is more soluble than guanidine picrate.

The *picrolonate*, $C_2H_7N_3 . C_{10}H_7O_5N_4$, dissolves in 4000 parts of cold water; m.p. 291° (Wheeler and Jamieson [1907]). The *aurichloride* $C_2H_7N_3 . HAuCl_4$, m.p. 198°, is soluble in ether. The *platinichloride* $(C_2H_7N_3)_2 . H_2PtCl_6$ forms monoclinic prisms and dissolves in 14·3 parts of water at 18-19°.

Benzene-sulphonyl-methyl-guanidine, $C_2H_6N_3 . SO_2 . C_6H_5$, m.p. 184°, soluble in 2500 parts of cold water, is suitable for the isolation (Ackermann [1906]). Aqueous mercuric chloride does not precipitate the nitrate of methylguanidine even in 5 per cent. solution; mercuric sulphate precipitates a 1 per cent. solution, phosphotungstic acid a solution of 1 : 9000 (Demjanowski [1912]).

Dimethylguanidine.

The *aurichloride*, $C_3H_9N_3 . HAuCl_4$, melts at 144°, decomposes at 150° and forms thin leaflets or plates.

The *picrolonate*, $C_3H_9N_3 . C_{10}H_7O_5N_4$, m.p. 275-278°, was probably obtained from human urine by Kutscher and Lohmann [1906, **3, 4**] and forms four-sided prisms. The *picrate*, $C_3H_9N_3 . C_6H_3O_7N_3$, forms small pointed needles or branch-like growths; m.p. 224° (Wheeler and Jamieson [1907]).

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