tion is allowed to flow into the descending colon; the outflow tube is then pinched and the patient is gradually rotated first to the dorsal position and then to the right side. As the next step in the procedure the shoulders are elevated above the level of the hips (the patient still

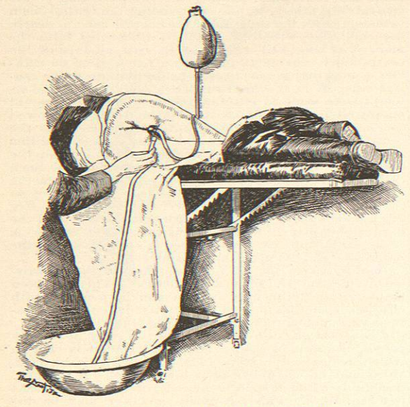

Fig. 1920.—Enteroclysis in the Sims Position.

being on the right side), in order that the fluid may gravitate into the caput coli. Then the steps just described are reversed, the outflow tube is released, and the fluid is permitted to escape. This method resembles Kussmaul's technique for irrigations with oil.

The indications for employing double-current enteroclysis may be deduced for the most part from the following paragraphs on the "Quantity and Temperature of the Solution."

Quantity and Temperature of the Solution.—In colitis, dysentery, typhoid, intestinal dyspepsia, the various forms of diarrhœa, constipation, fæcal impaction, etc., a quantity equal to from six ounces to one pint and a half may be allowed to flow into the bowel before escape is permitted. Enteroclysis may be employed once or twice daily, and the total amount of fluid used at each sitting should not be less than two quarts. In some cases as much as several gallons has thus been utilized. If there are no special contraindicating circumstances the flow should be kept up until the escaping fluid is quite clear. The temperature of the fluid used should be from 101° to 105° F. On the other hand, in sthenic cases, if there be a high temperature—as in dysentery, gastro-enteritis in infants, etc.—irrigation with a solution at from 90° to 75° F. may be employed, to aid in the reduction of temperature. Cold should be used cautiously, and I believe that it is contraindicated if there are renal complications.

For the relief of the spasm which accompanies renal, biliary, or intestinal colic, it is a good plan to allow from six ounces to one pint of a hot fluid (saline solution at a temperature of from 101° to 103° F.) to remain continuously in the bowel for fifteen minutes or longer. At times a solution having a still higher temperature (from 110° to 120° F.) acts better. This is often the case in intestinal colic, in which condition the hot solution aids in expelling flatus.

In all conditions in which it is desirable to stimulate the circulation, to increase renal secretion when insufficient, or to eliminate toxins through promotion of diuresis, etc., the quantity of the solution to be kept continuously in the bowel should average from a pint to a quart; and the enteroclysis should be kept up for from half an hour to an hour. If we wish to secure the best results under the conditions referred to, we should employ normal salt solution at a temperature somewhere between 110° F. and 120° F. In addition to the conditions last named and to those enumerated in the earlier part of the article, the following may be named: sepsis due to any cause, as from peritonitis, septic endocarditis, cholera, typhoid, diphtheria, etc.; malarial poisoning (to check or abort a chill); the various dropsical conditions, etc.

Enteroclysis, as may readily be supposed, has proved to be of use in gynæcology. Thus, for example, its usefulness in the case of an unmarried woman, as a substitute for vaginal douching, deserves special mention. It may be employed, in the form of hot enemata of normal saline solution (at a temperature of from 110° to 120° F.), for promoting the absorption of pelvic exudations, for the relief of leucorrhœas, of ovaritis, of shock, etc., and in many other conditions (C. Reginald Hyde).

In genito-urinary and rectal affections—such as inflammation of the prostate or bladder, retention of urine, spasmodic stricture of the urethra, chronic or acute inflammation of the urethra, internal hemorrhoids, etc.—enteroclysis often affords great relief. In cases of intestinal paresis and of intestinal obstruction injections of oxygen have been found useful by Dr. Clement Cleveland, of New York. (See New York *Medical Record*, January 5th, 1901.)

Solutions that may be Used in Enteroclysis.

Flaxseed tea, made thin and oily, of value in catarrhal conditions of the intestine, especially if accompanied by constipation; also aids absorption of exudations.

Fig. 1921.—Enteroclysis with Double Tube. Case of an infant.

Normal salt solution, a drachm of salt to a pint of water; useful for cleansing purposes; bland and especially safe for children; useful as a circulatory stimulant; as a diuretic, and to eliminate toxins.

Oil of peppermint or cinnamon (five to fifteen minims to the pint) may be added to the salt solution for cleansing purposes (William H. Thomson).

Boiled water, with boric acid (from half a drachm to a drachm to the quart, or even stronger), or with permanganate of potassium (from three to ten grains or more to two quarts of water); useful when there is marked fermentation or foul odor. A similar solution of bichloride of mercury (1 to 10,000) has been successfully employed (two quarts at each irrigation daily) in membranous (croupous) colitis of a septic type. In dysentery it is also of service. Stronger solutions do not appear to be necessary. Solutions of carbolic acid and of camphor have been found to be irritating. Rovacs and others employ quinine solutions (1 to 1,000) in amœbic dysentery. Solutions of tannin (one to two per cent.) are valuable in cholera. Tannic acid, tannin, witch hazel, and other astringents, are useful in dysentery. In chronic conditions injections of silver-nitrate solutions (gr. x.–xx. to the quart or even the pint), to be followed by normal saline solution to prevent overaction, are of service. Finally, solutions containing listerine, borolyptol, borax, bicarbonate of soda, or powdered alum (a drachm of the latter to the quart of water, or even a stronger solution), are of service.

Robert C. Kemp.

ENTEROL is a mixture of three isomeric creosols said to represent three bodies from the intestinal tract in normal proportion. It is slightly soluble in water, has an unpleasant odor, and is used as an intestinal antiseptic. It is given in pill or capsule, or in a solution of one grain to an ounce of water, usually combined with a laxative.

W. A. Bastedo.

ENTODERM, also called entoblast, and occasionally hypoblast, is the innermost layer of cells in the embryo; it is an epithelium which, in the adult, lines the digestive canal and its appendages, lungs, liver, pancreas, etc. (See *Germ Layers*.)

C. S. M.

ENURESIS (ἐν = in, and οὖρον = urine) is a condition in which the urine is passed involuntarily or unconsciously. It is not so much a disease as a symptom common to many diseases and disorders. In childhood, however, it may be considered a separate malady, since it is often found at this age without any other accompanying symptom or lesion; in infants, up to about three years of age, it is physiological.

Mechanism.—The bladder is a fibromuscular sac which acts as a temporary reservoir for the urine. The urine does not normally escape as it falls into the bladder, and without the knowledge of the individual; but, slowly and constantly secreted, it gradually accumulates in the bladder until it can be expelled. It is retained in the bladder by the sphincter vesicæ; it is expelled by the muscular contraction of the coats of the bladder and the detrusor urinæ, assisted by the diaphragm and abdominal muscles. Normal micturition is a reflex act. As the urine gradually flows into the bladder the intravesical pressure increases, until, on a sufficient quantity being present, afferent impulses are sent from the bladder to the centre for micturition in the lumbar enlargement of the spinal cord; from this efferent impulses are sent down strengthening the inhibitory force at the neck of the bladder, and thus causing delay in the act of urination. Further, there exist a motor tract and an inhibitory tract from the brain to the centre in the cord; and, as the intravesical tension continues to rise, impulses are sent to the brain, and other impulses, now conscious, are remitted to the neck of the bladder, causing either relaxation or further tightening till a suitable time and place present themselves. The act of urination is then brought about by the relaxation of the sphincter and the combined action of the detrusor urinæ and the abdominal muscles. Any interference with this mechanism may cause enuresis.

Etiology is frequently obscure, and many conditions that are considered causes are often probably only co-existing and not causal.

Any interference with the normal mechanism of micturition, as given above, will cause enuresis; such interference can take place at the lumbar centre, or in the afferent or efferent nerve tracts, or in the brain. Other causes are: 1. In the *urine:* increase in quantity or acidity. 2. In the *bladder:* malformation, increased irritability, muscular spasm of the detrusor urinæ, non-development or imperfect innervation of sphincter vesicæ, calculus. 3. In the *penis:* phimosis, paraphimosis, elongated or adherent prepuce, balanitis. 4. Stricture of urethra and enlarged prostate in advanced age. 5. Fissure or eczema of *anus*, and worms. 6. Lesions of the nervous system—shock, hysteria, locomotor ataxia, apoplexy.

In children this distressing condition is generally due to want of development of the muscles of the bladder, chiefly the sphincter vesicæ, and to lack of proper training. It must be remembered that up to about three years of age enuresis is physiological, and that after that age proper education can do much toward inculcating good habits. Often, too, there may be a diminished sensibility of the nerves of the bladder, when the sensations are not sufficiently strong to awaken the patient, or else the sleep is abnormally deep and prolonged. Children with a neurotic tendency are often afflicted with enuresis. The trouble generally disappears at or about puberty.

Symptoms.—Enuresis may be mistaken for the "overflow" or retention of urine common in enlarged prostate, but a catheter will settle the diagnosis. Otherwise the diagnosis is clear from the statement of the patient or parent.

In children the disease is classified as (1) enuresis nocturna, (2) enuresis diurna, and (3) enuresis continua. Of these the commonest is the nocturnal variety, in which the patient is troubled only at night, but regularly every night, and this in spite of the fact that he has emptied his bladder before being put to bed. The child is apt to be gloomy and downcast, and apparently much troubled about this sad condition which is indeed his misfortune and not his fault. In some cases the trouble continues even when the patient is aroused every few hours.

The diurnal variety is caused by muscular contraction, chiefly in laughing and coughing, and is apt to persist longer than enuresis nocturna; when found after the age of puberty it is more common in the female.

Prognosis.—In children the trouble generally disappears at puberty. In any case it is well to make a thorough examination and if possible remove the cause.

Treatment.—Whenever possible remove the cause or apparent cause. Look after the general condition of the patient. In the case of children punishment and reproaches are reprehensible; the little patient probably suffers considerably from the knowledge of his affliction. Many remedies have been tried, some with a little success. Raising the foot of the bed, so that the urine does not rest on the base of the bladder, has been recommended; so, too, has electricity—the faradic current,—one electrode in the rectum, the other on the perineum.

Drug medication is largely empirical, but cures have been reported from the use of strychnine, fluid extract of ergot, iron, atropine; also nitrate of silver applied locally.

In the *Medical and Surgical Reporter* for March, 1898, is reported the case of a boy who by mistake took, four times a day, a pill of extr. cannabis indica, gr. ¼; hyoscyamine, gr. $\frac{1}{150}$; zinc phosphide, gr. ¼; and was "cured," in three days, of enuresis.

R. J. E. Scott.

ENZYMES.—Enzyme (Gr. ἐν, in, and ζύμη, leaven), a name given to a class of chemically active substances widely distributed through the animal and vegetable kingdoms. They are chiefly of interest in medicine from the part they play in digestion, most of the chemical changes in this process being due to their agency.

In their action, enzymes present many points of striking resemblance to ordinary ferments, such as bacteria, yeast, moulds, etc. For example, both classes work best

at about the same temperature, viz., that of the human body, or a few degrees above it; each is destroyed by boiling; each will cause insoluble substances to be transformed so that they pass into solution. This similarity caused earlier observers to classify the enzymes as a special group of ferments. To distinguish them from the micro-organism ferments, they were called "unorganized ferments," "soluble ferments," "formless ferments," "unformed ferments," etc. All of these names are still in use at present, but the term *enzyme* has been the most favored one since the classical work of Kühne (1878), as it avoids confusion with ordinary fermentation. The advantage of this has been pithily expressed by Sheridan Lea, in "Foster's Physiology," as follows: "It appears advisable to use the term 'enzyme' to denote the soluble unorganized ferments generally, reserving the older name of 'ferment' for the organized agents such as yeast to which it was first applied. If this be done it will be convenient to use the expression 'zymolysis' to denote the changes produced by the enzymes in their action on other substances and to apply the term 'fermentation' to the action of the organized ferments. In this way 'zymolysis' corresponds to the German 'Ferment-Wirkung,' and 'fermentation' to 'Gährung.'"

In 1874, Musculus showed that an enzyme could be obtained from urine which contained the Micrococcus ureæ, and that the enzyme would produce the same decomposition of urea that was brought about by the living micrococcus. In 1885 Sheridan Lea found that this enzyme could be isolated from the micrococci. A number of similar discoveries have been made with other micro-organisms and moulds. Even the ordinary alcoholic fermentation produced by yeast has been brought within this category by the classical researches of Buchner; so that it is by no means improbable that the decomposition processes of fermentation may yet be shown to be those of zymolysis, the living micro-organism producing the enzyme, and the enzyme bringing about the chemical changes (see article *Fermentation*).

Chemical Composition of the Enzymes.—It is extremely difficult to get any of the enzymes even approximately pure, and it is doubtful whether an absolutely pure specimen has ever been obtained. For this reason the chemical nature of the enzymes is still undetermined. It is generally admitted that they contain nitrogen, and they have usually been classified as proteids. The purest specimens obtained a decade or more ago gave a number of reactions common to proteids, but it must be remembered that they are always obtained from close association with proteids in the cells from which they are derived, and the question has arisen whether or not the proteid did not cling to them as a contamination. As methods for their purification have improved, specimens have been obtained which gave fewer and fewer of the proteid reactions, until now the trend of opinion is away from the old theory of their proteid nature—but we have found nothing definite to take its place.

Theories widely different from the above find a place even in the most recent literature, the enzymes being regarded by some as imponderable, immaterial forms of energy "clinging to changing chemical substances, like electricity to a conductor" (Arthus, Thèse, Paris, 1896). The latest writer whose monograph has appeared on the subject (Oppenheimer, 1900) defines enzymes as follows: "An enzyme is the material substratum of a peculiar form of energy, which is produced by living cells and which clings to them more or less tenaciously without their action being bound up with the life process as such. This energy is able to bring about the freeing of the potential energy of chemical substances, and to transform this into kinetic energy (heat, light). The chemical substances are altered in such a way . . . that the products of the decomposition shall collectively represent less potential energy than is represented in the original substance," etc. He explains that he uses the term "material substratum" "because we are absolutely in a fog as to our knowledge concerning their chemical nature."

Mode of Action of Enzymes.—*Temperature.*—Each enzyme has a temperature at which it acts best. This varies with the enzyme: at a definite point of low temperature its action is suspended, but the enzyme is not destroyed; at a higher temperature its action is suspended, and at a still higher point the enzyme is destroyed. In the moist condition this destruction occurs at the boiling point, or below it, while some enzymes may be heated dry to 150° C. and still be active when placed again in solutions at the proper temperature.

Action of Enzymes Hindered by the Accumulation of the Products of Their Own Activity.—Most enzymes cease acting when the products of their activity reach a certain concentration. If pepsin be made to digest fibrin it stops acting when the proteoses and peptones reach a certain concentration, but will begin acting again if the mixture be simply diluted with water or with some other appropriate fluid.

Relation of Quantity of Enzymes to Work which they Do.—The quantity of enzyme will determine the rapidity of action but not the amount of action. One grain of pepsin will dissolve as much proteid as an ounce, but it will require a longer time.

Enzymes Not Used Up.—This is one of the most remarkable properties of enzymes. After causing an enzyme to transform a large amount of substance on which it acts, there is still so nearly as much enzyme present as there was at the beginning that it may be regarded as practically the same.

Nature of Action of Enzymes.—A discussion of the nature of zymolysis would lead us back to the classical discussion, between Liebig and Pasteur, of the action of ferments in general (see article *Fermentation*). The predominant action of the digestive enzymes is one of hydrolytic cleavage; that is, the enzyme will cause the substance acted upon to take up water and then split into two different substances, usually of the same class. This is well illustrated by the enzyme "invertin" of the intestinal juice in its action on cane sugar. The cane-sugar molecule, in the presence of invertin, takes up a molecule of water and then splits into dextrose and levulose as follows:

$$\underset{C_{12}H_{22}O_{11}}{\text{Cane sugar}} + \underset{H_2O}{\text{water}} = \underset{C_6H_{12}O_6}{\text{dextrose}} + \underset{C_6H_{12}O_6}{\text{levulose}}$$

It will be noticed that the enzyme is not shown in the reaction, as studied by its end products, and yet the reaction will not take place in simple aqueous solution without the presence of the enzyme; moreover, the enzyme is not used up, no matter how much cane sugar it converts. The exact mode of its action is not understood. The most plausible theory which has been advanced and the one which meets with most acceptance is that the enzyme acts as a go-between or carrier for water very much in the same way that NO acts for oxygen in the manufacture of sulphuric acid. Here sulphur is burnt in the air forming SO_2, which readily combines with water (steam) to form sulphurous acid, H_2SO_3. Sulphurous acid will unite with oxygen to form sulphuric acid:

$$\underset{H_2SO_3}{\text{Sulphurous acid}} + \underset{O}{\text{oxygen}} = \underset{H_2SO_4}{\text{sulphuric acid}}$$

but the process is too slow to be practicable. If, however, nitric oxide and air be introduced the action becomes rapid and continuous in the following way: The nitric oxide, NO, takes up oxygen from the air and becomes nitrogen peroxide, NO_2. This NO_2 readily gives up part of its O to the H_2SO_3, and is transformed back to NO.

$$\underset{H_2SO_3}{\text{Sulphurous acid}} + NO_2 = \underset{H_2SO_4}{\text{sulphuric acid}} + NO$$

As soon as it becomes NO it takes a fresh supply of O from the air, only to be robbed of it promptly again by a fresh supply of H_2SO_3, so that the process goes on continuously, the H_2SO_3 becoming transformed into H_2SO_4 the NO acting as an oxygen carrier and not being used up. In practice the process is even more striking: SO_2 from burning sulphur, H_2O (steam) and NO_2 are sent into a common receptacle where they mix with air and with each other. The reactions are then as follows:

(1) $SO_2 + H_2O + NO_2 = H_2SO_4 + NO$
(2) $NO + O = NO_2$

and so on continuously.

Sulphur and water are transformed, together with oxygen from the air, and the end product is sulphuric acid. A study of the end product does not show the intermediate product H_2SO_3, nor the action of the NO and NO_2 as a carrier of the oxygen. In the case of the enzymes we have been able to study satisfactorily only the substance transformed and the end products. The intermediate reactions have not all been determined, so we can reason, as to the rôle of enzymes, only by analogy with better known cases, such as the example chosen of sulphuric acid.

Determination of the Activity of Enzymes.—The various preparations—most of them proprietary—depending for their action on the enzymes which they contain, are mostly mixtures of enzymes with a certain amount of foreign matter. This is notably true of preparations from the stomach (pepsin) and from the pancreas. Much has been claimed for and against the relative merits of certain of these preparations. The method of investigation depends upon the enzyme which is being investigated, and for the details of standard methods the reader is referred to works on physiological chemistry. All methods, however, involve one fundamental procedure, viz.: to determine the amount of substance transformed by a given amount of enzyme in a given time; or to determine the amount of enzyme necessary to transform a given amount of substance in a given time; or to determine the time required for a given amount of enzyme to transform a given amount of substance; in other words, there are three factors in the process: time, strength of enzyme, and amount of substance transformed; if two of these are known the third can be determined. It must never be forgotten that all comparisons of enzyme-activity must be made under rigidly the same conditions as to the physical state of the substance acted on, the fluid medium in which it is placed, temperature, cleanliness of vessels, etc. Slight differences in these conditions, especially the physical condition of the substance acted on, will give results which are absolutely incomparable. This point should never be overlooked in comparing the results of one series of experiments with those of another.

Origin of Enzymes.—In the vegetable kingdom, enzymes may be extracted from the lower organisms such as bacteria, yeast, moulds, etc., while higher in the scale they play an important part in the plant economy, causing the solution of stored-up foods such as starch and even cellulose. This is especially well marked in germinating seeds. In some of the highest forms, such as certain pitcher plants (Nepenthes) there are well-developed cells resembling gland cells which secrete enzymes capable of digesting insects which the plant catches.

In the animal kingdom observations have been made which suggest that even in the lowest forms, such as the amœba, digestion is carried on by enzymes made by the cell and collected in vacuoles. Fredericq has isolated a digestive enzyme from sponges; and certain worms and cephalopods (snails) have been sufficiently well-studied to leave little doubt that digestion is carried on by enzymes secreted by glands. In the higher vertebrates, practically all the digestive enzymes are secreted by glands, and the preparation of these enzymes appears to be the chief work for which the glands are set apart. The enzymes do not exist as such in the glands, as is shown by the following experiments: If the stomach or pancreas of an animal be cut out, immediately after death and thrown into alcohol, it will yield very little enzyme. If, however, it be allowed to stand for several hours (twelve to twenty-four), the yield of enzyme will be considerable; hence we suppose that the living gland cells contain, not enzyme, but a substance which can readily be transformed into enzyme. To this substance the general name *zymogen* is given. In the case of specific enzymes, we use the specific name of the zymogen: thus, pepsinogen is the zymogen from which pepsin is formed, trypsinogen is the zymogen from which trypsin is formed, etc.

Microscopic investigations on the gland cells give still further support to the zymogen theory. If the cells of a gland be examined when the gland has been at rest for some time—that is, when it has not poured out an abundant secretion for several hours—the cells will be found to be packed full of granules, so as to obscure the nuclei and the boundary between the cells. After the gland has been actively pouring out its secretion, these granules disappear to a considerable extent, but the cell fills up with granules again on resting. These changes in the activity of the gland and in its histological appearance may be brought about by stimulating certain nerves to the gland, and those nerves which cause the greatest disappearance of granules make the gland produce a secretion richest in enzyme. This has been well demonstrated for the salivary glands for a number of years, and recent observations have confirmed the same for the stomach and the pancreas.

Fate of the Enzymes in the Human Body.—The urine contains a small amount of pepsin, amylolytic ferment, and rennin. (The occurrence of trypsin in normal urine is doubtful.) A certain amount of these ferments may therefore be regarded as gotten rid of normally in this way, but it is at best of secondary importance, as shown by Béchamps and Balthus for amylolytic ferments and by Schnappauf for pepsin. These observers injected the enzymes in question into the blood and found that there was no such increase of them in the urine as should have been produced if the kidneys had been active in excreting them. Most of the digestive enzymes are rendered inert or actually destroyed in the alimentary canal, and they or their residues pass out with the fæces. Enzymes are not dialyzable, so we should not expect them to get into the blood in any considerable quantity, and when injected into the blood they are destroyed in the system (probably to some extent in the blood itself). This process is probably a slow one as shown by the interesting researches of Hildebrandt. The enzyme emulsin breaks up the glucoside amygdalin from bitter almonds, with prussic acid as one of the decomposition products. Hildebrandt injected emulsin subcutaneously into rabbits, and found that it gradually disappeared from the blood without being excreted by the kidneys. At different intervals after injecting the emulsin he administered amygdalin, and the presence of the emulsin was indicated by symptoms of prussic-acid poisoning. He got this effect as long as six hours after injecting the emulsin, showing that the enzyme had not all been destroyed in this interval. After the blood had ceased to give the reaction for the enzyme, it was still found in the spleen and pancreas, and especially in the liver and in the lymph glands in the region where the hypodermic injection was made—showing that certain organs outside the blood could bind and hold the enzyme (and ultimately destroy it).

Classification of Enzymes.—Enzymes are classified in two different ways:

A. From a chemical standpoint, according to the kind of action they induce.

B. According to the class of substances on which they act.

Classification A is the more logical, but classification B is more convenient, and is by far the more generally used.

From a chemical standpoint, as above, Hoppe-Seyler gives the following classification (quoted, modified, from Halliburton):

(*a*) *Ferments which Act Like Dilute Mineral Acids at 100° C.:*

I. Change of starch or glycogen into dextrin and grape sugar:

$$\underset{(C_6H_{10}O_5)_4}{\text{starch}} + 3H_2O = \underset{C_6H_{10}O_5}{\text{dextrin}} + 3\underset{C_6H_{12}O_6}{\text{glucose}}$$

II. Change of cane sugar into dextrose and levulose (inversion):

cane sugar — dextrose — levulose

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$

III. Changes of various benzoyl glucosides into sugar, and simpler benzol derivatives by the action of emulsin. Example:

salicin — sugar — saligenin

$$C_{13}H_{18}O_7 + H_2O = C_6H_{12}O_6 + C_6H_4\begin{cases}CH_2OH\\OH\end{cases}$$

IV. Decomposition of sulphur-containing glucosides into glucose, sulphuric acid (acid sulphates) and oil of mustard by the action of myrosin. Example:

potassium myronate — glucose — mustard oil

$$C_{10}H_{18}NS_2O_{14}K = C_6H_{12}O_6 + HKSO_4 + C_4H_5NS$$

(*b*) *Ferments which Act Like Caustic Alkalies at a Higher Temperature. Fermentative Saponification:*

I. Decomposition of esters (fats) into an alcohol (glycerin) and an acid (fatty acid):

tristearin — stearic acid — glycerin

$$C_3H_5(C_{18}H_{35}O_2)_3 + 3H_2O = 3(C_{18}H_{36}O_2) + C_3H_5(OH)_3$$

II. Decomposition of amido compounds with absorption of water. Examples:

urea — ammonium carbonate

$$CO(NH_2)_2 + H_2O = (NH_4)_2CO_3$$

hippuric acid — glycine — benzoic acid

$$C_9H_9NO_3 + H_2O = C_2H_5NO_2 + C_7H_6O_2$$

The decomposition of proteids and albuminoids with the formation of leucin and tyrosin, as effected by the ferment trypsin from the pancreas, belongs in the above category. The proteids are in all probability amido bodies, but the formulæ are too complicated and the reactions too little understood to illustrate them as was done with the simpler compounds above.

The chemical classification given above, while classical, is difficult of general application, for in many instances our knowledge of the changes produced by enzymes is not sufficiently accurate to justify a direct comparison with well-determined chemical reactions. Again, in the biological field (whether animal or vegetable), where we meet enzymes, we have our attention concentrated on the substance affected (usually food) rather than on the chemical action of the enzyme. This has led to a somewhat mixed classification, based partly upon the character of the substance acted on by the enzyme, and partly upon the grosser and more apparent changes produced rather than upon delicate resemblances of chemical action.

In practice, therefore, biologists, almost universally, adopt some such classification as the following:

(1) Proteolytic enzymes.
(2) Amylolytic enzymes.
(3) Fat-splitting enzymes.
(4) Sugar-splitting enzymes.
(5) Coagulating enzymes.
(6) Oxidizing enzymes.
(7) Glucoside-splitting enzymes.

(1) Proteolytic enzymes. Those which act on insoluble proteids transforming them into soluble ones. In this group belong:

I. Pepsin in gastric juice.
II. Trypsin in pancreatic juice.

And these are often used generically for a whole group of enzymes resembling them. Thus one often hears of vegetable pepsins and vegetable trypsins according as the ferment in question resembles pepsin or trypsin in its final action. Pepsin (and the pepsins) transform insoluble proteids into peptone as the final stage of its action; peptone is a proteid. So pepsin never goes beyond the proteid class. Trypsin goes a step further and breaks the peptone down into a group of amido decomposition products of which leucin, tyrosin, and asparagin are the best-known examples.

III. Proteolytic enzymes in the vegetable kingdom. These are very widespread and various. They may be extracted from the lowest forms (bacteria) and in some cases produce the liquefaction of gelatin cultures. They are found in many stems, roots, leaves, and fruits of higher plants, and in certain pitcher plants they are secreted by a gland-like structure, and have the function of dissolving insects and other proteid food which the plant catches.

Two of these vegetable proteolytic enzymes—papain from the pawpaw and bromelin from the pineapple—have been very thoroughly investigated by Chittenden and his pupils, and may be said to be among the best understood of the enzymes.

(2) Amylolytic [diastatic] enzymes. Those which act on starch and carbohydrates of the starch group transforming them into soluble carbohydrates.

In this group belong:

I. Ptyalin in saliva.
II. Amylopsin in pancreatic juice.
III. Diastase in malt. This is one of the oldest and best-known members of the group and from it the name *diastatic* is derived as a generic term for the group.
IV. Enzymes of the diastase class have been discovered widely spread in the vegetable kingdom from bacteria to the highest plants. Among the organisms, of interest in medicine, which transform starch into sugars probably through the action of such an enzyme may be mentioned, the spirillum of cholera, Bacillus mesentericus vulgatus, and the moulds Penicillium glaucum, and Aspergillus niger. Some of the vegetable diastases are on the market in medicines to aid digestion.
V. Amylolytic enzyme in the liver. It is well known that the liver stores up carbohydrate food in the form of glycogen, and that it gives this out to the blood, as needed, in the form of glucose. It is also well known that after death glycogen rapidly disappears from the liver, glucose being found in its place. Some authorities attribute this transformation, both during life and after death, to an amylolytic enzyme in the liver. This point is still in dispute.
VI. Digestive juices, indicating by their action the presence of amylolytic enzymes, have been found in the animal kingdom as low as rotifers. The enzyme has been isolated in secretions from insects. In man, and in the mammalia generally, such ferments occur in appreciable quantities in the intestinal juice (succus entericus), the liver, and the muscles. In all these situations they have a rôle more or less important. Blood and lymph also contain them, as do nearly all the fluids and tissues of the body. Just how much of this is made in the tissues and how much is carried to them by the blood is an open question.

It is still a disputed point whether the animal and the vegetable amylolytic enzymes are the same. The weight of authority seems to be in favor of the view that they are different.

(3) Fat-splitting [steatolytic] enzymes. Those which break up fats into glycerin and fatty acid. The principal member of this class is—

I. Steapsin [pialyn, lipase]. This enzyme is found in pancreatic juice.
II. Enzymes similar to steapsin in their effects are found in the vegetable kingdom, especially in certain seeds which contain stored-up fatty substances. The enzymes bring these into solution at the proper time.
III. Fat-splitting enzymes are present in invertebrates as low as sponges (Fredericq). They are also found in the eggs of certain Crustaceans.
IV. Fat-splitting enzymes in the blood: Henriot has described an enzyme of this class in the serum. Cohnstein and Michaelis think such an enzyme exists in the red blood corpuscles.
V. Some observers claim to have found a fat-splitting enzyme in the stomach. This has not yet been generally accepted.

(4) Sugar-splitting enzymes. Those that split disaccharides, like cane-sugar, into monosaccharides, like dextrose and levulose.

I. Invertin [invertase], found in the intestinal juice, splits cane sugar into dextrose + levulose. Found also in plants, certain bacteria, and moulds.
II. Another enzyme [maltase], also found in the intestine, splits maltose into dextrose. Found also in plants, bacteria, yeasts, and moulds.
III. Lactase. Found in the intestine (?). Found also in the kephir organism. It splits milk sugar (lactose) into dextrose + galactose.
IV. Raffinase [melibiase], melizitase, trehalase. These are all new enzymes discovered and described within the last few years. They are found in yeasts and moulds, and have characteristic actions on rare sugars. They throw light on the composition of these sugars by breaking them up into well-known forms, and it is possible they may become of commercial value in connection with special forms of fermentation.

(5) Coagulating enzymes. Those whose action is marked by the production of a coagulum, curd or jelly. There are four well-known members of this group:

I. Rennin [rennet, chymosin], found in the mucous membrane of animals, especially young sucking animals; and in the pancreatic juice of many animals, including man. It is also found in the vegetable kingdom. It produces a sweet curd in milk which is different physically and chemically from the curd which is formed when milk sours. It is used to curdle milk in cheese manufacture. The rennin for this purpose is usually obtained from calves' stomachs, but it is interesting to note that in some rural districts plants (galium verum) are put into the milk to produce the curd, and in this case the action is due to vegetable rennin.
II. Fibrin ferment [thrombin, thrombase]. Found in shed blood during and after coagulation—absent from blood circulating in living blood-vessels. It causes the fibrinogen of blood to unite with calcium salts and to form a jelly-like clot which later becomes fibrous (fibrin). The origin and nature of fibrin ferment are still much disputed. Some deny that it should be classed with enzymes at all. Schmidt, who discovered it and described it (1872), classed it with the enzymes and said it was derived from the white corpuscles of the blood. Hayem (1878) and Bizzozero (1881), in classical researches on blood plates, claim that the blood plates break down when blood is shed and that the white corpuscles do not. The majority of observers who have studied coagulation under the microscope, with the greatest precaution, agree with Hayem and Bizzozero. On the other hand, those who have attacked the problem from a chemical standpoint particularly, tend to class fibrin ferment with the nucleoproteids and to derive it from leucocytes, because it may be so manufactured from tissue rich in these corpuscles. This fits several cleverly conceived theories of coagulation which are far from proved, and is, on the whole, attractive, but it should not outweigh the opinion of the majority of direct observers, viz., that in normal coagulation the leucocytes do not break down. It is commonly taught in text books that fibrin ferment is derived from blood plates and leucocytes, both of these elements breaking down when the blood is drawn, but this is at best an unsatisfactory compromise. The whole question must be settled by further research. (For fuller details on fibrin ferment see *Coagulation*.)
III. Myosin ferment, may be isolated from muscles which have gone into *rigor mortis*. It causes the plasma of muscle to coagulate as it does in *rigor mortis*, with the production of death-stiffening. This enzyme is found only in the animal kingdom.
IV. Pectase. Found only in the vegetable kingdom. It has to do with the formation of many of the vegetable jellies which may be made from fruits. It probably plays an important part in the plant economy, acting on pectose, a substance found associated with cellulose in the cell wall.

(6) Oxidizing enzymes [oxidases]. This class of enzymes was one of the latest to be discovered and investigated. A number of phenomena hitherto unexplained are now being attributed to oxidizing enzymes. The rôle of certain oxidizing enzymes in the vegetable kingdom has been fairly well studied. In the manufacture of wine, for instance, certain processes which were thought to be due exclusively to bacterial fermentation are now known to be produced by enzymes which can be extracted from the bacteria, and which can work independently of them. It has even been claimed that the delicate bouquets of certain wines may be produced in this way, without the presence of the micro-organism.

In the animal kingdom the presence and function of oxidases are not well determined. It has long been known that a certain amount of oxidation takes place in blood shortly after it is drawn. Certain reducing substances, such as dextrose, disappear; and less oxygen can be recovered *in vacuo* after the blood has stood free from exposure to the air. Within the last decade this has been shown to be due, in great probability, to an enzyme which oxidizes.

Removal of the pancreas causes a marked diabetes, and much can be said for Lépine's theory—that the pancreas manufactures an oxidizing enzyme which is taken up by the blood, and that when this is present it keeps the percentage of sugar in the blood so low that it does not appear in the urine. Abelous and Biarnés found that sugar was also destroyed by the testis, thyroid, liver, kidney, lungs, and spleen. Spitzer (1897) thinks these tissues owe their oxidizing power to nucleo-proteids.

Hammersten has described an enzyme (oxidase) in the stomach mucous membrane which oxidizes milk sugar (lactose) to lactic acid.

(7) Glucoside-splitting enzymes. Those which break up glucosides into glucose and various other substances depending on the glucoside. This group of enzymes is one of the most interesting, from a chemical standpoint, of all the enzymes. Some of its members were the first to be discovered and investigated. Myrosin, as the active principle of mustard seed, was pointed out by Lefèbre in 1660, and again by Boerhave in 1775, while the classic researches of Robiquet and Boutron-Chalard (1830) and of Liebig and Wöhler (1837) on myrosin and on emulsin were epoch-making in our knowledge of the action of enzymes in general. In later years they have played a no less important part in the work of E. Fischer on the synthesis and structure of sugars, which has led to some of the most brilliant and valuable discoveries of modern times in the field of organic chemistry. These enzymes do not occur in the human body, so they will not be described in detail. The members of the group are:

I. Emulsin—long known to exist in bitter almonds, found also widely in the vegetable kingdom from flowering plants to mosses, where it plays an important part in plant economy. Its presence in bacteria is disputed. It breaks up amygdalin, in bitter almonds, into glucose, prussic acid, and oil of bitter almonds. It also acts on a number of other glucosides.
II. Myrosin, found in mustard seed. It breaks up potassium myronate in the seed and liberates mustard oil.
III. Gaultherase, found in wintergreen. It liberates oil of wintergreen from the glucoside gaultherin found in the plant.
IV. Other enzymes of this group are: erythrozyme in madder root, rhamnase in Persian berry, and a new enzyme lately discovered in connection with the manufacture of indigo. It was formerly thought that bacteria were necessary for the decomposition of the glucoside indican in the manufacture of indigo, but the researches of Lookeren-Campagne (1894) and of Bréaudat (1898) make it appear probable that the action is one of zymolysis. Bréaudat gives the reaction in two stages: (*a*) the formation of indigo white by a hydrolytic enzyme, and (*b*) the oxidation of indigo white to indigo blue by the action of an oxidizing enzyme. He obtained these reactions in chloroform water which stops the activity of bacteria, but does not interfere with the action of enzymes (see article *Fermentation*).