finally reached on June 22, 1897, Vannutelli and Citerni, the two surviving members of the Second Bottegò Expedition were most cordially received by the Italian Envoys—Major Nerazzini and Captain Ciccodicola, and arrangements were quickly made for their return to Europe.

Among the perils and dangers of such a journey as this especially when the great difficulties of transport are taken into consideration, the collection of scientific specimens is by no means an easy task. Yet, as will be seen by reference to the Appendix to the present volume, the members of the Second Bottegò Expedition by no means neglected this part of their duties. After the geological, meteorological, and astronomical observations are given we find a summary of the zoological results prepared by Dr. Gestro of the Museò Civico of Genoa. These are based on specimens obtained during the first part of the journey between Brava and Lake Rudolf which, however, formed but a very small proportion of the whole collections. The Mammals have been described by Mr. Oldfield Thomas of the British Museum in two papers published in the Annals of the Museò Civico of Genoa, the first relating to 27 species and the second to 20, one of which (*Crocidura bottegi*) was new to science. The few birds saved from the wreck have been named by Count Salvadori, the Reptiles and Batrachians by Mr. Boulenger and the Fishes by Sigo Vinciguerra. Their reports have likewise appeared in the same well-known periodical. The more numerous specimens of Invertebrates have been worked out by various specialists of whose contributions the titles are given here, together with an abbreviated account of the principal novelties accompanied by many excellent illustrations. The value of this well prepared volume is further enhanced by the excellent series of maps attached to it, whereby every detail of the routes pursued may be followed with the greatest ease. The name of Giacano Doria attached to the preface is a guarantee that neither trouble nor expense has been grudged in the production of the present volume as is indeed at once evident to all that examine it.

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LONDON ZOOLOGICAL SOCIETY.

ON THE CHEMICAL NATURE OF ENZYMES.

THE enzymes form one of the most interesting groups of organic compounds from the physiological as well as the purely chemical point of view. Physiologically they may be classified as follows:

1. Enzymes which are intimately connected with nutrition, as diastase, pepsin, trypsin, lipase, etc.

2. Enzymes which cause oxidations—the oxidases.

3. Enzymes which bring on coagulations, the clotting enzymes: rennet, thrombase, pectase.

The first group has been known longest and best and has served certain authors for inferences and distinctions which at present are no longer tenable. Erroneous views as to the rôle of enzymes are however now and then entertained even at the present day, actions being ascribed to them which belong exclusively to the living protoplasm itself. Thus, in an article on 'Assimilation and Heredity' the hypothesis was formulated that "enzymes are the true bearers of heredity." Thus far it has been the well founded inference that the molecular arrangement, the invisible organization or tectonic of the chromosomes forms the foundation of the genetic differentiation and heredity. These chromosomes consist principally of a nucleoproteid (chromatin) of a very labile nature, that is easily converted into a stable proteid by injurious influences which cause their death. The chromatin of the chromosomes of different animals may not be identical, but only iso-

meric, or otherwise closely related (something chemically very difficult to prove), but there can be no doubt that the *tectonic* must be a different and a specific one in the chromosomes of every different kind of animal. This different construction or machinery causes those special differentiations in the further development of a fecundated egg which characterize a species,* while it is the chemically labile nature which confers the power of transforming and applying energy.

Moreover, the same author ascribes to enzymes the power to form living matter from the dead matter of the food. This, too, is not correct. The proteolytic enzymes merely provide the living animal cell with soluble protein (albumoses), but this inactive protein is converted into living matter by the living protoplasm itself (probably by the nucleus), but surely not by enzymes.

Besides the known enzymes that act on glucosides, carbohydrates, fats and true proteins, there exist certainly still others of however a rather narrow sphere of activity. Certain mites and a few fungi attack hair and horn and utilize therefore keratin as food, hence, they must be able to prepare an enzyme (keratinase), especially adapted to dissolve keratin. Certain fungi easily perforate the chitin structures of insects and a special enzyme (chitinase) has to be assumed also in this case. Still another group are the but recently recognized *bacteriolytic* enzymes, produced by certain kinds of bacteria themselves. These enzymes play an important rôle in the recovery from and immunization against infectious diseases.† Their powers of dissolving bacteria, however, are restricted to certain kinds and may in many cases act on one kind only. It is from the ecological standpoint certainly a remarkable fact, that an organism, as, *e. g.*, *Bacillus pyocganeus* produces an enzyme which, after reaching a certain concentration, dissolves the bacillus itself! The bacillus, so to speak, commits suicide by means of its own enzyme—certainly not a teleological working of nature for the maintenance of species!

As to the *chemical nature* of enzymes three questions above all have occupied the mind of investigators, viz.: 1. Are the enzymes proteins or not? 2. How is the fact to be explained that a very small amount of an enzyme can transform a relatively very large amount of another compound? 3. What is the cause of their quite specific action, the reason that they can only attack a specific compound and not others, even closely related ones?

The importance of the first question has been much overrated and while one author asserted they belong to the coagulable albumins, another ascribed to them the nature of nucleoproteids and still others claimed that enzymes are very different from any protein matter. It is true, special difficulties are encountered in the purification and isolation of enzymes, but it is also not less true, in many cases at least, that it is quite impossible to separate the enzymic activity from protein matter. The tendency of certain authors to infer from the nature of *one* enzyme the nature of all the others also, is not justified at all. There may exist enzymes in every group of proteins, and some may exist that are not proteins, although derived therefrom.

Wurtz* has recognized papayotin, the proteolytic enzyme of Carica papaya as an albumose and Chittenden† thinks the di-

* The various hypotheses treating this problem have been discussed by Ives Delage: *La structure du protoplasma et les théories sur l'herédité, etc.* Paris, 1895.

† Cf. Emmerich and Loew, Bacterolytische Enzyme als Ursache der erworbenen Immunität und die Heilung von Infections krankheiten durch dieselben; Zeitschrift für Hygiene, Vol. 31, May, 1899.

* *Comptes Rendus*, 90, 1379.

† *Transactions of the Conn. Acad. of Sciences*, Vol. 8 (1891).

gesting agent of the pineapple to be of the same nature. Pelkelharing* found that the activity of pepsin is intimately connected with a nucleoproteid and the same author as well as Halliburton declare thrombase (the clotting enzyme of the blood) to be a nucleoproteid. † Spitzer declared also the peroxidase of the animals to be a nucleoproteid, ‡ which however, the writer found not to be the case with the vegetal peroxidase, which in all probability has an albumose-like nature. Seegen and Kratschmer § inferred from their investigations an albuminous nature of the diastase of the liver, while the writer found the trypsin and diastase of the pancreas gland to be of peptone character; that is to say, when transformed to the *inactive state*, they behave towards the usual reagents like a peptone, while in regard to their *activity* they differ essentially from them (see on this point further below). As to the diastase of malt, Osborne as well as the writer, || has inferred its protein character. In the purest state it was prepared by Wróblewski, who showed that it was a proteose and was formerly obtained with an admixture of a carbohydrate, araban. This author recently also proved invertase to be of a proteose or peptone-like nature. Certain authors failed to obtain with their enzyme preparations either the reactions or the composition of protein matters, which may have been due in some cases to imperfect purification, while in others the enzyme might really be no protein at all, which is probably the case with the rennet, investigated by Hammarsten. The *active* character of an enzyme is not necessarily connected with a protein nature, since the ordinary soluble proteins have no such activity at all. In analyzing enzymes we can only find the composition of the *killed** enzyme, which in fact is no longer a real enzyme. This brings us to the second of the above questions, the cause of their chemical powers. The question how it is to be explained that a small amount of enzyme can transform a relatively very large amount of another substance has been answered in various ways, none of which have proved satisfactory. We shall not enter here on a critical review of all these hypotheses, which the reader will find treated in Green's recent work: 'The Soluble Ferments and Fermentation,' (chapter 24). † Only a few points, especially regarding recent views may be mentioned, before the view of the writer is discussed.

One author declared that enzymes are not bodies, but properties of bodies (which nonsense was called by several authors an 'ingenious,' hypothesis!); another said that small quantities of enzymes are merely attached to proteins, but are not proteins themselves; another declared that the enzymes act by repeatedly causing oxidation and reduction.‡ But even if this last mentioned view were correct (which cannot be, since most enzymes can be active also in the absence of oxygen), it does not explain the power that would cause the supposed oxidations and reductions. Saccharoff, who advanced this hypothesis, made experiments with papayotin only, in which he assumes a small quantity of 'bionuclein,' an active principle, containing iron, and associated with it a larger amount of another substance that has a mere promoting action. From some very vague

* *Zeitschrift physiol. Chem.*, Vol. 22, p. 233.

† *Arch. für Physiol.*, 1895, p. 213, and *Journal of Physiol.*, Vol. 9, p. 265.

‡ *Jahresbericht f. Thierchemie*, 1897.

§ *Jahresbericht f. Thierchemie*, 1877.

|| *Pflüg. Arch.*, Vol. 27, p. 206.

* The word 'killed' is used here as a short term for 'transformed to an inactive state.'

† Recently a review of this work was published in this JOURNAL.

‡ *Saccharoff, Centralbl. f. Bakteriologie*, Vols. 24. and 26.

trials this author draws far-reaching conclusions and even ascribes all actions of living protoplasm to the presence of an exceedingly small quanity of 'bionuclein,' present in albuminous matters of the cells.

The writer in 1882 proposed the view that enzymes are like the protein bodies of the *living* protoplasm distinguished by the presence of *chemically labile atomic groups** and said at that time: "it seems as if some remnant of the active powers of the protoplasm must be contained in the enzymes." Later on, somebody else called enzymes 'protoplasm splinters' and since then this phrase has been echoed by many who did not conceive or concede that the principle common to both consisted in chemical lability.

The principle of chemical lability (instability) has thus far been but little studied. The writer has recently suggested the desirability of distinguishing between kinetically-labile and potentially-labile compounds.† A *kinetically labile compound* is characterized on the one hand, by the easy change to a more stable, isomeric or polymeric modification or compound, and on the other, by the great facility with which it enters into reactions with various other compounds, especially with such as also possess labile properties, whereby result products with a less degree of instability. *Potentially labile* compounds behave differently, they do not pass into isomeric or polymeric modifications, do not easily yield various derivatives, but are inclined to sudden far-reaching decomposition or explosion. Examples of the former class are aldehydes, amido-aldehydes, amido-ketones; of the latter class, the diazo-compounds and the nitrates of polyvalent alcohols as nitroglycerol. Kinetic lability comprises free chemical energy while potential lability intra-molecular chemical energy of position to be well distinguished from the potential energy relatively to oxygen, a potential energy present in all organic compounds and liberated in the act of combining with oxygen.

Chemical energy consists in certain motions of atoms, motions of larger amplitudes than the motions of heat energy, although easily passing into the latter. We must infer the larger amplitudes of chemical energy from the fact that at the ordinary temperature the chemical energy can counteract the force of affinity in a much larger measure than heat energy can do it.

Free chemical energy in a labile compound is caused by a loose position of atoms in certain atomic groups, and this loose position is the consequence of a depression of affinities on account of one atom being under the simultaneous influence of two neighboring atoms. Such atoms in loose position are subjected to much more violent oscillations under the influence of heat energy than are the other atoms in stable position in the same compound. Thus, heat energy is easily transformed into chemical energy by labile atomic groups. As the writer first pointed out, such machines to transform heat into chemical action are, *the proteins of the living protoplasm* and *also the enzymes*, the latter, however, in a much less degree than the former.

The organized proteins of the living matter produce their own heat by respiration, whilst the enzymes utilize either the free store of heat energy in the atmosphere when they act at the ordinary temperature, or also the heat of other sources when they act at an elevated temperature.

Let us now review the general chemical

**Pflüger's, Archiv*, Vol. 27, p. 211. Also, *Journal für praktische Chemie*, Vol. 37, p. 103.

† A detailed account of this view, explained by numerous examples is contained in Chapter 11, of the treatise of the writer: "Der chemische Energie der lebenden Zellen," recently published in Munich by Dr. E. Wolff.

properties of enzymes. Although an increase of heat up to a certain point (the optimum temperature) promotes their actions, a further rise in temperature is injurious and a still further rise stops all their actions. This is in perfect accordance with the transition of a labile to a stable modification, or even to a still more different compound, produced by atomic migrations. The labile atoms approach by their larger oscillations too closely to other atoms, the affinity of which can exert now sufficient power to produce an 'atomic migration.'* The enzymes are 'killed' at this fatal degree of intensity of heat, in other words they have lost their labile, unstable atomic groups, by 'migration' of atoms into a stable position; lability and activity cease to exist. In further analogy to many cases of transformations of labile into stable compounds, enzymes are also 'killed' by a certain amount of alkalies or acids. Different enzymes are resistant in very different degrees, however, not only to these agencies but also to other injurious compounds. This indicates either differences in the *nature* of the labile atomic groups or, what appears more probable to the writer, different positions of the labile groups within the molecule. The closer to each other they are situated, the more easily the transformation to an inactive isomeric compound will take place. The greater the intensity of chemical energy at a given temperature the more activity is possible, and the more easily the point of destruction is reached.

It seems highly probable that there exist two or even more labile groups in one molecule of an enzyme, since Jacobson observed that by a cautious application of heat their power of decomposing hydrogen peroxide may be taken away, while their specific enzymatic action may be retained.*

A few instances will illustrate the differences of resistance of enzymes: trehalmse is killed at 64° C., while inulase at about 70°, emulsian at 75–80°, diastase at 80–86°. The temperatures, however, vary considerably with the acid or alkaline reaction of the liquid, with the degree of concentration and with the presence of neutral salts, or of some organic neutral compounds. Furthermore, while pepsin resists at the ordinary temperature 2 per mille hydrochloric acid, trypsin, emulsin, diastase and papayotin are killed by less than 0.5 per mille.† On the other hand, pepsin is more easily destroyed by sodium carbonate than trypsin and rennet. Invertin is very easily destroyed by dilute alkali (Wróblewski). Hydrogen sulphide easily kills the proteolytic enzyme of *Micrococcus prodigiosus* and *Proteus vulgaris*, not, however, that of *Bacillus Milleri*, nor pepsin, diastase or emulsin.‡

The writer has observed that prussic acid of 25 per cent. kills diastase (but not trypsin) at the ordinary temperature within 12 hours. Arsenious acid is reported to have no injurious effect upon enzymes, but in the writer's opinion this question deserves further study. Certain alkaloids have also been observed to have a destructive action on enzymes. Quinine, 1 per cent. has an inhibitory effect on the action of

* Organic chemistry abounds with interesting cases of this kind. Even the first synthesis of an organic compound, that of urea from ammonium cyanate, is due to such an interesting transformation.

**Zeitschrift f. physiol. Chem.*, Vol. 16, p. 340 (1892). Bourquelot assumes here the presence of an impurity with certain active properties which agrees with some recent tests of the writer.

† Organic acids act less energetically. Thus Wróblewski reports that invertin can resist even 4 per cent. acetic acid for some time.

‡ Cf. Fermi, *Archiv. f. Hygiene*, Vol. 14, p. 15. *Chem. Centralbl.*, 1894, I., p. 965. The writer has convinced himself that neither basic acetate of lead nor hydrogen sulphide, when applied for a short time in moderate quantities, injure diastase or trypsin, and therefore Wurtz's method may well be applied for the preparation of these enzymes, especially from the pancreas gland.

pepsin, while it does not injure diastase or oxidase. Atropine in moderate quantities makes diastase inactive.* Further, prolonged contact with alcohol injures the enzymes more or less.

The writer long ago tried to solve the question what kind of labile atomic groups cause the activity of enzymes, and had certain reasons for the supposition that the lability is due to the simultaneous presence of aldehyde and amido-groups in the molecule of an enzyme. Indeed amido-aldehydes (and amido-ketones) exhibit a high degree of lability. The usual tests for aldehyde groups failed however, but it may nevertheless be possible that these are present in the less active polymeric form.† It deserves to be mentioned in this connection that free hydroxylamnie which very easily enters into reaction with aldehyde groups, also renders diastase inactive in a diluted neutral solution. In regard to labile *amido-groups* it is to be expected that enzymes containing them would become inactive as soon as certain compounds combine with the amido-groups and change them. Such a substance is formaldehyde. Indeed, pepsin and diastase are rendered inactive when they remain for 24 hours in contact with a neutral solution of 5 per cent. formaldehyde. Other enzymes, as emulsin, papayotin, trypsin, etc., yield in its presence inactive precipitates.‡ These observations were later on, made also in the Institut *Pasteur* without, however, any attempt to draw a further inference. In the opinion of the writer however, this behavior makes the presence of labile amido-groups highly probable.

If we now take into consideration the fact that the study of the cleavage products, obtained by boiling with acids or alkalies, or the elementary analyses, can only clear up the composition of the *killed enzymes*, while it leaves us completely in the dark as to the nature of the labile active groups in the original enzymes, we must feel surprised at the attempts to find by simple analysis the true nature of the chemical power of enzymes.

The denial of the protein nature of enzymes on the ground that they are more easily changed by injurious influences than are the proteins is also a source of surprise. Several passages may here be quoted to show the opinions of recent physiologists. Thus we find in an article by a German physiologist the following passage: "There is no reason to doubt that as soon as an analysis of the enzymes has been accomplished, their synthesis will be accomplished too." And in a recent work of an English physiologist we read: "Some serious objections to the view that enzymes are proteids can be based upon the action of light upon them. Diastase is injured by direct sunlight, proteids are not." *Both these views are unqualifiedly erroneous.* Enzymes of protein nature are not ordinary passive proteins, but proteins with labile atomic groups. Only the changed (*killed*) enzymes can be classified with the *ordinary* proteins.*

As soon as we understand the close connection between lability and activity, and that enzymes are capable of transforming heat energy into chemical energy, we can also by means of Helm's principle of the intensity of energy understand that their chemical energy may be transferred to other compounds. And when these other compounds are of such a character that

* Detmer, *Landwirthshaftliche, Jahrbücher*, 1881.

† Nencki and Macfadyen observed with one enzyme only, viz., one derived from the cholera bacillus, a reduction of an alkaline silver solution (1891), while Brieger obtained a phenylhydrazone with a protein contained in a culture of the microbes of diphtheria.

‡ O. Loew, *Journ. f. prakt. Chem.*, Vol. 37, p. 704 (1888).

* The writer makes use of the proposed distinction between *protein* and *proteid*. Protein is the general name for all protein matter, while proteid signifies exclusively the more complicated kinds containing phosphoric acid, sugar, etc. (nucleins, mucins, etc.).

their atoms are easily set in motion, we can further understand that, by thus lessening certain affinities in them, another grouping of atoms may result.

It thus becomes intelligible why one molecule of an enzyme can, like a machine, change innumerable molecules, one after the other, of another compound. The chemical changes produced consist either in depolymerization, as in the production of dextrin from starch, or in hydrolytic action, as in the conversion of maltose into glucose, or in a further splitting combined with atomic migration, as in the production of amido-acids and bases from protein by trypsin.

Such chemical action produced by the mere transmission of chemical energy by a certain substance, which remains chemically unaltered, but acts like a machine, are called *catalytic*. We know that such actions are produced by finely divided metals, by alkalies and strong acids and that such are also produced by labile organic compounds. Thus, for instance, an aqueous solution of ethylaldehyde transforms dicyanogen rapidly in oxamid without undergoing a change itself (Liebig). Finely divided nickel splits acetylene into carbon and hydrogen,* finely divided platinum splits hydrogen peroxide into water and oxygen, etc.

We may now consider the third of the above questions: *How can the specific action of the enzymes be explained?* How is it, for example, that diastase can saccharify starch but not inulin, that inulase can saccharify inulin but not invert cane-sugar, that invertase can invert cane-sugar but not milk-sugar? Here the principle of the configuration of the molecules comes in. The closer the contact, the more perfect a transmission of energy is possible. The molecular adhesion, however, is enhanced by a certain coincidence of the surface features of the molecules. The writer in the year 1893 applied this principle to explain the fact that certain alkaloids have in very small quantities an effect only upon certain nerves, but not on all nerves, nor upon glands or muscles.* Later on, E. Fischer applied the same principle to the specific action of the enzymes, adopting the comparison to lock and key. However, *Fischer did not discuss at all the question* how enzymes can develop their energy nor did Green in his recent work: 'Soluble Ferments' devote a single line to it. The action of enzymes might be distinguished as *enzymations* to separate them from true fermentations which are such actions of bacteria as are intimately connected with, and directly dependent upon their living protoplasm itself and not upon enzymes secreted. From the recent observation of Eduard Buchner that alcoholic fermentation is not directly connected with the life of the yeast cell, it does not necessarily follow that lactic, butyric, or acetic fermentations are mere enzymations. Besides this, A. Wróblewski† has in a recent very interesting article pointed out important differences between zymase and the ordinary enzymes. The expressed juice of yeast is always *opalescent* and loses its fermentative action when filtered perfectly clear. It further soon loses its action upon mere dilution with water and also upon addition of 1½ per cent. of neutral salts. Formaldehyde, as well as sodium nitrite destroy the activity of zymase more easily than that of the true enzymes. Twenty per cent. ethyl alcohol destroys the zymase but not yet the known enzymes.

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* Moissan and Moureu, *Compt. Rend.*, Vol. 122, p. 1240.

* A natural system of poisonous actions, Chapter VI., Munich, 1893, Dr. E. Wolff, publisher.

† *Centralblatt für Physiologie*, September, 1899. He also showed that white diastase can be precipitated by saturation with sulphates, invertase can not.