

glucose. The glucose content of the solution may then be determined by any standard method.

This procedure is open to criticism in that the water extract will contain any water soluble substance originally present in the material unless it was dissolved out by the alcohol. Since pentosans are insoluble in alcohol and soluble in water they may be present in the water extract. These, if present, would be hydrolyzed to reducing sugars by acid, and recorded as starch.

It is, therefore, desirable to use, if possible, a hydrolyzing agent more specific than acid. Enzymes in general are specific in their actions, and taka-diastase is the most efficient of the enzyme mixtures which attack starch. Much work has been done upon the optimum conditions for enzyme activity. The optimum temperature range for taka-diastase is 30°–40° C.¹ The optimum pH is 4.5–5.0.² The hydrolysis curve begins to flatten at 50 hours³ and .2 gm. of enzyme is sufficient to dissolve .5 gm. starch.⁴ Most of this information is in Waksman and Davison, "Enzymes," but curiously, data could not be found indicating that any one had put it all together. Kuhn⁵ reports a curve for the hydrolysis of starch by taka-diastase at pH 4.5 for 50 hours at 36° C. in which he obtains 71.4 per cent. hydrolysis. He used 2 cc. of .5 per cent. enzyme solution for .603 gm. starch, so that it is probable that the quantity of enzyme was the limiting factor in that experiment. Horton⁶ working with unbuffered solutions reports that it is impossible to obtain consistent results with taka-diastase. In this laboratory, when 50 cc. of .3 per cent. starch solution was held at 40° C. for 36 hours with 1 cc. 10 per cent. taka-diastase and 5 cc. acetate buffer (pH 5.0), glucose values representing 98.5–101.1 per cent. hydrolysis were obtained consistently.

The substrate was potato starch which had been purified by repeated washings with distilled water and dried in a vacuum oven at 120° C. over phosphorus pentoxide to constant weight.⁷ The starch was not caramelized by this treatment. Furthermore, the analysis of undried starch of known moisture content gave concordant results. The reducing sugar was determined by the Schaffer-Hartmann⁸ modification of the Munson-Walker method. The amount of

reducing sugar produced by a maltose hydrolysis on another aliquot of the same solution was taken as the standard of complete hydrolysis.

Reducing the concentration of taka-diastase to $\frac{3}{4}$ did not decrease the amount of glucose formed, and $\frac{1}{2}$ the amount of enzyme gave 95.6–99.0 per cent. glucose. It was impossible to obtain consistent results at pH other than 4.5–5.0 with acetate buffers. A phosphate buffer at 4.5 gave 100.1 per cent. glucose.

In view of the present interest in the structure of starch it is interesting to note that both acid and enzyme hydrolysis consistently gave results equal to 93 per cent., the dry weight of the sample. It is possible that the starch was impure and contained only 93 per cent. hydrolyzable material. Davis and Daish⁹ working in England, report that acid hydrolysis of potato starch gives 93.8–94.5 per cent., the theoretical amount of glucose. They attribute the low results to destruction of glucose by the prolonged treatment with acid. If the enzyme hydrolysis be inaccurate, the fault probably lies in incomplete hydrolysis and the establishment of an equilibrium between dextrin, maltose and glucose. It seems improbable that this equilibrium point should coincide with the point reached by the destruction of glucose by acid hydrolysis. Noyes¹⁰ says that the analytical ratio between starch and glucose is .93. The agreement between these three figures may be coincidental, but the point should be further investigated.

The method seems to give concordant results with pure starch, and one series of determinations on grape wood gave values identical with those obtained by the maltose hydrolysis, but the method needs more work before it can be considered reliable. The specificity of taka-diastase for hexosans should be more carefully investigated. It is also possible that the time required for a determination may be shortened by the use of more concentrated enzyme solutions, or a different temperature.

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Studies in animal aggregations; protection of the individual by the mass: W. C. ALLEE (introduced by Frank R. Lillie). The injurious effects of crowding are easily demonstrated and have received due attention. Beneficial results from aggregations of animals without apparent social organization have escaped notice until recently,

¹ Euler, *Chemie de Enzyme* (Waksman); Kuhn, R.; *Berichte der Chem. Gesell.* 1924, 57: 2, 1965.

² *Ibid.*

³ *Ibid.*

⁴ This laboratory.

⁵ Kuhn, R.; *Berichte der Chem. Gesell.* 1924, 57: 2, 1965.

⁶ Horton: *J. Agr. Sci.*, 1921, 11: 240.

⁷ Walker: *J. A. C. S.*, 1907, 29: 54.

⁸ Schaffer and Hartmann: *J. B. C.*, 1920–21, 43: 371.

⁹ Davis and Daish: *J. Agr. Sci.*, 1914, 6: 152.

¹⁰ Noyes: *J. A. C. S.*, 1904, 26: 266.

when group survival value has been demonstrated for various land isopods and for the brittle starfish, *Ophioderma*, under starvation (Allee); for many animals when exposed to toxic reagents such as colloidal silver (Drzewina and Bohn, Bresslau, Allee and Schuett); and for certain marine turbellarians when exposed to hypotonic sea-water (Drzewina and Bohn). Groups of starving individuals show a modified rate of oxygen consumption as compared with wholly similar isolated animals. This appears to be correlated with changes in muscular tonus. Two explanations have been advanced for the protection furnished by numbers in toxic solutions: Drzewina and Bohn postulate an autoprotective secretion which enables the animals comprising the group to withstand the toxic action better than isolated individuals. The other workers find that with many reagents a large part of the protection is due to the greater fixation, by absorption or otherwise, of toxic material by the group. This leaves open the question of the production of a protective substance. Recent critical experiments demonstrate that the marine turbellarian, *Procerodes*, lives longer when a number are transferred to tap-water than do similar but isolated individuals in the same amount of tap-water. The greater survival value of the group is not wholly due to greater salt transfer, for greater protection is furnished by the filtrate from groups than by tap-water containing a larger amount of sea-salt. The protective material is given off both by living and by dead animals and once in solution will pass through ordinary filter-paper and will persist after the filtrate is brought to a boil. The results confirm the author's earlier suggestion of the existence of methods of integration in ecological animal communities more subtle than the food and space relations usually considered, and demonstrate a mechanism of cooperation among individuals comprising even loosely integrated animal aggregations.

The influence of a changed environment in the formation of new species and varieties: FRANK COLLINS BAKER (introduced by S. A. Forbes). In Barron County, Wisconsin, near Chetek, a series of small creeks and rivers were transformed into a number of large, shallow lakes by building a dam which ponded the waters for a distance of some eleven miles. The lakes were completed some sixty years ago for lumbering operations. The original molluscan inhabitants of this region were creek and small river species. Those mollusks that remained above the dam were quickly transferred from a creek habitat to a lake habitat. Many of these, such as the naiads, *Fusconaia*, *Elliptio lasmigon* and *Strophitus*, as well as the gastropod, *Campelona*, either migrated upward to the remaining part of the creeks, Moose Ear and Pokegoma, where the fauna was unchanged, or became exterminated. Certain other species of both naiades and gastropods, *Anodonta grandis plana*, *Lampsilis siliquidea*, *Amnicola limosa*, *Stagnicola catascopium*, *Helisoma antrosa* and *Helisoma trivolvis*, remained in the newly ponded waters and in the course of sixty years have become modified into recognizable varieties, several of which occur widely distributed in the lakes formed after

the retreat of the ice following the Wisconsin stage of glaciation. The changes in the form of the shell resulting from the change in environment have been, in the naiades, a shortening of the shell and an increase in obesity, and in one case, *Lampsilis*, of a greatly increased height. In the gastropods, *Amnicola*, *Helisoma*, *Stagnicola*, the result has been an increased globoseness and a widening of the umbilical region, in short, a loosening of the coiling of the whorls, whereas in the creek forms the whorls are coiled more tightly, leaving a smaller umbilical perforation. The result of this unintentional experiment, conducted on so large a scale and during such a long period of time, is conclusive proof, it would seem, that the environment does have a modifying effect on such plastic animal life, the degree of differentiation varying with the inherent variability of the organism. What has taken place in the Chetek Lake region is without doubt similar to the changes that occurred in the animal life of the waters following the different glacial invasions of the Ice Age, when the life, which has been driven southward by the ice, returned after its recession to find, instead of river systems, like that now existing in the Driftless Area of Wisconsin and Illinois, a vast system of lakes, which it invaded and in which it changed from one species or variety to another by the process of small variations like those shown to have taken place in the Lake Chetek region during a period of less than a century.

Energy metabolism as related to the plane of nutrition of cattle: E. B. FORBES. A study of the economy of utilization of the energy of feed by cattle was made at the Pennsylvania Institute of Animal Nutrition by means of a respiration calorimeter. This calorimeter, which is the only such apparatus in the world of the size necessary for large farm animals, permits of the direct measurement of the heat given off by the animal, as well as the determination of all factors of material outgo. In a duplicate series of energy balance studies with two steers, at five planes of nutrition, from fast to full feed, it was shown that the loss in heat per unit of weight of feed (the heat increment), increases with each increase of feed, but the rate of increase in heat increment is such as to be expressed not by a straight ascending line but by a curve falling from such a line. By the subtraction from the gross energy of the feed of all losses and expenses of utilization—namely, the potential energy of the visible excreta and of the methane produced by fermentation in the paunch, and the increased heat loss due to the consumption of the feed, the net energy available to the animal for maintenance and body increase was derived. The total net energy per unit of feed was found to decrease in a simple proportional manner with increase in feed; but in order to be able to compute separately the feed requirements for maintenance and production, for guidance in feeding practice, it is necessary to determine separate net-energy values of feeds for these purposes. Using the heat production of the fasting animal as the measure of the maintenance requirement of net energy at all planes of nutrition, it is proposed that one net-

energy value be used as the basis for computing the entire maintenance requirement of feed. Then a second lower net-energy value would represent all feed used for body increase. Net-energy values of alfalfa hay and corn meal, for maintenance and for body increase, computed by a new procedure, are presented.

The origin and destiny of prairies: HENRY C. COWLES (introduced by John M. Coulter). Many theories have been advanced to explain the treelessness of prairies. None of these has been generally accepted as adequate, and yet it is likely that most of them have some degree of validity. One of the major difficulties has arisen from lumping together prairies of many sorts and seeking a common explanation for them all. In this paper the attempt is made to sort out the major prairie types into three groups, which may be called climatic types, edaphic types and tension line types. The edaphic and tension line prairies are regarded as having a forest density, but the climatic prairies are believed to be more permanent. Recent soil studies show interesting interchanges between prairies and forests, and shed much light on various aspects of the prairie problem.

Reflection of light from the surface of leaves: CHARLES A. SHULL.

The isolation of a crystalline protein with tuberculin activity: FLORENCE B. SEIBERT. In the case of tuberculin, as with other biologically active principles, toxins, etc., there has always been the question as to whether the specifically potent factor is a protein or merely an infinitesimal amount of some very highly active substance attached to the protein. The difficulty in solving these problems has been due to the fact that the purification of proteins is one of the most difficult chemical tasks there is. Many previous investigators, beginning with Koch himself, have thought the active principle of tuberculin, which is responsible for the skin reaction in tuberculous subjects, was protein and all of the evidence from our laboratory during the last three years, *i.e.*, experiments based upon chemical analyses, dialysis, precipitation and hydrolysis, have consistently confirmed this view. Chief among the evidences is the fact that when the water-soluble protein obtained from tuberculin, that had been made in a non-protein medium, is treated with the proteolytic enzymes, pepsin and trypsin at the proper reaction, there is a loss in biological activity, paralleling a chemical break-down in the whole protein molecule to proteoses, peptones and amino acids. This evidence, together with the fact that the water-soluble protein, when not denatured, will crystallize into needles or burrs which after five or ten recrystallizations are still potent biologically, would seem to leave very little further doubt as to the protein nature of the active principle. The protein is crystallized by Hopkins' method for crystallizing ovalbumin at an optimum hydrogen-ion concentration point of pH 4.9. One lot of crystals, after fourteen crystallizations with solution in distilled water and filtration through hardened filter-paper between each crystalliza-

tion, gave the following protein tests. They were heat coagulable, gave positive biuret, Millon's, vanillin, ninhydrin and glyoxylic tests, a negative Molisch test and stained blue with methylene blue. The crystalline protein is purer and therefore more potent than the original water-soluble fraction of tuberculin from which it is made, as indicated by the following test. One tenth of a milligram of the original fraction is required to produce a maximum skin reaction in tuberculous guinea pigs, whereas, of the protein obtained from it and recrystallized ten times, as little as 0.04 mgm., measured as coagulable protein, sufficed to give an equally strong reaction. Throughout the experimentation evidence has accumulated for the following interpretations. The protein in tuberculin in its most natural and unchanged form, is crystallizable into needles and burrs, but it is an extremely labile protein very readily becoming less soluble in distilled water and then not crystallizable. This less soluble protein is still biologically active, but loses all or most of its activity with a further change to complete insolubility in distilled water. This denaturation and instability of the protein in water explain the small yield of crystals obtained and the great losses in activity during isolation of the protein and emphasize the importance of using the quickest and least drastic methods possible when the highest yields of most potent material are desired. In the case of tuberculin, therefore, there seems to be sufficient evidence to warrant the conclusion that the active principle is protein. It is a specifically toxic bacillary product and a protein obtainable in crystalline form, thus passing the first criterion of purity from a chemical standpoint. This work has been aided by a grant given to Dr. E. R. Long by the National Tuberculosis Association, for whom the H. K. Mulford and the Parke, Davis Co. have supplied the tuberculin in large quantities.

Are arginine, glutamic acid and aspartic acid necessary components of the diet during growth? W. E. BUNNEY and W. C. ROSE. In continuation of the investigations in this laboratory regarding the nutritive importance of the amino acids, growth studies have been made with diets practically devoid of (a) arginine, and (b) arginin, glutamic acid, and aspartic acid. The nitrogenous portion of each ration consisted of hydrolyzed casein from which the amino acids in question had been precipitated by appropriate methods. White rats have been maintained for periods of 100 days upon food mixtures containing the modified casein at levels of 12 and 9 per cent., respectively. During these periods excellent growth occurred. Animals which were furnished the 12 per cent. rations grew at a perfectly normal speed, while those which received the 9 per cent. diets increased in weight at a slightly subnormal rate. That the less rapid growth under the latter circumstance was not due to a deficiency of arginine, glutamic acid or aspartic acid is shown by the fact that the incorporation in the diets of the amino acids in question entirely failed to accelerate growth. The above results point very strongly to the conclusion that arginine, glutamic acid and

aspartic acid are probably not indispensable for normal nutrition. On the other hand, this fact does not exclude the possibility that some other component of the protein molecule may assume vicariously the functions of the missing amino acids. The chemical similarity of proline, glutamic acid, and the ornithine portion of arginine suggests that these three compounds may be closely associated physiologically, or even interchangeable in metabolism. This possibility is being investigated.

Relationship between the structure and bactericidal properties to B. Leprae of certain organic acids: ROGER ADAMS and associates (introduced by W. A. Noyes).

Power series expansions in the neighborhood of a point on a surface: ERNEST P. LANE. In ordinary space an analytic non-ruled surface referred to its asymptotics may be defined, except for a projective transformation, by a completely integrable system of partial differential equations in Fubini's canonical form

$$x_{uu} = px + \theta_u x_u + \beta x_v, \quad x_{vv} = qx + \gamma x_u + \theta_v x_v, \quad \theta = \log(\beta \gamma).$$

The four homogeneous coordinates of a point on such a surface referred to the covariant tetrahedron x, x_u, x_v, x_{uv} , with suitably chosen unit point, are such that, if the ratios of the first three of them to the fourth are denoted by x, y, z , there exists an expansion of the form

$$z = xy - \frac{1}{3}(x^3 + y^3) + \dots$$

This paper presents a new method for calculating the coefficients of this expansion to as many terms as desired, and computes for the first time the coefficients of the terms of the fifth order. Then various geometrical applications are made. Since every term of the series is an absolute invariant it is easy to show how to write the equations of many curves and surfaces covariantly connected with the original surface and the geometrical nature of this connection is explained. In particular the algebraic surface of order n obtained by truncating the series after degree n , which is a generalization of the canonical quadrics and cubic surfaces used by Wilczynski, Green and Fubini, is characterized geometrically.

Groups generated by two operators of order three, the cube of whose product is invariant: G. A. MILLER.

Non-monogenic functions (by title): EDWARD KASNER.

Multiply transitive groups of prime-power degree: R. D. CARMICHAEL (introduced by G. A. Miller).

On a property of frequency distributions of the powers and roots of variates of a given distribution: H. L. RIETZ (introduced by G. A. Miller).

Geometric properties of triple systems: A. EMCH (introduced by G. A. Miller).

On the theory of ideals in an algebra of finite order: G. A. WAHLIN (introduced by G. A. Miller).

Light quanta and interference: A. J. DEMPSTER. The

reconciliation of the interference of light with the quantum theory of atomic processes leads to great difficulties. Hitherto no experiments have been made in which the light from a single atomic emission was examined. In this paper interference experiments are described in which only one atom at a time was excited. Interference patterns were observed just as when the light is produced by many atoms radiating at the same time, showing that a single light quantum follows the classical laws of division at a half silvered mirror and of subsequent recombination with the phase difference required by the wave theory of light.

The structure of atoms as a periodic function and its relation to ion-formation and valence: ARTHUR A. NOYES.

X-ray methods and results in the examination of fibrous materials, particularly asbestos: GEORGE L. CLARK. (1) Typical X-ray diffraction patterns are presented for the following fiber-crystalline materials: natural cellulose, regenerated cellulose (rayon), silk, cobwebs, wood, stretched rubber, balata and gutta-percha, γ -polyoxymethylene (methyl ether), worked grease, drawn and rolled metals, and 7 varieties of asbestos. (2) A new modification of the monochromatic pinhole diffraction method is exemplified for the case of chrysotile asbestos. (3) The method of calculating the identity period in the direction to the fiber axis is shown to be entirely independent of any assumptions concerning crystallographic system or arrangement of atoms and molecules in the unit crystal cell. (4) The practical significance of fiberizing in terms of behavior is indicated, with particular reference to the use of the X-ray method in testing, specification and research. The patterns for the varieties of asbestos are correlated with action of acids, heat and most desirable properties when used as catalyst base (contact sulfuric acid process), Gooch filter, brake bands, etc.

Surfaces: W. D. HARKINS.

The photochemical decomposition of hydrogen-iodide; the mode of optical dissociation: BERNARD LEWIS (introduced by W. A. Noyes). The photochemical decomposition of hydrogen-iodide was studied at low gas pressures where the collision frequency is comparable with the mean life of the excited state. The quantum efficiency of the process was found to be about two, which agrees with Warburg's value for high gas pressures. This indicates that hydrogen-iodide dissociates in an elementary act as a result of absorption of radiation without the necessity of a collision. From the continuous absorption spectrum it is shown that dissociation takes place into a normal hydrogen atom and an excited iodine atom in the $2p_1$ state, the excess energy, if any, being dissipated as kinetic energy. It is pointed out that the time between absorption and dissociation is shorter than 2×10^{-12} second.

The volcano problem: ARTHUR L. DAY, of the Geophysical Laboratory, Carnegie Institution, Washington.
(To be continued)