

THE AMERICAN CHEMICAL SOCIETY

DIVISION OF BIOLOGICAL CHEMISTRY

J. S. Hughes, *chairman*W. T. Bovie, *secretary*

At the sixty-sixth meeting of the American Chemical Society held in Milwaukee, Wisconsin, September 10 to September 14, 1923, the Division of Biological Chemistry held a symposium on Biophysical Chemistry. The symposium was followed by the regular scientific program of the division.

There were six papers on the symposium program, the first three of which dealt with the use of physical-chemical methods in biological investigations:

C. H. Bailey, of the University of Minnesota, discussed the use of electrolytic conductivity measurements, hydrogen-ion concentration measurements, viscosity measurements, and the determination of colloidal properties in the study of cereals.

J. Arthur Harris, of the Carnegie Institution of Washington, Station for Experimental Evolution, discussed the use of osmotic concentration measurements, specific electrical conductivity measurements, hydrogen-ion concentration measurements, etc., in investigations on the geographic distribution of plants.

C. L. A. Schmidt, University of California, Berkeley, California, described a method for the separation of the hexone bases from certain protein hydrolysates by electrolysis.

The other three papers on the symposium program dealt with the physical chemistry of the structure of protoplasm:

W. T. Bovie, of Harvard University, discussed the molecular organization of protoplasm and pointed out the importance of the colloid interfaces in determining this organization. He pointed out that protoplasm has a molecular organization in the three dimensions of space, and since it is the seat of a series of regulated and coordinated chemical events it has a dynamic organization in the dimension of time. It is necessary to consider these four dimensions in explaining the activity of living things.

W. D. Harkins, of the University of Chicago, presented a theory of emulsions and their inversion, according to which the stability of emulsions of the type produced by soap, is dependent upon the existence around the droplets of a film of molecules with the polar ends oriented toward the water and the non-polar ends toward the oil. A second and independent part of the theory considered the molecules as capable of representation by the frustra of cones (or in a plane, by wedges). The water or the oil will be the continuous phase of the emulsion accord-

ing as the polar or the non-polar ends of the molecules form the bases of the frustra.

H. H. King and J. S. Hughes discussed the part played by surface tension in the organization of living matter and presented a theory of emulsions similar to that of Harkins. They invoked von Baeyer's theory of tension to account for the shape of the molecule forming the emulsion interfaces and thus determining which phase of the emulsion is continuous.

There were thirty-two papers on the general program, one third of which were concerned with vitamins. Many of them elicited lively discussion.

The following officers were elected: *Chairman*, W. T. Bovie; *secretary*, R. Adams Dutcher; *executive committee*, W. T. Bovie, J. S. Hughes, R. Adams Dutcher, Carl L. A. Schmidt, L. S. Palmer, G. E. Holm.

SYMPOSIUM PROGRAM

Physical chemistry in the cereal industries: C. H. BAILEY.

Physico-chemical properties of the tissue fluids of agricultural plants: J. ARTHUR HARRIS.

The application of certain physical-chemical principles to the separation of the hexone group of amino acids: C. L. A. SCHMIDT.

On the nature and structure of protoplasm: W. T. BOVIE.

A theory of emulsions and the inversion of emulsions: WILLIAM D. HARKINS.

The part played by surface tension in the organization of living matter: H. H. KING and J. S. HUGHES.

The nutritive value of the proteins of milk from the standpoint of reproduction: BARNETT SURE. On rations containing 12 to 20 per cent. milk proteins, and in the presence of a liberal supply of the fat-soluble and the water-soluble vitamins, and dextrin; and an adequate salt mixture, no successful reproduction was secured. All rations contained 0.4 per cent. cystine, and during the breeding and lactation periods certain rations were fortified with lysine and proline. Still all the females remained sterile. That another as yet unidentified dietary syndrome might play a prominent part in reproduction is suggested from the following paper.

Suggestive evidence for the existence of a specific vitamin for reproduction: BARNETT SURE. In connection with studies on the rôle of cystine in reproduction it has been observed that on a 40 per cent. velvet bean pod meal ration, containing a liberal supply of dextrin, 9 per cent. casein, 2 per cent. cod liver oil, an adequate salt mixture, and dextrin carrying alcoholic extracts of 10 grams of ether-extracted wheat embryo in 100 grams of ration, fertility and a certain degree of success in reproduction is always obtained, the degree of success of reproduction increasing with the concentration of the water-soluble B vitamin. The experimental evidence suggests that the fertility and partial success of reproduction is to be ascribed to a specific vitamin that controls reproduction other than water-soluble B vitamin.

The relative vitamin and mineral values of cow pea, soy bean, alfalfa, lespedeza and prairie hays as ordinarily cured: J. W. READ. The rat seems to utilize satisfactorily as high as 40 per cent. of these hays. Cow pea, alfalfa and prairie hays were good sources of vitamin A at a 20 per cent. level. Soy bean and lespedeza were almost as good at 10. Regarding vitamin B, prairie hay was poor at 40, cow pea and soy bean were good at 20, but alfalfa and lespedeza were fairly good only at 20. As a source of minerals, when fed at a 10 per cent. level prairie and cow pea hays were poor, lespedeza fair, while soy bean and alfalfa were good.

Studies of the vitamin potency of cod liver oil. VI. The effect of storage of livers on the vitamin A potency of cod liver oil: ARTHUR D. HOLMES. Cod liver oils were extracted from fresh cod livers; from livers that had been stored six months; and from livers stored one year. The results of three series of comparable tests in which albino rats suffering from vitamin A starvation were fed graduated amounts of the three oils noted above, showed that livers which had been stored at a low temperature out of contact with air had as high a vitamin potency as oil rendered from fresh livers. One milligram per day of each of the oils contained sufficient vitamin A to insure good growth in albino rats.

On the cystine deficiency and the vitamin content of the lentil, Lens esculenta Moench: D. BREESE JONES and JOSEPH C. MURPHY. Feeding experiments with albino rats showed that the proteins of the lentil are deficient in cystine. Young animals fed a diet, the sole source of protein of which came from the lentil, rapidly declined in weight and died in 17 to 49 days. On the same diet, after addition of 0.36 per cent. of cystine, the animals grew at an average rate not far from normal. Slightly better results were obtained when the lentil had been cooked than when raw. Two grams daily of raw lentil furnished about the minimum quantity of vitamin-B required by the albino rat for growth at the normal rate. The lentil also contains appreciable quantities of vitamin-A.

Dried yeast as a supplement to a good poultry laying ration as a means of increasing egg production: A. J. SOUBA, H. C. KNANDEL and R. ADAMS DUTCHER. Ten pens (45 birds per pen) of white leghorn pullets and hens were fed for ten months on wet and dry mash rations, with and without yeast and with and without artificial lights. Biometrical methods were used in interpreting the data with regard to body weight, egg production, size and weight of eggs and food intake. Practically all groups, receiving yeast, showed a response, although in some cases the differences were not significant. When pullets were subjected to longer feeding periods by use of electric lights, significant differences were obtained in favor of the yeast-fed pens.

The influence of heat on certain physical and chemical properties of cow's milk: J. ROY HAAG, STANLEY R. SHIMER and R. ADAMS DUTCHER. It was impossible to demonstrate a calcium deficiency in pasteurized milk by the use of feeding methods. Raw and heated milks were passed through an ultra filtration apparatus under a pressure of 200-250 pounds, using nitrogen gas. No

significant differences could be noted between the sera of raw and heated milks with regard to freezing point depression, hydrogen ion concentration, and the amounts of calcium and phosphorus present. When raw and heated milks were centrifuged at high speed and the intermediate layer of skim milk analyzed for calcium and phosphorus no significant differences could be noted.

The storage of vitamin B in the albino rat: EMMA FRANCIS and R. ADAMS DUTCHER. A general description of the technique adopted at the Pennsylvania State College. It was pointed out that some animals eat very large proportions of their fecal matter. Such animals may grow normally for months on a diet deficient in vitamin B, due to the fact that the vitamin is apparently utilized again and again. When screens were introduced this trouble was eliminated. Rats can not store large quantities of this vitamin for long periods. The importance of accurate food intake records was emphasized.

The application of the Benedict-Osterberg method to the quantitative estimation of carbohydrates in plant tissues: WALTER THOMAS and R. ADAMS DUTCHER. The colorimetric method of Benedict and Osterberg (slightly modified) was far superior to the usual copper reduction methods. Glucose, fructose, l-arabinose, l-xylose, maltose, sucrose and starch can be satisfactorily determined by this method. It was possible to obtain practically theoretical recovery of these carbohydrates when they were added, in quantities approximating 5 to 10 milligrams, to plant extracts.

Experiments on the isolation of the antineuritic vitamin by picric acid precipitation: ATHERTON SEIDELL. Addition of picric acid to a concentrated vitamin extract, prepared from brewer's yeast by the fuller's earth method, yields a highly active precipitate which appears to contain at least two picrates. By solubility differences in 95 per cent. acetone, there can be separated a well crystalline picrate melting at about 200°, but showing very little activity, and a less well crystalline product, which has been found to be active in doses of one milligram per day, when given to pigeons fed only on polished rice.

Studies in the genus Mentha. 8. The oil of Mentha piperita L., 1922: ROLAND E. KREMERS. The oil of peppermint produced by the Wisconsin Pharmaceutical Experiment Station in 1922 was examined for its constituents. Phellandrene was present in traces only, if at all. The presence of pinene and limonene was not conclusively proved. Cineol (cineolic acid m.p. 206°), 1-menthol (m.p. 42°), and 1-menthone (semicarbazone m.p. 184°) were found as expected. Two menthenones, d-piperitone (semicarbazone m.p. 215°) and d-pulegone semicarbazone m.p. 171° were isolated. The cause for the difficulty experienced in crystallizing 1-menthol from American peppermint oils was studied and is thought to be due to another alcohol occurring in the menthol fractions.

Studies in the genus Mentha. 10. A preliminary study of the reduction of d-pulegone by palladium and hydrogen: ROLAND E. KREMERS. d-Pulegone from *M. arvensis* was reduced by Pd-H until action had practically ceased.

The oil rectified by steam had $[\alpha]_D + 21.19^\circ$ and no longer reacted with neutral sulfite. It distilled largely between 205 and 210°C .; b.p. menthone is $207-8^\circ \text{C}$. The semi-carbazone consisted largely of a fraction m.p. $183-4^\circ$. The regenerated ketone had $[\alpha]_D - 7.4^\circ$; semi-carbazone from regenerated oil melted at 184° . A lower fraction of semi-carbazone regenerated an oil, $[\alpha]_D + 25.7^\circ$. Semi-carbazone from this oil melted at $175-6^\circ \text{C}$. A mixture of menthones had accordingly resulted.

Studies in the genus Mentha. 11. *The occurrence and significance of menthenones in mentha oils:* ROLAND E. KREMERS. A menthenone-3 is known to occur in all investigated *Mentha* oils except *M. spicata* L. and *M. citrata* Ehrh. Since d-menthone-4 (8) one-3, (pulegone) and l-menthanol-3 (menthol) are the only two compounds which become preponderant constituents in mentha oils, a genetic relationship is suggested. It is conceivable that menthone and menthol are formed by successive reductions. Charabot found that oil production is associated with chlorophyll, *i.e.*, reducing, functions. Brooks could not oxidize menthol with peppermint oxidase. Since piperitone reduced by Pd-H gives an isomenthone and pulegone a menthone, it may be possible to follow the plant syntheses.

Biochemistry of plant diseases VI. Physical and chemical basis of resistance to brown rot in plums: J. J. WILLAMAN and N. C. PERVIER. Mechanical tests have been devised for measuring toughness of skin and firmness of flesh of plums. It has been found that those varieties of plums which are resistant to brown rot have firmer flesh, tougher skin, and higher content of fiber and pentosans than the susceptible varieties. The crude fiber relation is so sharp that any variety having 6 per cent. or more of crude fiber on the dry basis can be classed as resistant. As ripening progresses, the texture of the resistant varieties remains firm, while that of the susceptible becomes softer. The crude fiber content also is less in ripe plums and at this stage they are the most susceptible to the rot.

The multiple nature of bios: E. I. FULMER, W. W. DUECKER and V. E. NELSON. Alfalfa extract was fractionated into four fractions with ethyl alcohol. No. 1 insoluble in 40 per cent.; No. 2 insoluble in 70 per cent.; No. 3 insoluble in 95 per cent.; No. 4 soluble in 95 per cent. Each precipitate was analyzed for nitrogen and protein and the optimum concentration for yeast growth determined. The basal medium had the following composition; Ammonium chloride, 0.236 gms; di-potassium phosphate, 0.1 gm; calcium chloride, 0.1 gm; calcium carbonate, 0.04 gms; cane sugar, 10 gms, per hundred cc. Combination of the optimum concentrations of 1 and 3 produced greater stimulation of yeast growth of either alone. Fractions 1 and 4 are different and Bios is not a single substance.

Determination of iodine in natural waters: J. F. McCLENDON. The method of reduction of iodate to iodide and the oxidation of iodide to iodine, shaking this out with carbon-tetrachloride and determining colorimetrically is adapted to quantities of .001 milligrams or greater by the use of a Bausch and Lomb colorimeter, cups holding 1 cc at 2 cm depth. Water residue is first

ignited in pure oxygen in a silica combustion tube with the end bent down into KOH solution.

Determination of iodine in large quantities of food-stuffs: J. F. McCLENDON. The method is similar to the method for water except that the sample is burned in two stages. The first stage in an apparatus similar to the silica tube described before but large enough to burn several kilograms. KOH solution is evaporated down and together with the ash is returned in the smaller tube and analyzed as in the preceding.

Relation of distribution of iodine to simple goitre: J. F. McCLENDON. There are two endemic centers for goitre in the United States. One centers in Michigan and the other in northern Idaho. The goitrous regions decrease in severity from these centers, the goitre-free region being the broadest in the Gulf States with only a narrow strip on the Atlantic coast and a still narrower strip on the Pacific coast. Analysis of iodine in drinking water from about one hundred localities shows an inverse ratio between the iodine and goitre prevalence. It would take one thousand years for a person to drink enough of Lake Superior to obtain the iodine which is in his thyroid gland.

The effect of vitamin-carrying additions to the diet of baby chicks: ALVIN R. LAMB and CHARLES W. KNOX. Chicks were fed immediately after hatching in small outdoor lots with no vegetation under the care of hens, which were fed separately. A basal ration of cereal grains and by-products, some meat scrap, and 5 per cent. of alfalfa meal, comparable to practical chick rations, was used. Cod liver oil, butter-fat, yeast, tomato juice and buttermilk were fed as vitamin carriers. The yeast and buttermilk lots grew more rapidly and economically, and the lots receiving the fats were not so good as the control lots.

Further chemical studies on the placental hormone: PAUL M. GIESY. A method has been developed for preparing a concentrated material containing the hormone; yield, 0.02 per cent. This concentrate contains cholesterol and other lipoidal impurities, but contains no nitrogen, sulfur or halogens. The hormone is lipoidal in solubility. It diffuses through rubber in acetone, benzene, ether and chloroform solution, and through collodion in 75 per cent. and 62½ per cent. alcohol solution. From the effect of various reagents on its activity, it appears to be an unsaturated compound of ester-like nature, containing neither the carbonyl, amino, imino, nor hydroxyl grouping.

The importance of iron in reproduction: J. S. HUGHES. White rats reared from weaning time to maturity on a diet of cows' milk failed to produce any young. The addition of vitamins had no beneficial results. The addition of iron in the form of iron citrate, however, brought about normal reproduction.

The relation of sunlight to rickets (weak legs) in chickens: J. S. HUGHES. Chickens receiving a standard scratch feed and mash, supplemented with sprouted oats and buttermilk, developed rickets (weak legs) when deprived of direct sunlight. Chickens receiving same feed but given sun bath developed normally, although they were confined in very small pen, with little opportunity

to exercise. Light from ordinary electric bulb had very little, if any, beneficial action. Light from Hereus mercury arc lamp was very beneficial. Cod liver oil also proved to be effective in preventing rickets in chickens as in mammals.

Cereal values as determined by number, fertility and composition of eggs: C. B. POLLARD and R. H. CARR. Twelve pairs of pigeons were fed with as many different kinds of grains, supplemented with grit, bone, shell, charcoal and salt. A chemical study was made of both eggs and feces by the Van Slyke method. It was found that wheat, rye, kaffir, corn, oats, barley, peas, soy beans and hemp were efficient in egg production in about the order named, whereas no eggs were obtained from buckwheat, pop corn or sunflower seed. Great differences were noted in the egg proteins from different cereals, especially in those from wheat, rye, oats and corn which contained high (3 to 4 per cent.) melanin (tryptophane) and were hatchable, whereas the others were not fertile.

Colorimetric test for arsphenamine: M. X. SULLIVAN. Arsphenamine reacts with beta naphthoquinone monosulphonate with the formation of a dark red solution and a dark red precipitate in concentrations of 25 parts per million and up. By proper attention to the reaction and to the proportionality of reagents and with proper control, one tenth milligram of arsphenamine in 20 cc of water can be detected. Fresh neoarsphenamine gave no color, even in concentration of 100 milligrams in 20 cc of water. Old, somewhat brownish neoarsphenamine is a little more reactive but gave very much less color than arsphenamine, and the mixture with beta naphthoquinone monosulphonate is yellow at a concentration that arsphenamine gives a vivid red solution and a heavy precipitate.

Carbon monoxide hazards from tobacco smoke: G. W. JONES, W. P. YANT and L. B. BERGER. Previous investigators have shown that undiluted tobacco smoke contains 0.5 to 12.0 per cent. CO. Three or more subjects, in a non-ventilated room of 1,000 cu. ft. capacity, smoked 1½ to 2 hours—cigar, cigarette, stogie or pipe. The smoke was 4 to 6 times more concentrated than would be permitted under ventilated room conditions; the eyes had to be protected by goggles. The highest CO content of the room was 0.02 per cent. CO saturation of the blood was not greater than 5 per cent., even in two subjects who each inhaled the smokes of 20 cigarettes. Tests showed that the concentrated smoke does not enter the alveoli.

Isolation from plants and vegetables of a substance possessing properties similar to those exhibited by insulin: HARRY E. DUBIN and H. B. CORBITT. From common plants, by a simple inexpensive method, a substance can be isolated exhibiting typical insulin-like action. A reduction of 50–60 per cent. in blood sugar has been obtained by injecting this into rabbits. Certain dosages produce convulsions which, like those produced by insulin, are abated by injection of glucose. Clinical experiments are to be undertaken with this substance. "Glucokinin" found in a variety of plants, as recently reported by Collip, produces first a rise in blood sugar, followed by a fall in 11–42 hours. But the product

described here produces a rapid fall in blood sugar similar to that produced by insulin, without any preliminary rise.

A metabolic response of wheat to variation of temperature: W. E. TOTTINGHAM. A nutrient solution was devised to permit separate varying of the plane of supply of nitrogen and potassium. Growth of wheat seven weeks from the seed showed that for production of dry matter and storage of carbohydrates the ratio of potassium to nitrogen should be high at 14 to 15° C. and low at 23 to 25° C. On exposure at 23° C. during the seventh week to the lowest plane of nitrogen supply, without renewal, the plants returned to the solution an amount of nitrate equivalent to over 30 per cent. of the original supply, indicating a peculiarly critical relation between temperature and assimilation of nitrates.

The mechanism of the physiological action of radiation: W. T. BOVIE. This discussion is entirely theoretical and is presented in order to emphasize the importance of interfacial organizations in protoplasm. A mechanism of photosynthesis is described in which the colloidal interfaces of the protoplasm play an important part. The theory is compatible with all the experimental facts of the photosynthetic process of which the author is aware.

Photo cytotoxicity as a measure of metabolic activity: W. T. BOVIE and C. E. BARR. When an active amoeba is exposed to ultra violet light of a suitable intensity, cytotoxicity occurs. The protoplasm having the greatest physiological activity cytotoxicizes first. The pseudopods cytotoxicize in the inverse order in which they were formed. These results are similar to those of Miss Hyman, who exposed active amoebas to various poisons. The results are interpreted as indicating that the exposure to radiation interferes with the normal course of metabolic changes so that the normal sequence of physiological events does not occur. Organization in the dimension of time is destroyed and the organizations in the dimensions of space are no longer maintained. The disorganization in the dimensions of space is cytotoxicity.

The relation between the physiological effects and the absorption index of radiation: W. T. BOVIE and O. L. INMAN. Mast's experiments with the illuminated square were repeated except that the square was illuminated with ultra violet instead of visible light. The negative photo-tropic reaction of the amoeba is much more precise than the reactions reported by Mast. This is in agreement with Grotthus' law of photochemical reaction. The normal course of physiological change is interfered with. Physiological dominance of the advancing pseudopod is lost and the direction of locomotion is reversed. This interpretation of the results gives a physiological meaning to the negative photo-tropic response of amoebae.

The significance of indican in constipation: LILLIAN SEGAL KOPELOFF and NICHOLAS KOPELOFF.

Urinary acidity as influenced by bacillus acidophilous milk and lactose: LILLIAN SEGAL KOPELOFF and NICHOLAS KOPELOFF.

W. T. BOVIE,
Secretary