losses, the excessive increases of agar in dilute solutions would be accentuated.[4]

The results illustrate in no uncertain manner that the chlorides of Na, K. Ca and Mg. at concentrations from 0.001 to 0.0001 M cause an excessive hydration of such sections of agar, that the effects of the kations are distinctive, and the differential effects of the anions, nitrate and sulphate, are also apparent. Excessive swelling was found by the use of HCl 0.0001 N, and in weak hydroxides, while still more marked effects were secured with solutions of amino-compounds such as glycocoll, histidine, phenyl-alanin, asparagin, alanin, etc.[5] These increases took place in the various solutions under a total range from Ph. 4.2 to Ph. 11. Mixtures of agar and gelatine also showed a great increase over that in water, especially in amino compounds.

Fairbrother and Mastin,[6] apparently unacquainted with these results, published similar data with regard to the effect of the common acids and alkalis on the swelling of agar.

Dokan's experiments dealt with the agar gel through only a third of the total range and took no account of losses by solution. Consequently the differential effects were lost and diminished totals found. The auxographic measurements recorded hydration increases of agar plates from a fresh air-dry condition at 15 to 20° C. to approximate satisfaction. The increased delicacy and effectiveness of this method extends the differential effects of kations and anions to extremely dilute concentrations. The range of swelling followed in such measurements is four times as great as in the method of Dokan, which was that employed by many workers. The measurement of the changes in volume of an agar plate from the air-dry condition in which it holds one fourth its weight in water to one in which it holds as much as forty times its volume of water doubtless comprises the changes in such a reversible gel of direct biological interest. It is by no means, however, the whole story of the hydration of agar.[7] If the water content of agar plates be reduced to a minimum by drying still further in a desiccator over phosphorus pentoxide, it will be found that their hydration capacity has been reduced so that an increase of only a few hundred per cent. is exhibited when they are placed in water as determined by weighing. In what manner the differential effects of kations would be shown if such desiccated material were placed in solutions of electrolytes is not known.

Professor Michaelis cites some hydration reactions of Konyaku, a polysaccharide of mannose and glucose obtained from the tubers of *Amorphophallus Konaku* measured by Dokan, which does show excessive and differential swelling in solutions of some electrolytes and hydroxides, but not in others. These comparative effects are reminiscent of the hydration increases of agar-gelatine mixtures in suites of solutions and suggest that the substance in question will be found to include a relatively high proportion of proteinaceous material: Glycolipins may also be present.

The results of scores of workers show that the action of ions on colloids, and consequently on permeability of walls and plasmatic layers, are not to be accounted for solely by electrostatic effects, determined by sign and valency. Such effects are due to the direct action of ions on colloidal particles. Differential or lyotropic effects among univalent ions, for example, are to be attributed to the varying attraction of the different elements for water molecules, thus exerting an indirect effect on the hydration of the colloidal particle, as is well described by Professor Michaelis, who suggests that the nature of such attraction depends on atomic radius. As noted above, he believes these effects are not seen at low concentrations, except in the case of the hydrogen ion. The results cited in this note establish well-marked differential action of common univalent and bivalent ions at extremely low concentrations in conformity with the lyotropic series, and give greater value to the proposal of Michaelis as to the physical basis of such effects.

D. T. MACDOUGAL
B. L. CLARKE

DESERT LABORATORY

[4] MacDougal, D. T., and Spoehr, H. A.: "The solution and fixation accompanying swelling and drying of biocolloids and plant tissues." *Plant World, 22,* 129, May, 1919.

[5] MacDougal, D. T.: "Auxographic measurements of the swelling of biocolloids and of plants," *Bot. Gaz., 70,* 126, 1920; "Action of bases and salts on biocolloids and cell-masses," Proc. Amer. Phil. Soc., *60,* 15, 1921; and Spoehr, H. A., "The components and colloidal behavior of plant protoplasm," 59, 150, 1920.

[6] Fairbrother and Mastin, Trans. Chem. Soc., *123,* 1412 (1923).

[7] Clarke, B. L., *Journ. Amer. Chem. Soc., 47,* 7, (1925).

THE AMERICAN CHEMICAL SOCIETY

DIVISION OF BIOLOGICAL CHEMISTRY

R. Adams Dutcher, *chairman*
R. J. Anderson, *secretary*

The fate of intarvin in the normal and diabetic economy: MAX KAHN and HATTIE L. HEFT. Intarvin, the synthetic neutral odd-carbon fatty-acid fat (glyceryl trimargarate), is absorbed in the animal organism to the extent of about 95 per cent.; and upon catabolism yields

approximately eight calories of heat per gram of fat. It is oxidized in the normal and diabetic organism without the production of beta-oxy-butyric acid, diacetic acid, acetone or alpha or beta lactic acids. It thus lends itself to the feeding of diabetic individuals, in quantities sufficient to bring up the total caloric intake to a maintenance diet. Five successive generations of rats have been fed with intarvin as one of the main food ingredients, with no observation of any toxic or detrimental effects. The successive generations are normal in size, weight and behavior. Studies are now under way to determine the nature of the fat deposited.

Some chemical properties of vitamine B: ATHERTON SEIDELL. The picric acid present in the antineuritic picrate, prepared from brewers' yeast by the method previously described, can be quantitatively removed with the aid of nitron. The resulting free base retains the antineuritic properties of the picrate. It protects pigeons from loss in weight on polished rice in daily doses of 43 milligrams. The activity is not destroyed by drying in a vacuum at moderate temperature. The molecular weight of the base, determined by lowering of the freezing point of acetic acid, is 201. Combustion analyses give results corresponding to the formula $C_8H_{15}O_3N_3$. When titrated with acid, using methyl red as indicator, a fairly sharp end-point occurs at approximately one molecule of acid to two of base. A Van Slyke determination shows one amino nitrogen.

A contribution to the chemistry of grape pigments. IV. The anthocyans of Isabella grapes: R. J. ANDERSON and FRED P. NABENHAUER. The anthocyans occurring in grapes, so far as determined, are glucosides of methyl delphinidin. American grapes contain mainly delphinidin monomethyl ether, while *Vitis vinifera,* as well as hybrids of American varieties with vinifera, contain principally the dimethyl ether. Isabella grapes (labrusca × vinifera) contain delphinidin dimethyl ether as a monoglucoside. The anthocyanin crystallizes in prisms and is identical with oenin. When hydrolyzed with hydrochloric acid, the glucoside yields glucose and anthocyanidin chloride, identical with oenidin chloride. Oxidation of the acetyl derivative of oenidin gives acetyl syringic acid and by hydrolyzing the latter syringic acid is obtained. This fact is of importance in determining the constitution of oenidin.

Reactions of bb dichlorethyl sulphide with compounds containing amino groups: WALTER E. LAWSON and E. EMMET REID. This comprises an investigation of the reactions of bb dichlorethyl sulphide, sulphoxide and sulphone with primary, secondary and tertiary amines and direct condensation of the sulphone with two amino acids, in an effort to obtain information bearing on a "condensation" theory of vesicant action, that is, the disturbing of the equilibrium maintained within the cell by a reaction between the toxic compound and portions of the protein molecule. Thirty-six new compounds, exclusive of hydrochlorides or platinum salts, were prepared, and several new series of compounds were discovered. The evidence secured was not opposed to the theory.

The physiologic properties of some unsaturated hydrocarbons: LLOYD K. RIGGS. Hydrocarbons of the olefine, acetylene and diolefine series have been administered to white rats by inhalation, and the anesthetic potency and toxicity of each hydrocarbon has been determined. Ethylene 90 per cent., propylene 40 per cent., butylene 20 per cent., amylene 6 per cent. and acetylene 78 per cent. induce anesthesia in 15 to 18 minutes. Propadiene 20 per cent. and methylacetylene 5 per cent. produce prostration, not true anesthesia, in 15 to 18 minutes. Propylene 65 per cent., butylene 20 per cent., amylene 6 per cent, acetylene 90 per cent., methylacetylene 5 per cent. and propadiene 15 per cent. cause respiratory failure in about two hours. The potency and toxicity of each hydrocarbon has been related in the form of an index thus: $\frac{\text{Potency} \times 100}{\text{Toxicity}}$ = Anesthetic Index. The anesthetic indices of propylene, butylene, amylene and acetylene are 100, 61 and 71, respectively.

Studies of the physiologic action of propylene: HAROLD D. GOULDEN and LLOYD K. RIGGS. The time in which various concentrations of propylene induce analgesia, anesthesia and respiratory failure has been measured. A number of mixtures of propylene, oxygen and nitrogen were made up in different proportions and tested for anesthetic effect. The time required to produce analgesia, anesthesia and finally respiratory failure was measured. The results indicate that propylene is a potent anesthetic which possesses a wide margin of safety.

Physico-chemical studies on proteins. II. Alkali binding. A comparison of the electrometric titration of proteins and of phosphoric acid with sodium and calcium hydroxides: WALTER F. HOFFMAN and ROSS AIKEN GORTNER. H_3PO_4, casein and durumin have been titrated electrometrically with NaOH and $Ca(OH)_2$ and "back titrated" with HCl. The alkali titration curve of casein resembles that of a weak acid, such as NaH_2PO_4, while that of durumin resembles much weaker acids. NaOH and $Ca(OH)_2$ give the same type of curves with proteins but not with H_3PO_4, where both the secondary and tertiary hydrogens are replaced by calcium at the pH at which Na_2HPO_4 is formed. The difference between the alkali $^{\circ}$ H_3PO_4 or protein and alkali + H_3PO_4 or protein + HCl curves is shown to be due to $Ca_2H_2(PO_4)_2 + 4HCl = 2CaCl_2 + 3H_3PO_4$ not going to completion.

A study of the effect of the concentration of sodium carbonate in the Benedict quantitative sugar method: ARMAND J. QUICK. The glucose equivalent of Benedict's quantitative sugar reagent is found to be markedly influenced by the concentration of sodium carbonate. A minimum and constant value is obtained when the final concentration of sodium carbonate exceeds 25 g per 100 cc; below this concentration the glucose equivalent increases as the sodium carbonate decreases. The minimum and constant value of the reagent prepared according to the

directions of the author is found to be 1.88–1.90 mg of glucose per cc instead of 2.0 mg, the value now used.

The effect of additions of fluorine to the diet of the rat on the quality of the teeth: E. V. MCCOLLUM, NINA SIMMONDS and J. ERNESTEIN BECKER. The addition of 220 parts per million of fluorine in the form of sodium fluoride to a diet which produces good bones and teeth was sufficient to cause pronounced damage both to skeleton and dental structures. The persistently growing incisors in the rat were especially damaged. Being soft and chalky, the lower teeth wore to the gum line and from lack of attrition the upper ones grew into grotesque shapes.

Further studies on the cause of ophthalmia in rats produced with diets containing vitamin A: E. V. MCCOLLUM, NINA SIMMONDS and J. ERNESTEIN BECKER. An intensive study designed to analyze the problem of factors concerned in producing ophthalmia by diets containing excessive amounts of inorganic elements in the presence of sufficient vitamin A indicates that no single element is responsible. Experimental conditions were devised under which the appearance of ophthalmia was determined by the vitamin B content of the diet. The interpretation of the results is complex, and reference must be made to the original publication.

The action of sodium phosphate on hexose sugars: H. A. SPOEHR and PAUL C. WILBUR. Solutions of hexose sugars in the presence of disodium hydrogen phosphate undergo mutual conversion resulting in an equilibrium mixture of aldoses, 2-ketohexoses and 3-ketohexoses. The rate of change depends upon the concentration of sodium phosphate and the temperature. With sodium phosphate there is no indication of saccharinic acid formation from the sugars, nor is there any evidence of polysaccharide formation. At lower temperature (38°) the action of sodium phosphate is confined to the interconversion reaction, while at higher temperature (70°) there is evidence of more complex reaction of the sugar molecule and the phosphate.

A further report on cholesterol and phytosterol activated by irradiation: A. F. HESS and MILDRED WEINSTOCK. Irradiated cholesterol prevents rickets when given subcutaneously. It is of value when animals are fed a ration low in calcium or low in phosphorus. Experiments with selective filters demonstrated the wave-lengths of ultra-violet which are able to bring about activation. Human or calf's skin can be rendered antirachitic by irradiation, owing to their cholesterol content. Dihydrocholesterol, dihydro-phytosterol and certain unsaturated terpenes were of no protective value following irradiation. Spectrograms of the various irradiated substances showed an alteration in transmission which paralleled their biological activity.

The influence of the bacterial flora on the biological test for vitamine B: V. G. HELLER, C. H. MCELROY and BERTHA GARLOCK. Steenbock, Sell and Nelson demonstrated that the growth periods of rats fed on a vitamine free diet was considerably prolonged when they had access to their feces. Various investigators have found certain microorganisms to be synthesizers of vitamines. We have attempted to find that the prolonged growth period might be due to the presence of such organisms in the intestinal flora. Rats were divided into lots. The first placed on shavings, the second on screens and others on screens with access to various types of roughage; all were fed rations deficient in vitamine B. Examinations of the feces from the various lots show that the spore-forming organisms present vary directly with the observed extension of the growth period, disappearing from the feces somewhat prior to the time that growth ceases. These organisms have been cultured and grown upon vitamine free media and their vitamine content measured to verify the theory.

A chemical and nutritive study of the grain sorghums: V. G. HELLER and ROBERT A. GREENE. The grain sorghums thrive in the semi-arid cattle country of the southwest, where corn can not be raised. Considerable argument exists in regard to their feeding value. A few nutritive studies have been made of isolated varieties by different investigators with contradictory evidence in regard to the vitamine and protein content. Our chemical and nutritive studies of twenty-two members of the family show small individual variations. The proteins are of high quality, but like corn are deficient in certain amino acids. These deficiencies may be overcome by the addition of small amounts of certain goods, the resulting ration then produces normal growth, reproduction and successful rearing of young through at least three generations. A complete study of the vitamine contents are reported. Its wider use for feeding should be recommended.

Soft pork and its causes: J. O. HALVERSON and EARL HOSTETLER. The problem of what constitutes soft pork is discussed. Methods of investigations are given. The ration fed is the largest factor causing soft pork. This may be divided into two classes: (a) the "softening" ration, which is high in protein and in oil, and (b) the "hardening" ration, which does not contain a high percentage of oil but consists chiefly of starch. The "softening" rations of the south, which are largely high in protein and in oil, are the peanut and the soy bean. The cereal rations of the north are the "hardening" rations. These consist chiefly in starch or carbohydrates.

Results of soft pork investigations: J. O. HALVERSON and EARL HOSTETLER. The effect was studied of various methods of feeding hardening and softening feeds, especially the peanut, and Brewer's rice, which consists chiefly of starch with very little ether extraction. Equal total amounts of oil were fed individually throughout to each pig. To some were given equivalent amounts of starch (as Brewer's rice) at the same time. To others the oil-feeding period was followed by the Brewer's rice.

All pigs consumed the same amount of peanut oil and equivalent amounts of starch throughout the experiment, according to the energy relation of fat to starch, 2.24. The hardening period of Brewer's rice following peanuts resulted in a harder carcass in every instance. All pigs throughout these experiments were individually fed a "standard" ration of equal energy, total digestible protein and total oil intake. The work indicates that to harden a peanut-fed hog the pig must consume a starch equivalent to 2¼ to 2½ times the total amount of oil consumed.

Soft pork studies: Formation of fat in the pig on a ration moderately low in fat: N. R. ELLIS and O. G. HANKINS. In connection with cooperative soft pork studies conducted by the department of agriculture and a number of state experiment stations, a quantitative study was made of the progressive hardening of pigs on a ration of corn and protein supplement. From pigs slaughtered at various stages of development, determinations were made of the amounts of fat consumed and the quantity and composition of the fat deposited. Fat formed and deposited by an animal is usually hard. Ingested fat tends to be deposited with but slight modification of its characteristics. An increase in hardness in these experiments was accompanied by an increased rate of deposition. The soft fat of the corn had less and less diluting effect on the harder synthesized fat. The saturated acids of the lard increased, the oleic acid remained constant, and the linolic acid decreased.

Proteins of wheat bran. III. The nutritive properties of the proteins of wheat bran: JOSEPH C. MURPHY and D. BREESE JONES. Chemical investigation of the proteins of wheat bran has shown that these proteins are relatively rich in the so-called nutritionally essential amino acids. These results have now been corroborated by feeding experiments with young rats, using a diet in which bran constituted practically the sole source of protein. Growth at a rate better than normal has resulted when the bran was fed at a crude protein intake level of 9.9 per cent. ($N \times 6.25$). Better results were obtained with clean commercial bran than with the same bran after having been washed. Utilization of the proteins was found to be decidedly improved by grinding the bran.

A pure culture apparatus for laboratory use: F. M. HILDEBRANDT. The apparatus consists of a Pasteur flask for holding sterile growing solution and a tube in which the organisms are grown, the two vessels being so connected that liquid may be passed from the flask to the tube without danger of contamination. Provision is made for withdrawing the culture and refilling the flask with sterile solution when necessary.

The chemistry of blood clotting: A. P. MATHEWS and C. A. MILLS. There are two normal physiological mechanisms of blood clotting: thrombin clotting and tissue fibrinogen clotting. These are independent processes. Tissue fibrinogen was prepared free from uncombined cephalin. It clotted blood plasma very quickly. The serum from this clot had all the prothrombin or serozyme in unchanged amount in it. No thrombin was formed. We adsorbed the prothrombin on calcium phosphate, thus giving a plasma free from prothrombin. This was clotted as quickly by tissue fibrinogen as if all the prothrombin had been present. Also clotting by thrombin does not involve tissue fibrinogen. The essential basis of the process consists in making an electro-positive colloid in the plasma. This then unites with the electro-negative fibrinogen to make the complex protein fibrin. There are two different fibrins.

A biochemical study of the false blossom of the cranberry: CHAS. P. SPAETH and H. R. KRAYBILL. False blossom plants are higher in free reducing sugars, sucrose, starch, lipoid substances and dry matter than the healthy plants. This condition is similar to that of the mosaic disease of spinach. No consistent differences were found in total nitrogen content of healthy and diseased plants. An increase in anthocyanin pigment accompanies the accumulation of carbohydrates in the leaves and stems of the diseased plants. The chemical data indicate that the disease known as false blossom of the cranberry may be similar to the mosaic diseases of other plants.

The relationship between chemical structure and physiological action of halogen and hydro derivatives of 2 oxo, 3 indole propionic acid: E. C. KENDALL and A. E. OSTERBERG. A chemical study of the derivatives of 2 oxo, 3 indole propionic acid has shown that an ethylene imine linkage can exist between number seven carbon and the nitrogen. A series of substances containing this group will be described. These substances have physiological activity when they are injected with the ethylene imine linkage if the pyrrolidone ring is closed. Their activity is not manifested when the pyrrolidone ring is open. Oxidizing potentials agree with the physiological behavior. The imine linkage of the closed pyrrolidone ring has a higher oxidizing potential than the same compound with the pyrrolidone ring open. The relationship between physiological activity and this ethylene imine group will be discussed and the occurrence of this same grouping in other substances having similar chemical and physiologic activity will be pointed out.

Autoclaving and scorbutic diets: EDWARD F. KOHMAN and WALTER H. EDDY. We have demonstrated that autoclaving canned cabbage 30 minutes at 100° or 30 minutes at 127° F. produces no difference in its vitamine C content; autoclaving canned spinach 70 minutes at 115° or 120 minutes at 115° produces no difference in the vitamine C content; autoclaving canned peas 25 minutes at 120° or 50 minutes at 120° produces little if any difference in the vitamine C content. Until some one demonstrates that traces of vitamine C in soy bean and similar products are actually destroyed by autoclaving, the investigations in question do not meet the issue. In the light of our findings it appears that the only way to meet it is to fall back on synthetic materials to secure a diet free from vitamine C.

R. J. ANDERSON,
Secretary