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ABSTRACTS OF PAPERS

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The establishment of precise spectrophotometric constants for hemochromogens and Cytochrome C, upon an iron basis, and the analysis of the Cytochrome C spectrum: DAVID L. DRABKIN[1] (introduced by W. M. Clark). A new method[2] has been modified appropriately and adapted successfully to the accurate determination of iron in such organic complexes as hemin and Cytochrome C. The iron, freed from the complex and reduced under controlled pH conditions, is converted into ferrous orthophenanthroline, which has an absorption maximum at wave-length 500 mμ. The light transmission (from which the extinction is calculated) was determined by precise, visual spectrophotometry, or by means of a photoelectric filter photometer of new design (to be described elsewhere). 10 mg samples of Cytochrome C, the purest preparations of which contain 0.43 per cent. iron, were sufficient for accurate analysis. The precision of the method was equal to that of spectrophotometry under optimal conditions. Using the iron content as the basis for the concentration of total pigment, spectrophotometric constants, ε[3] (C = 1 mM per liter (iron equivalent basis), d = 1 cm, spectral interval = 1.5 to 2 mμ), were established for dipyridino ferroporphyrin (a synthetic hemochromogen) and Cytochrome C.

The kinetics of the enzyme-substrate compound of peroxidase and their relation to the Michaelis theory: BRITTON CHANCE, J. G. BRAINERD, F. A. CAJORI and G. A. MILLIKAN (introduced by A. N. Richards). A study of the kinetics of the formation and breakdown of the enzyme-substrate compound of peroxidase has been carried out by means of a photoelectric method for measuring rapid changes in the violet absorption spectrum of the enzyme. Under the same conditions, photoelectric measurement of changes in the red absorption spectrum of malachite green were recorded as leuco-malachite green was oxidized by peroxidase and hydrogen peroxide. The complete cycle of enzyme operation was recorded: the rapid formation of the enzyme-substrate compound, the enzyme operation at constant concentration of enzyme-substrate compound and the decrease in concentration of this compound when the substrate was used up. Correspondingly, the rate of formation of malachite green started slowly, accelerated to a large constant value, and then fell to zero. Thus the operation of the enzyme-substrate compound has been directly related to the over-all enzyme action. The Michaelis affinity for peroxidase, determined by direct measurement of the concentration of enzyme-substrate compound as a function of substrate concentration, was found to be in agreement with the values obtained elsewhere by measurement of the rate of malachite green formation. Solutions of the differential equations representing the Michaelis theory were obtained by the University of Pennsylvania differential analyzer and fitted the observed data in curve shape, time scale and concentration values over a part of the experimental range.

The synthesis of chroman derivatives: RALPH CONNOR and PETER L. DE BENNEVILLE (introduced by M. H. Jacobs). The synthesis of chromans received relatively little attention until it was discovered in 1938 that the tocopherols (Vitamin E) were chroman derivatives. In the last two years the chemistry of this series has been studied in a number of laboratories with the result that good methods are now available for the synthesis of 2,2-dialkylchromans. These methods, however, are not applicable to the synthesis of chromans with substituents in the *3* and *4* positions. The present report describes a new synthesis of chromans which should be applicable to the synthesis of any chromans except those with substituents in the *2* position. The hydrogenation of coumarin over copper chromite at 250° and under a hydrogen pressure of 2,000–3,000 pounds per square inch gave o-(γ-hydroxy-propyl)-phenol (85–90 per cent.). This phenolic alcohol, upon treatment with phosphorus tribromide, gave chroman (85–90 per cent.). By the application of this method to substituted coumarins, 4,7-dimethylchroman, 6-methylchroman, 7-methylchroman, 7,8-benzochroman and 7,8-tetrahydrobenzochroman were obtained. From 4-methyl-6-hydroxycoumarin the product was 4-methyl-6-hydroxy-hexahydrochroman. When nickel was used as a catalyst at 250°, hydrogenated chromans were the chief products obtained from coumarins. This is the first synthesis of compounds of this type. By this method the completely hydrogenated derivatives corresponding to all the chromans named above were isolated. These results indicate that coumarins, which are fairly readily prepared by known methods, may be readily converted to either chromans or hexahydrochromans by selection of the appropriate catalyst.

Sulfonyl ureas: EDWARD H. COX (introduced by C. E. McClung). Only two methods for the preparation of sulfonyl ureas are recorded in the literature. One, the ammonolysis of sulfonyl isocyanates is dangerous to carry out and gives low yields of desired product, the second, the reaction of potassium cyanate on sulfonamides is not duplicatable. We, therefore, set out to find our own method. Attempts to treat urea with sulfonyl chlorides under varying conditions, acid and alkaline ($Ba(OH)_2$) hydrolysis of sulfonyl quanidines, and acid hydrolysis of sulfonyl alkylthioureas, failed. Ammonolysis of sulfonyl urethanes also failed. We have found that the sulfonyl alkyl isoureas can be hydrolyzed to the ureas in good yields. The base, ethyl isourea is prepared as the hydrochloride salt when cyanamide dihydrochloride and cyanamide is treated with ethyl alcohol under pressure. The sulfonyl isoureas are prepared by treating the sulfonyl chloride and the isourea salt with the proper amount of alkaline solution. The sulfonyl isoureas are then hydrolyzed to the corresponding ureas by hydrochloric acid.

[1] Fellow of the John Simon Guggenheim Memorial Foundation at the Department of Physiological Chemistry, Johns Hopkins School of Medicine. Part of this work was done during tenure of the fellowship.

[2] O. Schales, Personal communication. W. Lintzel, *Z. ges. exp. Med.*, 86: 269, 1933. G. Barkan and B. S. Walker, *Jour. Biol. Chem.*, 131: 447, 1939; 135: 37, 1940.

[3] ε, unmodified, is the symbol for *extinction* and is equal to $-\log \tau$ (*transmission*, expressed as a fraction).

This work is preliminary to the preparation of sulfonyl ureas derivatives in the sulfanilamide series.

Mono- and polyhydroxyprogesterones: MAXIMILIAN EHRENSTEIN and THELMA O. STEVENS (introduced by A. N. Richards). The hormones of the adrenal cortex represent hydroxyprogesterones in which the side chain of the steroid molecule carries a primary alcohol group. In addition, one or two hydroxyl groups may be attached to the nucleus. It has been possible to obtain by synthetic chemical procedures hydroxyprogesterones which are different from the naturally occurring compounds and to subject them to a physiological examination. These compounds will be briefly discussed with reference to the subject of steroid structure and biological activity.

The gonadotropic hormone of urine of pregnancy: SAMUEL GURIN, CARL BACHMAN and D. WRIGHT WILSON (introduced by A. N. Richards). The gonadotropic hormone of pregnancy urine has been prepared in a high state of purity. It is a glycoprotein and has been studied by physical and chemical methods. The crude hormone was obtained from fresh urine collected from women about two months pregnant by Katzman and Doisy's adsorption method on benzoic acid. This material assaying 30–50 minimal effective doses (Friedman Units) per mg was extracted with 50 per cent. ethanol at pH 6.0 and the derived product extracted with the same solvent at pH 4.8. Preparations assaying from 1,000 to 3,000 Friedman units were obtained. Further purification was accomplished by shaking a solution of the hormone with chloroform and dialyzing the resulting solution. The material obtained by precipitation with acetone had an activity of 4,000 Friedman units. All manipulations were carried out at about 0° C. Our best material was apparently well purified because it showed homogeneity in the ultracentrifuge and the Tiselius apparatus. The protein contains 15 per cent. carbohydrate. Two thirds of the carbohydrate is galactose and one third a hexosamine. The material is unstable in dilute solution at 0° C. An approximation suggests that the minimum molecular weight is about 80,000. Comparisons of our assay method with the International Standard indicate that our preparation which assays 4,000 Friedman units per mg contains about 8,000 international units per milligram.

Preparation of crystalline pepsin having the properties of a pure protein: ROGER M. HERRIOTT, VICTOR DESREUX and JOHN H. NORTHROP. The three most commonly accepted tests for the purity of a protein are homogeneity in the ultra-centrifuge and in the electrophoresis cell and constant solubility. The solubility method is based on the phase rule which states that the solubility of a pure substance must be constant and independent of the amount of solid present in the system. This method is quite analogous to the classical melting point test for purity used in organic chemistry but which is not applicable to proteins. Very few proteins have been shown to be pure by all three tests. Crystalline pepsin prepared by the method originally described shows only one protein component when analyzed in the ultra-centrifuge or in the electrophoresis cell. The solubility of such preparations, however, usually varies with the amount of solid protein present, indicating the presence of more than one component. These preparations may be separated by fractional precipitation into at least two proteins of slightly different solubility and enzymatic activity. A method has been worked out for the isolation and crystallization of the more soluble of these proteins. Such preparations of crystalline pepsin have constant enzymatic activity and their solubility in several solvents is independent of the amount of solid phase present.

A simplified procedure for isolating the polysaccharides and nucleic acid of tuberculin using electrophoresis: FLORENCE B. SEIBERT and DENNIS W. WATSON (introduced by D. H. Tennent). A simple scheme was devised for making a rough separation of the protein, nucleic acid and polysaccharide fractions of tuberculin. The protein was precipitated from the heated culture filtrate of tubercle bacilli, grown upon synthetic medium, by half saturation with ammonium sulfate at pH– 7.0 and 5° C. On standing in the cold the filtrate deposited a sediment, designated S2. The filtrate was carefully adjusted to pH– 4.0 with hydrochloric acid, yielding a flocculent precipitate, designated S3. Alcohol (95 per cent.) was then added to the filtrate until a brown sticky mass appeared at the interface between the two liquids. This mass was further separated into two fractions; one designated S4, which was readily soluble in water, and another designated S5, which was much less soluble. To the filtrate from these, more alcohol was added to remove ammonium sulfate and the remaining solution was concentrated in vacuo to a syrup, dialyzed in an extra heavy membrane, and dried in the cryochem, yielding a product designated S6. Electrophoretic studies in the Tiselius apparatus showed that all six fractions consisted of varying proportions of nucleic acid, protein and polysaccharide, a variance which probably accounted for their different physical properties. Chemical analyses confirmed the conclusions. Two types of polysaccharides were isolated in a Tiselius macroelectrophoresis cell, as well as by chemical separations. One type, which predominated in the S6 fraction, was immobile, homogeneous, colorless and contained only about 0.2 per cent. nitrogen. The other polysaccharide had a low mobility and was present in much larger quantities in the S4 fraction; its nitrogenous content, consisting of protein and nucleic acid, could never be reduced to less than 1.6 per cent. nitrogen, even by prolonged electrophoresis. Moreover, the component always remained diffuse, indicating that it is probably not a true compound. Pure nucleic acid was also isolated in the macroelectrophoresis cell.

Concerning the internal organization of the streptococcal cell: STUART MUDD (introduced by O. T. Avery). New insight is being gained into the structure of the bacterial cell through the use of the new electron microscopes available at the RCA Laboratories. The chain structure of the hemolytic streptococcus, for instance, is seen to be due to the continuity from cell to cell of a solid surface membrane which is differentiated from an interior protoplasm. Cytolysis, as, for instance, under the action of sonic vibration, may cause this inner protoplasm to escape, leaving an empty membrane analogous to the stroma of a hemolyzed red cell (Mudd and Lackman). A single strain of Streptococcus pyogenes may occur in forms varying from the virulent mucoid va-

riant with its extracellular envelope, to the naked pleomorphic cell of the rough variant, which is only just recognizable as a streptococcus. Chemically the streptococcal cell has been shown by Sevag, Smolens and Lackman to be about 80 per cent. protein and nucleoprotein. From 18 to 24 per cent. of the dry weight of mucoid and smooth variants is nucleic acid, and from 14 to 17 per cent. of the rough variants; of the nucleic acid 10 to 30 per cent. gives the color reactions characteristic of the desoxyribose type and the remainder the reactions characteristic of the d-ribose type. In higher animals and plants the desoxyribose type of nucleic acid is characteristically found in the nucleus and the d-ribose type in the cytoplasm—yet no indication of the existence of a nucleus within the streptococcal cell has been found nor has any phenomenon resembling karyokinesis been observed in this or other pathogenic bacterium. The nutritional requirements of the streptococcus are extremely exacting. Thus Pappenheimer and Hottle in this laboratory have found that a certain strain of streptococcus requires, besides a source of carbon and necessary minerals, several particular amino acids, and the six characterized members of the vitamin B complex—thiamin, riboflavine, pyridoxine, pantothenic acid, nicotinic acid and biotin. The metabolic potentialities of the streptococcal cell are equally complex. For instance, beside the protein, nucleic acid, carbohydrate and lipoid of its own protoplasm, the streptococcal cell has been shown to elaborate the erythrogenic toxin, a protein hemolysin, a fibrinolysin, a leucocidin and a newly detected lethal substance (Harris). The nature of the organization within a cell of only about 1 μ diameter, which makes possible such complex syntheses and transformations of matter, is not even definitely suggested by present-day knowledge.

The relation of hereditary constitution, allergic irritability, antibody production and the development of local immunity in resistance to tuberculosis: MAX B. LURIE (introduced by E. L. Opie). By brother and sister inbreeding of rabbit groups, under strictly similar environmental conditions, six families have been obtained in which the genetic constitution alone determines a characteristic inherited resistance to tuberculosis of each family, generation after inbred generation. Two of these families are highly susceptible to the disase. One is of exceptionally high resistance to the infection and three are intermediate in their susceptibility to tuberculosis. The fundamental variant in the disease developed by these three family groups is the degree to which the infection is limited to the portal of entry of the bacillus. In tuberculosis naturally acquired by respiratory contagion, the most resistant family confines the disease to the lungs where the infection originates and where it slowly progresses. There is little if any dissemination to the rest of the body. In the most susceptible families there is a rapid spread by the lymph and blood from the fulminating primary pulmonary lesion. The families of intermediate resistance exhibit an intermediate degree of generalization of the disease. This suggests that resistance to tuberculosis is largely a function of those constitutional factors which determines the host-parasite relationship at the portal of entry. When members of these three family groups were treated with heat-killed tubercle bacilli intracutaneously it was found that the most resistant family rapidly developed a high degree of tuberculin sensitivity, which was maintained at a high level. Likewise antibodies (agglutinins) against the tubercle bacillus soon appeared in the blood and attained a considerable concentration therein. The most typical and constantly susceptible family developed allergic sensitivity very slowly. Similarly in this family, antibodies appeared in the blood later and attained a concentration considerably below that of the resistant family. The families of intermediate resistance developed tuberculin sensitivity even more rapidly than the resistant one. However, the momentum of this increment soon abated and sank below that of the resistant rabbits. The antibody production of these families was intermediate in rate and intensity between those of the most susceptible and most resistant families. If rabbits thus treated with killed tubercle bacilli are now infected with virulent living microorganisms in the skin the most resistant family develops a lesion which rapidly attains a maximum and soon heals. It quickly develops a local immunity. The mononuclear phagocytes rapidly acquire an effective capacity to inhibit the growth of the bacillus in their cytoplasm, for bacilli are rarely found in them. In the most susceptible families the local lesion develops apace with but retarded and feeble healing. The phagocytes do not acquire sufficient power to inhibit the growth of the bacilli. Their cytoplasm swarms with them. An intermediate degree of local immunity developed in the rabbit families of intermediate resistance.

Studies on air-borne infection: WILLIAM FIRTH WELLS (introduced by E. B. Wilson). An apparatus for study of the bacterial behavior of air has revealed a mechanism of air-borne infection leading to a hypothesis that the semi-enclosed atmospheres of our habitations provide a vehicle for the epidemic spread of contagion. *Air as a vehicle of contagion:* Evaporation of minute droplets expelled in expiratory processes enables infection to ride these droplet nuclei on air-currents. Quantitative experiments upon animals, inhaling nuclei-infected air, demonstrate the penetration of these nuclei to the depths of the lung with consequent production of disease. The transfer of nuclei infection from infector to infectee depends upon conditions of sanitary ventilation, and the equilibrium concentration predicted by the ratio of elimination to addition rate of test organisms in semi-enclosed spaces corresponds to the concentration of nasopharyngeal organisms determined by sanitary air analysis. Aggregation within the same enclosed atmosphere implies the proximity in time and space which dominates the idea of contagion, and is implied by the term "contacts." Air-borne infections thus become contagious infections. *Dynamics of epidemic contagion spread:* Contagious epidemics are characteristically dynamic, waxing or waning with infection velocity depending upon atmospheric density of susceptibles. If susceptible density depends upon immunity conferred by infection, then the magnitude of an epidemic

will be double the initial excess density of susceptibles over the "threshold density" determined by critical sanitary ventilation. *Experimentum crucis:* Bactericidal irradiation of air provides an instrument for making the experimentum crucis, *i.e.*, the effect of air disinfection upon the incidence of contagion introduced into susceptible aggregations, first to determine whether the epidemic spread of childhood contagions through aggregations of susceptible children can be prevented by raising the sanitary ventilation above a critical value. For three successive years classes in irradiated rooms of the primary department of the Germantown Friends School have been spared the epidemic spread of mumps or chicken-pox suffered by comparable classes in unirradiated rooms in each of these years. *Conclusion:* We intend to pursue the theory to scientific proof but believe that enough is now known to warrant the recommendation that ultraviolet radiation apparatus be installed in buildings designed to house large companies of men.

Fat metabolism in diabetes mellitus: WILLIAM C. STADIE, JOHN A. ZAPP, JR., and F. D. W. LUKENS (introduced by D. D. Van Slyke). The current theories of fat metabolism in diabetes mellitus are: 1. Knoop's hypothesis of successive beta oxidation. 2. Hypothesis of obligatory coupled ketone body-carbohydrate oxidation. (3) Fatty acids are converted by the liver into carbohydrate. Evidence in the literature and our own experiments convince us that these hypotheses are no longer tenable. That ketone bodies are utilized by the peripheral tissue of the diabetic animal was shown by four independent methods in the depancreatized cat. Therefore the depancreatized cat must oxidize ketone bodies without coupling with carbohydrate oxidation. The multiple alternate oxidation hypothesis postulates that fatty acids are simultaneously oxidized along the entire length of the chain at alternate carbon atoms with the formation of four molecules of ketone bodies per molecule of fatty acid and no acetic acid. Further experiments conformed to the hypothesis of multiple alternate oxidation rather than the Knoop hypothesis. By means of the multiple alternate oxidation hypothesis the ketone utilization in human cases of diabetes was calculated. The calculations show an abundant utilization of ketone bodies independent of carbohydrate oxidation. A new hypothesis for the metabolism of fats in the diabetic was formulated: Up to a certain level of fat catabolism (about 2.5 gm. of fat per Kg per day) fats are utilized completely and there is no ketonuria. When total fat catabolism exceeds this amount only a part of the fat is completely oxidized. The balance is excreted as ketone bodies. There is no obligatory chemical coupling of fat and carbohydrate oxidation. In further experiments with liver slices from depancreatized cat the important oxidative reactions were measured. The results show that there was no oxygen available for the conversion of fat to carbohydrate. It was concluded that the overproduction by the liver of carbohydrate from fat does not occur.

Qualitative differences in the biological activity of adrenal cortical steroids: DWIGHT J. INGLE (introduced by A. N. Richards). Extracts of the adrenal cortex have a number of measurable physiologic properties. They are capable of maintaining life in adrenalectomized animals, effect the distribution of electrolytes in the body, the amounts of carbohydrates in the body; they will maintain the ability of the adrenalectomized animal to work, and when administered in large amounts to normal animals will cause the atrophy of the adrenal cortices. A large number of pure compounds have been isolated from extracts of the adrenal cortex. Some of these compounds are biologically inactive and the active compounds show qualitative differences in their biologic properties. Thus it is found that those compounds which are the most active in prolonging the life of adrenalectomized animals are incapable of maintaining normal carbohydrate metabolism. On the other hand, compounds which have the greatest effect upon the carbohydrate metabolism of normal or of adrenalectomized animals are only weakly active for maintaining life and in effecting the distribution of electrolytes.

(To be continued)

REPORTS

A NEW PAN AMERICAN TREATY

"WASHINGTON, October 12—American Republics Sign Convention on Nature Protection." Such was the heading of a news release sent out by the Pan American Union. This was the announcement of the inception of an International Nature Protection Treaty designed to meet the international wildlife problems of the twenty-one American Republics. This is the first treaty of its kind ever to be proposed on this continent. It is planned to include twice as many countries as the only other International Convention of a similar nature. I refer to the London-African Convention of 1933, which included no provisions for migratory bird protection.

This Pan American Convention marks the third great cooperative step taken by the United States to further wildlife protection by international treaty on the American Continent.

In 1916 we ratified the Migratory Bird Treaty with Great Britain with respect to Canada. This treaty became effective in 1918. In 1936 we ratified a somewhat similar treaty with Mexico. In addition to these treaties we have entered into international agreements regarding seals, whaling, and fisheries. The Pan American Convention on Nature Protection and Wildlife Preservation in the Western Hemisphere covers a broad virgin field for international agreement.

It establishes a basic pattern for a scheme of parks and reserves throughout the Americas which our own experience has taught us is thoroughly sound. It